VOLUME 3
NUMBER 2
2019

Khazar Journal of Science and Technology

KHAZAR
UNIVERSITY PRESS

Vol. 3, №2, 2019 ISSN 2520-6133

KHAZAR JOURNAL OF SCIENCE AND TECHNOLOGY (KJSAT)

Hamlet Isayev/Isaxanli *
Editor-in-Chief
Khazar University, Azerbaijan

*Also H. Issakhanly, H. Isakhanly, H. Isakhanli, H.A.Isayev, G.A.Isayev, or G.A.Isaev due to differences in transliteration. I use Hamlet Isayev in mathematics and science fields and Hamlet Isakhanli/Isaxanli in humanities.

Khazar University
41 Mehseti str., Baku AZ1096
Republic of Azerbaijan
Tel: (99412) 4217927
Website: www.kjsat.com

KHAZAR UNIVERSITY PRESS

Contents

Malihe Eshaghzadeh, Samaneh Sardashti, Maryam Seyyedi Noosh Abad
Evaluation of the Relationship Between Reflux Esophagitis and Helicobacter Pylori Stomach Infection in Patients Referring to Zahedan Hospitals, Sistan and Baluchistan Province, Iran *5*

Safada Taghiyeva
Determination of Antibacterial Properties of the Bacillus Cereus ATCC 14579 Strain for Application in Synthesis of Bacteriocins by New Methods *14*

Samaneh Sardashti, Malihe Saghzadeh, Hamideh Yaghoobi
Use of Paracetamol and Ibuprofen in Combination for Pain Relief in Children after Oral Surgery *21*

Mohammad Yadegari1, Hasan Ghahri, Mohsen Daneshyar
The Effects of Savory (Satureja khuzistanica) Extract on Performance, Organ Weight, Blood Parameters and Immune Function in Heat Stressed Broilers *28*

Konul Gasim Hasanova
Changes to Some Hormones and Glucose in the Blood of Rabbits Subjected to Physical Exertion and Regimes of Darkness and Light, and Subfetal Hypoxia of Ontogenesis *41*

Kamala Asadova
Bioecological Features of Some Feed, Poisonous and Noxious Plants on the Winter Pastures of the Mil Steppe in Azerbaijan *56*

Abdul-Rahman Balogun Muhammed-Shittu
An Endowment (Al-waqf) Concept for the Supply of Medical Care: an Exploratory Study of the Sustainability of Health Insurance at Higher Institutions *63*

Narmin Mahmudova
The Importance of Using Database Management Systems in Hospitals *73*

Nigar Mammadova
The Importance of Health Information Systems and Implementation of an Electronic Healthcare System *80*

Information to Contributors 87

Khazar Journal of Science and Technology Volume 3 №2 2019, 5-13

DOI: 10.5782/2520-6133.2019.3.2.5

Evaluation of the Relationship Between Reflux Esophagitis and Helicobacter Pylori Stomach Infection in Patients Referring to Zahedan Hospitals, Sistan and Baluchistan Province, Iran

Malihe Eshaghzadeh*, Samaneh Sardashti, Maryam Seyyedi Noosh Abad

Department of Nursing, School of Nursing and Midwifery, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran
Corresponding author: m.eshaghzadeh93@gmail.com

Abstract

Gastro-oesophageal reflux disease is a common disorder of the digestive tract. Exposure of the oesophageal mucosa to gastric acid can lead to mucosal damage as in oesophagitis. The aim of this study was to determine the relationship between Helicobacter pylori infection in the stomach and reflux oesophagitis in patients, referring to the endoscopy units of hospitals in Zahedan between 2015 and 2019. In this cross-sectional study, all patients (under 15, 15-25, 25-35, 35-45 and 45 years and older) who referred to the Endoscopy units of two randomly selected hospitals in Zahedan during 2015-2019, were studied. H. pylori infection was diagnosed from pathological specimens. Information on all patients, including age, sex, presence of oesophagitis and its grade, and H. pylori infection was recorded on questionnaires and their relationships with oesophagitis and its grade as well as H. pylori infection were analyzed statistically. Tables and charts were used to illustrate the data. T-tests and Chi-squared tests were used to investigate the relationships between variables. The results showed that the frequency of oesophagitis in our patients was 52.8%, with the highest frequency being obtained for grade B oesophagitis (48%). In this study, the prevalence of H. pylori infection was 57.5%. This rate was 55.4% in patients with oesophagitis, with a statistically significant difference from the group without oesophagitis. There was a significant relationship between grade A oesophagitis and H. pylori infection. In conclusion, we were able to demonstrate a significant relationship between H. pylori infection and oesophagitis in affected patients.

Keywords: *H. pylori* bacteria, Reflux oesophagitis, Endoscopy unit, Hospital, Zahedan

Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral, microaerophilic bacillus that can be isolated only from humans and other primates (Aydogdu *et al.*, 2009). It has the ability to reach the protective mucus layer at the surface of gastric mucosa and to survive the extreme acid content of the stomach thanks to its 4-6 flagella (Aziz et *al.*, 2015), and by avoiding low pH areas using chemiotaxis it first colonizes the antrum, where there are no acid-producers cells (Bore *et al.*, 2017). Approximately, half of the world's population is infected with the bacterium and the prevalence of its infection varies in different countries (Camilo *et al.*, 2017), with about 25 per cent in developed countries, and around 80 per cent in developing countries, including Iran (Queiroz, 2014). It is colonized in the gastric mucosal epithelium and, through the formation of oxidative stress, causes damage and apoptosis, thereby causing changes in the gastric epithelial tissue (Ciclitira, 2001, Feighery, 1999). Symptoms of the infection include decreased appetite, abdominal pain during the night or while eating, weight loss, pallor and other abdominal symptoms (Camilo, 2017; Diamanti, 1999). The H. pylori infection also leads to gastritis, peptic ulcers and chronic pathological changes in the gastric mucosa, such as atrophy, metaplasia and adenocarcinoma (Ghoshal & Chourasia, 2010). Gastro oesophageal reflux disease is a common disorder caused by an increase in the frequency or duration of oesophageal contact with the contents of the stomach. Oesophageal mucosa contact with gastric acid can lead to visible mucosal lesions in the endoscopy, including erosive oesophagitis, oesophageal stricture, and Barrett's oesophagus (Haruma, 2004; Jozefczuk *et al.*, 2005). Since the prevalence of H. pylori infection and gastro-oesophageal reflux disease is high (Kandulski & Malfertheiner, 2014), some correlation is expected to exist between them. The results of studies show that H. pylori may be directly correlated with oesophagitis and gastro-oesophageal reflux disease through various mechanisms. These mechanisms include increased acid secretion, direct infection of the columnar epithelium in the distal oesophagus with H. pylori, and indirect damage due to release of harmful substances into the gastric juice (Konturek *et al.*, 2000). Colonization of the gastric mucosa with H. pylori can lead to diffuse and atrophic gastritis, which reduces gastric acid and therefore oesophagitis (Konturek *et al.*, 2000). The association between H. pylori infection and oesophagitis has been controversial over recent decades. H. pylori infection, despite increasing the risk of peptic ulcer and gastric cancer, has been associated with a substantial reduction in the risk of oesophagitis, Barrett’s oesophagus and oesophageal adenocarcinoma (Diamanti *et al.*, 1999; Luzza *et al.*, 1999). Referring to several population studies, there is a noticeably inverse relationship between H. pylori and gastro-oesophageal reflux, but when considering the single patient this relationship is difficult to explain, since gastro-oesophageal reflux is a disease determined by several

concomitant factors. Some studies have examined the association between H. pylori infection, Barrett's oesophagus and adenocarcinoma, with contradictory results (Memeo *et al.,* 2005). The results of some studies have indicated that eradication of H. pylori may cause oesophagitis reflux and exacerbate the symptoms of oesophagitis (Konturek *et al.,* 2000; Nejad *et al.,* 2011). Studies have shown a low prevalence of H. pylori in patients with gastro-oesophageal reflux disease and oesophagitis (Villanacci *et al.,* 2006). Considering the high prevalence of oesophagitis and H. pylori in endoscopic patients and the importance of H. pylori infection treatment and eradication, the aim of the current study was to determine the relationship between H. pylori stomach infection and reflux oesophagitis in patients referring to the Endoscopy Units of two hospitals in Zahedan from 2015-2019.

Materials and Methods

All patients who underwent endoscopy for various reasons, such as gastrointestinal bleeding, dyspepsia, abdominal pain, anaemia, and other signs of risk and indications, were included in the study if their information was complete. Reports with incomplete data were excluded. In this cross-sectional study, all patients who referred to the Endoscopy Units of two hospitals in Zahedan for endoscopy during 2015-2019 were studied. Prior to pathological sampling and before endoscopy, informed consent for the use of patient information in relevant research was obtained from all patients. All patient information was also kept confidential, and disclosure of information was avoided. Identification codes were used to prevent registration of the patient's name and surname. The data were recorded on SPSS software as numerical codes. The severity of oesophagitis was determined by Los Angeles classification, consisting of 4 grades, such as A, B, C and D. The endoscopy was performed by two gastrointestinologists in the endoscopy units of the hospitals mentioned. Additionally, endoscopic reports were drawn from the hospital system and information including age, sex, cause of endoscopy and current lesions in different parts of the digestive system were recorded on the checklist. Further, pathologic reports on these patients were obtained from pathology laboratories. Diagnosis of H. pylori infection was based on pathological specimens. All patients' information, including age, sex, oesophagitis and its grade, and H. pylori infection, was recorded on the questionnaire. After data collection, the data were entered into the SPSS version 15 and analyzed for association between oesophagitis and its grade, and H. pylori infection.

Data analysis

T-tests and chi-squared tests were used to investigate the relationship between H. pylori stomach infection and reflux oesophagitis in patients referring to the Endoscopy Units of two hospitals in Zahedan during 2015-2019 and the level of significance in this study was considered to be ($p \leq 0.05$).

Results and Discussion

The demographic characteristics of the patients are shown in Table 1. This shows the variables, cumulative frequency and percentages. Lower and the higher frequency corresponded to the under 15s and 35-45-year-old patients respectively.

Table 1. Demographic characteristics of patients

Variables	Frequency	Percentage
Under 15	9.5	9.6
15-25	35.5	26.2
25-35	56.2	20.2
35-45	76.2	20.1
More than 45 years	100	23.5
Male	50	50
Female	90	50
Fischer (Negative)	81.5	81.5
d2 positive	95	18.2
Negative H. pylori	54.5	54.5
Positive H. pylori	100	45.2
Negative scalping	81.5	81.5
d2 positive	96	18.6
Negative atrophy	52.5	52.5
Positive	92	47.2
Marsh 1 Negative	87.5	87.2
d2 positive	100	12.2
Marsh 2 negative	96.6	96.5
Positive (d2)	94	3.5
Marsh 3 Negative	90.2	90.2
Positive (d2)	98	9.2
Negative d. mild. atrophy	62.5	62.5
Positive (d2)	96.4	33.5

Positive (Bulb)	98.1	1.5
d2 and bulb	99	2.1
Negative d. mod. atrophy	95	95.5
Positive (d2)	92	4.5
Negative d. severe. atrophy	94.2	93
Positive (d2)	97	5.4

The results of relationships between oesophagitis and H. pylori infection are shown in Table 2. The data showed a statistically significant difference in the group without oesophagitis.

Table 2. Relationships between oesophagitis and Helicobacter pylori infection

Variables	SEM	Total	H. pylori	
			Positive (%) No	Negative (%) No
Age 15 or under	0.52	(100) 14	(50) 7	(50) 7
15-25		(100) 39	(35.9) 14	(64.1) 25
25-35		(100) 30	(56.7) 17	(43.3) 13
35-45		(100) 30	(46.7) 14	(53.3) 16
More than 45 years old		(100) 35	(42.9) 15	(57.1) 20
Male	0.01	(100) 74	(55.4) 41	(44.6) 33
female		(100) 74	(35.1) 26	(64.9) 48
Fischer Negative	0.54	(100) 121	(45.5) 55	(54.5) 66
Positive (d2)		(100) 27	(44.4) 12	(55.6) 15
Negative scalping	0.29	(100) 121	(43.8) 53	(56.2) 68
Positive (d2)		(100) 27	(51.9) 14	(.48.1) 13
Negative atrophy	0.08	(100) 78	(51.3) 40	(48.7) 38
Goodness		(100) 70	(38.6) 27	(61.4) 43
Marsh 1 Negative	0.03	(100) 130	(40.8) 53	(59.2) 77
Positive (d2)		(100) 18	(77.8) 14	(22.2) 4
Marsh 2 negative	0.41	(99) 143	(44.8) 64	(55.2) 79
Positive (d2)		(100) 5	(60) 3	(40) 2
Marsh 3 Negative	0.53	(100) 134	(45.5) 61	(54.5) 73
Positive (d2)		(100) 14	(42.9) 6	(57.1) 8
Negative d. mild. atrophy		(100) 93	(51.6) 48	(48.4) 45
Positive (d2)	0.05	(100) 50	(32) 16	(68) 34
Positive (Bulb)		(100) 2	(100) 2	(0) 0

d2 and bulb		(100) 3	(33.3) 1	(66.7) 2
Negative d. mod. atrophy	0.39	(100) 141	(44.7) 63	(55.3) 78
Positive (d2)		(100) 7	(57.1) 4	(42.9) 3
Negative d. severe. atrophy	0.53	(100) 140	(45) 63	(55) 77
		(100) 8	(50) 4	(50) 4

The results of some reliable studies have been conducted on the association between oesophagitis and H. pylori infection for example, different studies in Asia have shown an inverse correlation between H. pylori infection and reflux oesophagitis (Haruma, 2004; Kandulski & Malfertheiner, 2014; Villanacci *et al.*, 2006; Rostami-Nejad *et al.*, 2016). In a Diamanti study, the prevalence of erosive oesophagitis and Barrett's oesophagus were reported to be 4.3% and 1%, respectively (Diamanti *et al.*, 1999), suggesting that H. pylori infection is inversely correlated with erosive oesophagitis (Luzza *et al.*, 1999). The result of another study showed that in patients undergoing upper endoscopy, the group with erosive oesophagitis had a higher prevalence of H. pylori infection than the control group, and the prevalence of the infection was inversely correlated with oesophagitis to different degrees (Villanacci *et al.*, 2006). A study that was performed on endoscopies showed that 49.5% had oesophagitis, 67.8% of whom were confirmed pathologically, and 77.7% of patients diagnosed with oesophagitis by endoscopy, had H. pylori (Rostami-Nejad *et al.*, 2006). In some studies, the prevalence of erosive oesophagitis and Barrett's oesophagus with a high prevalence of H. pylori infection was low (Kandulski & Malfertheiner, 2014; Memeo *et al.*, 2005; Nejad *et al.*, 2011; Shavalipour *et al.*, 2017). Studies in western countries have also shown that there is an inverse correlation between H. pylori infection and reflux oesophagitis (Shavalipour *et al.*, 2017). And this relationship in our study was significant. A study by Villanacci showed that there was an inverse correlation between oesophagitis and H. pylori infection, and that the infection had a protective role, countering the incidence of oesophagitis (Villanacci *et al.*, 2006). However, in some studies, a direct correlation between H. pylori infection and oesophagitis has been observed. For example, Fatin et al., in a study of patients with upper endoscopy, divided the patients into two groups: H. pylori-infected and non-H. Pylori infected. It was observed that H. pylori infection and oesophagitis and its grade were directly correlated (Jozefczuk *et al.*, 2015). In addition, in another study, the prevalence of H. pylori infection in gastro-oesophageal reflux disease patients and was compared with a control group; this showed that the prevalence of the infection was significantly higher in the gastro-oesophageal reflux disease patients group than in the control group (Rostami-Nejad *et al.*, 2006; Vakil, 2006). The results of these studies are consistent with our study (Villanacci

et al., 2006; Rostami-Nejad *et al.,* 2006). Meanwhile, a study of Konturek's patients with reflux symptoms used manometry, oesophageal pH measurements and biopsy. In that study, no association was observed between H. pylori infection and lower oesophageal sphincter pressure, oesophageal manometric waves and oesophagitis in pathological examinations (Konturek *et al.,* 2000). A study conducted of patients with gastro-oesophageal reflux disease showed that H. pylori infection had no significant association with gastro-oesophageal reflux disease and erosive oesophagitis (Yaghoobi *et al.,* 2010). In a study by Queiroz of gastro-oesophageal reflux disease patients, the eradication of H. pylori infection did not affect the clinical, endoscopic and manometric characteristics of the patients (Queiroz, 2004). Additionally, other investigations showed that there was no difference between H. pylori prevalence in gastro-oesophageal reflux disease patients and the controls conducted, but the prevalence of the cag A gene of H. pylori and the co-existence of cag A and cag E were significantly higher in the control group (Rostami-Nejad *et al.,* 2006; Rostami-Nejad *et al.,* Queiroz, 2004).

Conclusion

In conclusion, we were able to demonstrate that there was a significant relationship between H. pylori infection and oesophagitis in patients with oesophagitis, and the highest frequency of H. pylori infection was observed in grade B oesophagitis.

Acknowledgements

The authors are very grateful to the patients and clinical staff of the two hospitals for their cooperation with us in running this test.

References

Aydogdu, S., Cakir, M., Yuksekkaya, H.A., Tumgor, G., Baran, M., & Arikan, C. (2009). Helicobacter pylori infection in children with celiac disease. Scandinavian journal of gastroenterology.

Aziz, I., Key, T., Goodwin, J.G., & Sanders, D.S. (2015). Predictors for celiac disease in adult cases of duodenal intraepithelial lymphocytosis. Journal of clinical gastroenterology.49 (6):477-82.

Bor, S., Kitapcioglu, G., & Kasap, E. (2017). Prevalence of gastro esophageal reflux disease in a country with a high occurrence of Helicobacter pylori. World J. Gastroenterol. 23:525–532.

Camilo, V., Sugiyama, T., & Touati, E. (2017). Pathogenesis of Helicobacter pylori infection. Helicobacter. 22: 12405.

Ciclitira, P.J. (2001). AGA technical review on celiac sprue. Gastroenterology.120 (6):1526-40.

Diamanti, A., Maino, C., Niveloni, S., Pedreira, S., Vazquez, H., & Smecuol, E. (1999). Characterization of gastric mucosal lesions in patients with celiac disease: a prospective controlled study. The American journal of gastroenterology. 94 (5):1313-9.

Feighery, C. (1999). Fortnightly review. Celiac disease. BMJ: British Medical Journal. 319 (7204):236.

Ghoshal, U.C., & Chourasia, D. (2010). Gastro esophageal Reflux Disease and Helicobacter pylori: What May Be the Relationship? J. Neurogastroenterol. Motil. 16:243–50.

Haruma, K. (2004). Review article. Influence of Helicobacter pylori on gastro esophageal reflux disease in Japan. Aliment. Pharmacol. Ther. 20:40–44.

Jozefczuk, J., Bancerz, B., Walkowiak, M., Glapa, A., Nowak, J., & Piescikowska, J. (2015). Prevalence of Helicobacter pylori infection in pediatric celiac disease. 6:30.

Kandulski, A., & Malfertheiner, P. (2014). Helicobacter pylori and gastro esophageal reflux disease. Curr. Opin. Gastroenterol. 30:402–407.

Konturek, P., Karczewska, E., Dieterich, W., Hahn, E., & Schuppan, D. (2000). Increased prevalence of Helicobacter pylori infection in patients with celiac disease. The American journal of gastroenterology. 95 (12):3682.

Luzza, F., Mancuso, M., Imeneo, M., Mesuraca, L., Contaldo, A., & Giancotti, L. (1999). Helicobacter pylori infection in children with celiac disease: prevalence and clinic pathologic features. Journal of pediatric gastroenterology and nutrition. 28 (2):143-6.

Memeo, L., Jhang, J., Hibshoosh, H., Green, P.H., Rotterdam, H., & Bhagat, G. (2005). Duodenal intraepithelial lymphocytosis with normal villous architecture, common occurrence in H. pylori gastritis. Modern pathology. 18(8):1134-44.

Nejad, M.R., Rostami, K., Emami, M.H., Zali, M.R., & Malekzadeh, R. (2011). Epidemiology of celiac disease in Iran: a review. Middle East Journal of Digestive Diseases. 3(1):5; 55(6):728-32.

Queiroz, D.M.M. (2004). IL1B and IL1RN polymorphic genes and Helicobacter pylori cag A strains decrease the risk of reflux esophagitis. Gastroenterology. 127:73–79.

Rostami-Nejad, M., Ehsani-Ardakani, M.J., Assadzadeh, H., Shahbazkhani, B., Ierardi, E., & Losurdo, G. (2016). Pathological and clinical correlation between celiac disease and helicobacter pylori infection; a Review of Controversial Reports. Middle East journal of digestive diseases. 8(2):85.

Rostami-Nejad, M., Villanacci, V., Mashayakhi, R., Molaei, M., Bassotti, G., & Zojaji, H. (2009). Celiac disease and H. pylori infection association in Iran. Revista Espanola de Enfermedades Digestivas. 101(12):850.

Shavalipour, A. et al. (2017). Prevalence of cytotoxin-associated genes of Helicobacter pylori among Iranian GERD patients. Gastroenterol Hepatol from bed to bench. 10:178–183.

Vakil, N. (2006). The Montreal definition and classification of gastro esophageal reflux disease. A global evidence based consensus. American Journal of Gastroenterology. 101:1900–1920.

Villanacci, V., Bassotti, G., Liserre, B., Lanzini, A., Lanzarotto, F., & Genta, R.M. (2006). Helicobacter pylori infection in patients with celiac disease. The American journal of gastroenterology. 101(8):1880-5.

Yaghoobi, M., Farrokhyar, F., Yuan, Y., & Hunt, R.H. (2010). Is there an increased risk of GERD after Helicobacter pylori eradication? A Meta-Analysis. Am. J. Gastroenterol. 734.

Khazar Journal of Science and Technology Volume 3 №2 2019, 14-20
 DOI: 10.5782/2520-6133.2019.3.2.14

Determination of Antibacterial Properties of the Bacillus Cereus ATCC 14579 Strain for Application in Synthesis of Bacteriocins by New Methods

Safada Taghiyeva

National Academy of Sciences of Azerbaijan, Institute of Microbiology, Department of Biotechnology

E-mail: safada.tagiyeva@yahoo.com

Abstract

Nowadays bacteriocins occupy an important niche among the wide range of various antimicrobial matter, including synthetic antibiotics, pesticides, disinfectants, bacteriofages, and so on. Sources of those substances are different bacteria capable of producing such matter for self-protection against bacteria in conditions connected with limitations of nutrients and inappropriate environmental changes. Bacteriocins are characterized as safe for human health and the welfare of animals; they do not cause side effects and resistance by microbes like ordinary antibiotics. That is why scientific investigations into the development of new bacteriocins, as well as the implementation of new antibacterial substances in medicine, veterinary treatment, food production, plant sanitary are necessary. But the number of bacteria really suitable for such syntheses is very small. Bacillus cereus and Bacillus subtilis bacteria are most convenient for such process. Thus, this study was dedicated to investigating the antimicrobial properties of the bacterium Bacillus cereus strain ATCC 14579 as a possible producer of new bacteriocins. Especially for this investigation the author developed two original methods. The aforementioned strain was opposed to different Gram-positive and Gram-negative bacteria in laboratory conditions, and a certain antagonism was found. In particular, in experiments Bacillus cereus inhibited the growth of such Gr+ bacteria as Staphylococcus Epidermidis, Streptococcus pyogenes and Streptococcus agalactiae, as well as Gr- bacteria Escherichia coli and Pseudomonas aeruginosa. Subsequenced research should be continued to obtain antimicrobial agents from ATCC 14579.

Keywords: bacteriocins; *Bacillus cereus*; antagonism of bacteria; new method

Introduction

The Bacillus cereus bacterium combines more than 100 various strains. Some of the strains are harmful to humans and animals and able to cause toxic infection - food poisons - but others are harmless saprophytes. This bacterium is an intestine and food inhabitant that can active multiply in meals like vegetables, eggs, meat and dairy products and is capable of producing, in food and livestock feed, two types of toxins: emetic toxin and enterotoxin **(**Turnbull *et al.*,1983) Immunocompromised people and animals are more susceptible to Bacillus cereus bacteremia, and the endocarditis, meningitis, pneumonia and endophthalmitis caused by this bacterium. Conversely, other strains of these bacteria are known as sources of probiotics that activate growth and development in cattle and sheep (Charalampopoulos *et al*, 2009). They can also provocate abortion in cattle, related to placental necroses (Schuh & Weinstock, 1989) During intestinal colonization the opportunistic human pathogen *Bacillus cereus* demonstrates antimicrobial activity against concurrent Gram-positive and Gram-negative bacteria by excreting antimicrobial matter. For these properties, Bacillus cereus may be used for the production of natural antibacterial substances (bacteriocins). Generally, bacteria of the genus Bacillus justified themselves as producers of bacteriocins and other biologically active substances, not only on a scientific, but also on an industrial scale. Moreover, they are quite accessible, since they are widely distributed in the environment: water, soil, dust and air, and are easily cultivated on ordinary nutrient media, requiring no special conditions for reproduction, and are stable. For example, Bacillus cereus bacteria can multiply within a wide temperature range from 4 ° C (39.2 ° F) to 55 ° C 131 ° F and within acidity of pH 5.3 to 9.3. As potential producers, being facultative aerobes, they do not require special conditions for reproduction, and due to their spore formation, they are well preserved in the environment. (Duaa, 2005).

The metabolism of Bacillus cereus strains has been studied. For example, in a study by American scientists, “Biological Activity of Two Fungiostatic Antibiotics Isolated from Bacillus cereus UW85,” antifungal activity was observed in the form of inhibition of budding of the herm-tube and in Petri dishes by diffusion on nutrient agar. Then isolated eluate was tested for antifungal activity. In addition, Bacillus cereus UW85 was mutated and the mutant strains’ ability to inhibit the growth of fungi was tested, too (Laura *et al.,* 1994).

Practical applications of particularly strain ATCC 14579 were completed with the production of restriction enzyme endonuclease Bce14579 food testing (as a control strain for bacteriological tests) (https://lifescience.invitro.com.au/products /s/ATC14579/Bacillus-cereus/).

Publications describing the antibacterial metabolites of B. cereus ATCC 14579 also known as the Frankland and Frankland strain are extremely limited. Only one Norway study reports that scientists obtained a bacteriocin-like inhibitory substance (BLIS) with molecular mass 3.4 kDa. (Risøen, 2004)

That is why our scientific investigations were dedicated to confirming the ability of particularly the strains that inhibit growth of other bacteria in laboratory conditions, for a determination of ATCC 14579 as an appropriate strain for syntheses of bacteriocins. A determination of the antibacterial properties of the bacillus cereus ATCC 14579 strain for application in the synthesis of bacteriocins by new methods were conducted.

Material and Methods

For the detection of bacterial antagonism, two original methods were developed by the author. For the first method (See Figure 1), during the experiments Bacillus cereus ATCC 14579 was co-cultured with five Gram-positive and five Gram-negative bacteria: Bacillus cereus on the "upper" semicircle, the other bacteria on the "lower" semicircle of the Petri dish. The second method (See Figure 2) was similar to the identification of bacteria using a bacteriophage, dropped onto bacterial culture, but in our method Bacilus cereus and Bacillus subtilis inoculates were added as two different drops to agar, freshly cultured and dried for a few minutes, and not to ready colonies as in the bacteriophage typing method. Biosafety and biosecurity rules were strictly followed, as well as good laboratory practice (World Health Organisation) and principles too.

For the purity of the experiment, lyophilized ATCC (American Type Culture Collection) strains delivered from USAMRİİD (The United States Army Medical Research Institute of Infectious Diseases) were customized. No wild strain was obtained from the environment. From lyophilized bacteria suspensions in Soybean Broth, supplemented with 10% glycerol to McFarland standard 4.0, were prepared, aliquoted and frozen. The first part of the experiments proved the viability of the bacterial strains and their suitability for laboratory studies. Bacillus cereus (ATCC 14579), Bacillus subtilis (ATCC 6633), Gram-positive bacteria Staphylococcus aureus (ATCC 29213), Staphylococcus epidermidis ATCC 12228), Streptococcus pyogenes (ATCC 19615), Streptococcus aqalactiae (ATCC 13813), Enterococcus faecalis (ATCC 29212) and Gram-negative bacteria Escherichia coli (ATCC 15922), Pseudomonas aeruginosa (ATCC 27853), Salmonella typhimurium ATCC 14028), Proteus mirabilis (ATCC 25933), Shigella flexneri (ATCC 12022) were

cultured and tested. https://www.atcc.org/en/Products/Cells_and_Microorganisms/Bacteria.aspx

Figure 1. First original method for the determination of bacterial antagonism between Bacillus cereus and Enterococcus faecalis. Bacillus Cereus colonies covered the whole plate, but Enterotococcus colonies were not visible at all.

To verify the suitability of the cultured bacteria, they were re-identified for confirmation of bacterial strain and pure culture by colony morphology, microscopy, biochemical and other additional test. For supporting manual tests, strains were also identified on the Vitek® 2 Compact 30 (French bioMerieux) instrument. Here, suspensions of bacterial cultures were added to disposable cartridges and the machine tested the cultures over 5 hours. The results of the manual and automatic identifications confirmed the suitability of the bacteria for the study.

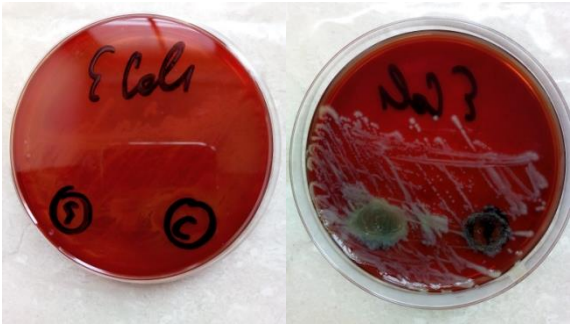

Figure 2. Second original method for determination of bacterial antagonism between Bacillus cereus (C) and Bacillus subtilis (S). Look at the zone under and around the dropped area. Here Bacillus Cereus have grown under and far around the drop. In the left figure - outer side, on the right figure - inner side of the same plate.

In the main course of the laboratory experiment for identification of the antibacterial properties of Bacillus Cereus, all bacteria were cultured on Meat Peptone Agar and Blood agar (with the addition of sheep red blood cells) and tested. As well as bacterial cultures, culture media were pre-tested, that is, their sterility and ability to provide normal bacterial growth were confirmed. In the first method, each Petri dish was divided on the outer lower side by a marker into two equal semicircles and labelled with the names of the bacteria. On each plate, Bacillus Cereus was streaked onto one semicircle and one of the Gram-positive or Gram-negative bacteria onto the other semicircle. The experiment was carried out repeatedly using both nutrient media. At the same time, all biosafety rules were followed to prevent contamination and false results. After cultivation at 37 ° C for 18 hours, the joint growth of bacteria was analysed. Inhibited growth (rare or absence of colonies) of some bacteria near the line of intersection was recorded. In the second method Bacillus cereus and Bacillus subtilis inoculates (suspensions) were added as two different drops to agar, freshly cultured and dried for a few minutes, then incubated at 37 ° C for 18 hours and a “clear zone” around the Bacillus of other bacterial colonies was detected. Most of the Petri dishes were photographed and images systematized. As a result of both methods it was found that bacteria of the genus Bacillus, especially Bacillus cereus, actually demonstrated antibacterial properties in vitro. That means that Bacillus cereus ATCC 14579 slows down, and sometimes completely inhibits, the growth of other bacteria. The outcomes of the experiments are shown in Table 1. The text describing the character of antagonism detected is shown in italics.

Table 1. Analysis of joint bacteriological culture of bacteria of the genus Bacillus with other bacteria in order to detect antagonism.

No	Bacterial name	*Bacillus Cereus*
Gram-positive bacteria		
1	*Staphylococcus aureus*	Antagonism not observed
2	*Staphylococcus epidermidis*	On both the Blood and Meat Peptone agar, Bacillus cereus interferes with the normal growth of Staphilococcus Epidermidis
3	*Streptococcus pyogenes*	On the Meat Peptone agar, Bacillus cereus interferes with the normal growth of Streptococcus pyogenes and some colonies of B.C. growing on the side of Str.P. On Blood agar near B.C. Str.P colonies are very rare
4	*Streptococcus aqalactiae*	On Meat Peptone agar, Bacillus cereus interferes with the normal growth of Streptococcus agalactiae and some colonies of B.C. growing on the side of Str.A. On Blood agar, Str.A colonies are very rare and small
5	*Enterococcus faecalis*	Antagonism not observed

Qram-negative bacteria		
6	*Escherichia coli*	Bacillus cereus interferes with the normal growth of E. Coli, part of the colonies of Bacillus Cereus grow on the side of E.C.
7	*Pseudomonas aerogenosa*	Bacillus cereus inhibits normal growth of Ps.A.
8	*Salmonella typhimurium*	Antagonism not observed
9	*Proteus mirabilis*	Antagonism not observed
10	*Shigella flexneri*	Antagonism not observed

Results and discussion

Thus, the bacteria Bacillus Cereus were repeatedly plated on Blood and Meat-Peptone agars in pairs together with one of ten bacteria. During the experiments, it was proved that these bacteria actually have the property of inhibiting the growth of other bacteria by some biologically active substances secreted by them into the nutrient medium. Moreover, in some cases, they completely or partially suppressed the growth of colonies of another bacterium; in other cases, Bacillus colonies “crossed” the conditional border intersection and grew on the semicircle where the non-Bacillus bacteria were cultured. Sometimes between the colonies of Bacillus and other bacteria, a colony-free “clean zone" had appeared, which once again confirmed the presence of an antimicrobial substance in nutrient media around Bacillus cereus. The influence of Bacillus cereus ATCC 14579 on different bacteria was significantly different. Thus, in our experiments, Bacillus cereus prevented the growth of Staphylococcus epidermidis, Streptococcus pyogenes and Streptococcus agalactiae. Bacillus cereus also showed antagonism to Escherichia coli and Pseudomonas aeruginosa. For other bacteria tested, antagonism was not observed.

Thereby, based on the above facts, it can be assumed that bacteria Bacillus cereus ATCC 14579 was characterized by a wide spectrum of antibacterial activity against staphylococci and streptococci and some Gram-negative bacteria. To confirm this theory, as well as to obtain new antibacterial substances, further studies are expected using microbiological and biotechnological methods.

References

Charalampopoulos, D. et al. (2009.) Prebiotics and probiotics in science and technology. 33-78.

Duaa, S. Al-Baqer. (2005). Bacillus Cereus King Saud University College of Pharmacy Department of Pharmaceutics, Section of Microbiology, 5-6.

Laura, A. Silo-Suh, et al. (1994). "Biological Activities of Two Fungistatic Antibiotics Produced by Bacillus cereus UW85. Applied and environmental microbiology, 2023-2030.

Risoen, P.A., Rønning, P., Hegna, I.K. & Kolstø, A.B. (2004). Characterization of a broad range of antimicrobial substance from Bacillus cereus. J. Appl. Microbiol. 96(4):648-55.

Schuh, J. & Weinstock, D. (1985). Bovine abortion caused by Bacillus cereus. J Am Vet Med Assoc.187(10):1047-8.

Turnbull, P. et al. (1983). Non-gastrointestinal *Bacillus cereus* infections: an analysis of exotoxin production by strains isolated over a two-year period. J Clin Pathol. Oct, 36(10): 1091–1096.

Khazar Journal of Science and Technology Volume 3 №2 2019, 21-27

DOI: 10.5782/2520-6133.2019.3.2.21

Use of Paracetamol and Ibuprofen in Combination for Pain Relief in Children after Oral Surgery

Samaneh Sardashti, Malihe Saghzadeh, Hamideh Yaghoobi
Department of Nursing, School of Nursing and Midwifery, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran.
Corresponding author: m.eshaghzadeh93@gmail.com

Abstract

The aim of this study was to determine the use of paracetamol and ibuprofen in combination for pain relief in children after oral surgery. Children under 10 years old having one or more wisdom teeth removed under general or local anaesthesia were instructed to take two tablets before the operation then, every 4 h for up to 48 h, two tablets of a combination of acetaminophen 250 mg and ibuprofen 150 mg per tablet and acetaminophen 250 mg per tablet alone and ibuprofen 150 mg per tablet alone. The primary outcome measure was the area under the curve of the 100 mm visual analogue scale of pain measurements taken for up to 36 h after surgery, divided by time, at rest and in activity. The result showed that although all four secondary endpoints favoured the combination treatment, only the global pain rating reached statistical significance. More participants experienced 'nil' or 'mild' pain with the combination (68.4%) than with either other group; this difference was significant for acetaminophen (37.5%; P=0.008), but not for ibuprofen (54.3%; P=0.263). The use of any rescue medication also favoured the combination treatment, but this did not reach statistical significance. In conclusion, we demonstrated that patients using the combination of acetaminophen and ibuprofen experienced less pain during the first 48 h after oral surgery than those using the same daily dosage of either agent alone and we think the difference was clinically relevant.

Keywords: Paracetamol; Ibuprofen; Pain relief; Surgery; Children

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have fewer regulatory restrictions (Cousins *et al.,* 2004), but they too have important adverse effects which are more likely at higher doses or with longer courses (Merry, 1995).

Acetaminophen is often used with a non-steroidal anti-inflammatory drug for acute pain (Altman, 2004). These drugs have had to be given separately, typically at different time intervals (Mehlisch, 2002). This drug is also used to treat mild to moderate pain from headaches, menstrual periods, toothaches, backaches, osteoarthritis, aches and pains from colds and flu, and to reduce fever (Anderson & Cranswick, 2005). Acetaminophen, also known as paracetamol, is commonly used for its analgesic and antipyretic effects (Barden *et al.,* 2004). Its therapeutic effects are similar to salicylates, but it lacks anti-inflammatory, antiplatelet and gastric ulcerative effects. Ibuprofen is a medication in the none-steroidal anti-inflammatory drug (NSAID) class that is used for treating pain, fever (Rainsford, 1999), and inflammation (Cooper *et al.,* 1989; Desmeules *et al.,* 2003). This includes painful menstrual periods, migraines and rheumatoid arthritis. It may also be used to close a patent ductus arteriosus in a premature baby (Viitanen *et al.,* 2003). It can be taken by mouth or intravenously (Menhinick *et al.,* 2003). It typically begins working within an hour. Common side effects include heartburn and a rash (Prescott, 2001). Compared to other NSAIDs, it may have fewer side effects such as gastrointestinal bleeding. It increases the risk of heart failure, kidney failure, and liver failure (Hyllested *et al.,* 2002; Henry *et al.,* 2002). It is used primarily to treat fever, mild to moderate surgery pain, painful menstruation, osteoarthritis, dental pain, headaches, and pain from kidney stones (Davies *et al.,* 1998). Ibuprofen combined with paracetamol is considered generally safe for children for short-term usage (Dahl *et al.,* 2004). The combination of paracetamol and ibuprofen has been found to be efficacious in a variety of acute pain states, including postoperative pain, dysmenorrhoea and musculoskeletal pain (Gazal & Mackie, 2007). The authors concluded that paracetamol and ibuprofen in combination provide better analgesia than the same dose of either drug alone, with fewer patients taking the combination requiring rescue analgesia or experiencing an adverse event (Derry *et al.,* 2013). The aim of this study was to explore the effect of taking paracetamol and ibuprofen in combination for pain relief in children after oral surgery.

Material and methods

This study was conducted at a publicly-funded teaching hospital and a private day-surgical clinic in Zahedan, Iran. It was conducted on children undergoing extraction of at least one lower wisdom tooth with or without one or more upper wisdom teeth by one of three participating surgeons. Patients were excluded from the study if: they were under 10 years old; weighed under 25 kg; had taken any NSAID within 36 h of the operation; had taken medicines containing

acetaminophen or acetaminophen within 12 h of the operation; were taking an angiotensin converting enzyme inhibitor, warfarin, steroid or any immunosuppressive drug; were intolerant of any NSAID or acetaminophen; were suffering from a severe local infection; had a history of peptic ulceration, asthma, or severe haemopoetic, renal or hepatic disease; were participating in the investigation of another experimental agent; or if the clinician believed for any other reason that participation in the study might not be in their best interests. Patients were first approached by the surgeon and then by the study nurse. They were given written and spoken information about the study, and invited to participate. If they consented, patients were then randomized into one of the three study groups in a sequential order to receive one of these formulations, in blinded packs. The randomization sequence was computer generated by the study statistician as a 1:1:1 allocation ratio to the three treatments in a sequence of permuted blocks with stratification for anaesthetic type such as local or general and study centre. Stratification by anaesthetic type ensured a balance between treatments in terms of the number of teeth extracted, as most patients having more than two teeth extracted have a general anaesthetic. Only the statistician had access to the schedule of patient numbers by drug allocation. Participants and investigators were blinded and the randomization code was not broken until the final database had been checked and locked. Children under 8 years old having one or more wisdom teeth removed under general or local anaesthesia were instructed to take two tablets before the operation, then two tablets every 4 h for up to 36 h of a combination of acetaminophen 250 mg and ibuprofen 150 mg per tablet, and acetaminophen 250 mg per tablet alone, and ibuprofen 150 mg per tablet alone. Participants were asked to take two tablets of the study medication before the operation, as close as possible to the start of surgery and then every 4 hours if possible, up to every 6 hours, for up to 48 hours after surgery. All participants were given bupivacaine local anaesthetic blocks by the surgeons. For those participants undergoing general anaesthesia, this was induced with propofol and maintained with isoflurane and nitrous oxide in oxygen. Monitoring was in accordance with the guidelines of the Australian and New Zealand College of Anaesthetists. All extractions were carried out by one of three surgeons, each using his normal technique. If participants required additional postoperative pain relief while in hospital, a rescue dose of fentanyl 10 μg was given as required. After discharge to home, codeine was provided in 30 mg tablets, one to two to be taken as needed up to 4 hourly. Blood samples were obtained from the 38 participants undergoing general anaesthesia in order to have evaluable pharmacokinetic data for at least 30 patients. The first sample was obtained 30 min after the first dose of study medication, the second sample at the end of anaesthesia, and additional one or two samples after the operation in the hospital. The plasma concentration of

acetaminophen and ibuprofen were measured by the sponsor and used to form individual time–concentration profiles. The analytical method used an HPLC Diode Array Detector assay for the simultaneous determination of acetaminophen and ibuprofen in plasma. Precision and accuracy of the acetaminophen and ibuprofen assay were validated over the concentration range 0.5–50 μg ml−1 for both drugs. The intra- and inter-batch precision of the assays at low, medium, and high concentrations of acetaminophen and ibuprofen varied from theoretical values by less than 15%. The lower limit of quantification for each drug was 0.5 μg ml−1. The sponsor monitored all data collected during the study and queries and corrections were made when any inaccuracies or inconsistencies were identified. The primary outcome measure was the area under the curve of the 100 mm visual analogue scale of pain measurement taken for up to 36 h after surgery, divided by time, at rest and in activity. Pharmacokinetic data were collected from a subset of patients. The data obtained were analysed using SAS version 9.1. Efficacy analyses were conducted on an ITT basis with the additional provision that there were at least three VAS measurements over at least 12 h available to calculate the primary endpoint. All participants who were randomized into the study were included in the safety evaluations. As the first dose of study medication was taken before the operation and while under the supervision of the surgeon, all randomized patients took at least a single dose of study medication. A last observation carried forward approach was used for those subjects who left the study prematurely for non-AUC based variables. We compared the primary endpoint between the combination group and each of the acetaminophen and ibuprofen arms, at rest and in activity, using a general linear model (GLM) of SAS software version 9.1 which included terms for treatment, the centre, and anaesthetic stratum. Additionally, to confirm the consistency of treatment effects across strata, the stratum treatment interaction terms were tested and included in the final model. The analysis was also checked with the number of teeth extracted as an additional factor. Continuous secondary efficacy endpoints were tested for significance using the same models as used for the primary endpoint. A one tailed ($P \leq 0.05$) test was pre-specified to indicate statistical significance.

Result and Discussion

The time adjusted AUCs were substantially and significantly lower at rest and in activity in the combination group than in either of the other two treatment groups (Table 1) with all four ($P<0.01$).

Table 1. Mean of time-adjusted AUC of visual analogue pain scores at rest and in activity by treatment groups

------	Paracetamol N=40	Ibuprofen N=40	Combination N=40
Rest	34	35	23
Activities	45	41	29

The differences between combination and each constituent were significant at rest (vs acetaminophen P=0.007 and vs ibuprofen P=0.003) and on activity (vs acetaminophen P=0.006 and vs ibuprofen P=0.007

Although all four secondary endpoints favour the combination treatment (Table 2), only the global pain rating reached statistical significance. More participants experienced 'nil' or 'mild' pain with the combination (68.4%) than with either other group; this difference was significant for acetaminophen (37.5%; P=0.008), but not for ibuprofen (54.3%; P=0.263). The use of any rescue medication also favoured the combination treatment (Table 3), but this did not reach statistical significance.

Table 2. Secondary efficacy endpoints by treatment groups

Global pain rating [n (%)]	Paracetamol	Ibuprofen	Combination
Nil	3(7.5)	4(11.4)	4(10.5)
Mild	22(30.0)	14(42.9)	12(57.9)
Moderate	12(55.0)	15(40.0)	22(31.6)
Severe	3(7.5)	2(5.7)	0(0.0)
Global pain rating [n (%)]			
Nil	26(65.0)	25(71.4)	30(79.0)
Mild	10(25.0)	8(22.9)	7(18.4)
Moderate	3(7.5)	2(5.7)	1(2.6)
Severe	1(2.5)	0(0.0)	0 (0.0)
Vomiting episodes (*n*)	5	0	0

The only significant difference was between the global pain ratings for combination and acetaminophen (P=0.008, Mann–Whitney U-test)

Table 3. Rescue analgesia by groups

Rescue analgesic	Paracetamol	Ibuprofen	Combination
Fentanyl in hospital	5 (11.6%)	9 (23.7%)	6 (15.4%)
Codeine in the first 24 h	21 (47.70%)	16 (43.20%)	13 (32.50%)
Codeine in the second 24 h	22 (53.70%)	14 (42.40%)	16 (42.10%)
Any rescue medication over 48 h	25 (62.5%)	18 (58.10%)	21 (56.8%)

We demonstrated that patients using the combination of acetaminophen and ibuprofen experienced less pain during the first 48 h after oral surgery than those using the same daily dosage of either agent alone and we think the difference was clinically relevant. There was no evidence of any pharmacokinetic interaction between acetaminophen and ibuprofen. Patients receiving ibuprofen alone reported the lowest frequency of adverse events, but the numbers are too small for meaningful comparisons between the groups, and we saw no cause for concern in any group. The current study obtained data that were consistent with previous evidence showing that a combination of ibuprofen and acetaminophen provides better analgesia than acetaminophen alone. However, two of these studies were on children, so data on adults are relatively limited. On the other hand, there are many studies supporting the more general point that the addition of various NSAIDs improves the pain relief obtainable from acetaminophen alone. More importantly, our data add convincingly to the sparse evidence supporting the more controversial proposition that this combination is superior to ibuprofen alone. In a smaller study in an orthopaedic pain model which was positive for the combination in comparison with acetaminophen, Dahl and colleagues (2004) showed that there was no such benefit whereas Viitanen and colleagues (2003) showed an advantage for the combination only in the period after discharge from hospital. The similarity in efficacy between ibuprofen and acetaminophen on their own seen in our study contrasts with the findings of superior pain relief from ibuprofen after dental surgery by Cooper and colleagues (1989) but theirs was a single-dose study.

Conclusion

In conclusion, we could also demonstrate that the doctors treating pain after oral surgery, in hospital and at home, and probably pain in many other situations should consider using acetaminophen and ibuprofen together 6 times a day, provided there are no contraindications to either drug, and taking into account the known risks of NSAIDs. The combination formulation studied here simplifies this regimen. Additionally, more studies in the future are needed for further explanation.

References

Anderson, B. & Cranswick, N. (2005). The placebo (I shall please)-is it so pleasing in children? Paediatr Anaesth. 15:809–13

Altman, R.D. (2004). A rationale for combining acetaminophen and NSAIDs for mild-to-moderate pain. Clin Exp Rheumatol. 22:110–7.

Barden, J., Edwards, J., Moore, A. & McQuay, H. (2004). Single dose oral paracetamol (acetaminophen) for postoperative pain. Cochrane Database Syst Rev.

Cooper, S.A., Schachtel, B.P., Goldman, E., Gelb, S. & Cohn, P. (1989). Ibuprofen and acetaminophen in the relief of acute pain: a randomized, double-blind, placebo-controlled study. J Clin Pharmacol. 29:1026–30.

Cousins, M.J. & Brennan, F. & Carr, D.B. (2004). Pain relief: a universal human right. Pain.112:1–4.

Dahl, V., Dybvik, T., Steen, T., Aune, A.K., Rosenlund, E.K. & Ræder, J.C. (2004). Ibuprofen vs. acetaminophen vs. ibuprofen and acetaminophen after arthroscopically assisted anterior cruciate ligament reconstruction. Eur J Anaesthesiol. 21:471–5.

Davies, N.M. (1998). Clinical pharmacokinetics of ibuprofen. The first 30 years. Clin Pharmacokinet. 34:101–54.

Desmeules, J., Rollason, V., Piguet, V. & Dayer, P. (2003). Clinical pharmacology and rationale of analgesic combinations. Eur J Anaesthesiol Suppl. 20:7–11.

Gazal, G. & Mackie, I.C. (2007). A comparison of paracetamol, ibuprofen or their combination for pain relief following extractions in children under general anaesthesia: a randomized controlled trial. Int J Paediatr Dent. 17:169–77.

Henry, D. & Mc Gettigan, P. (2003). Epidemiology overview of gastrointestinal and renal toxicity of NSAIDs. Int J Clin Pract. 135:43–9.

Merry, A. & Power, I. (1995). Perioperative NSAIDs: towards greater safety. Pain Rev. 2:268–91.

Mehlisch, D.R. (2002). The efficacy of combination analgesic therapy in relieving dental pain. J Am Dent Assoc. 133:861–71.

Hyllested, M., Jones, S., Pedersen, J.L. & Kehlet, H. (2002). Comparative effect of paracetamol, NSAIDs or their combination in postoperative pain management: a qualitative review. Br J Anaesth. 88:199–214.

Menhinick, K.A., Gutmann, J.L., Regan, J.D., Taylor, S.E. & Buschang, P.H. (2004). The efficacy of pain control following nonsurgical root canal treatment using ibuprofen or a combination of ibuprofen and acetaminophen in a randomized, double-blind, placebo-controlled study. Int Endod J. 37:531–41.

Rainsford, K.D. (1999). Ibuprofen: A Critical Bibliographic Review. London: Taylor and Francis. The pharmacokinetics of ibuprofen in humans and animals, 92–5.

Viitanen, H., Tuominen, N., Vääräniemi, H., Nikanne, E. & Annila, P. (2003). Analgesic efficacy of rectal acetaminophen and ibuprofen alone or in combination for paediatric day-case adenoidectomy. Br. J. Anaesth. 91:363–7.

Khazar Journal of Science and Technology Volume 3 №2 2019, 28-40
© Khazar University Press 2019 DOI: 10.5782/2520-6133.2019.3.2.28

The Effects of Savory (*Satureja khuzistanica*) Extract on Performance, Organ Weight, Blood Parameters and Immune Function in Heat Stressed Broilers

Mohammad Yadegari[1], Hasan Ghahri[1*], Mohsen Daneshyar[2]
[1]Faculty of Veterinary Medicine, Urmia Branch,
Islamic Azad University, Urmia, Iran
[2]Department of Animal Science, Faculty of Agriculture,
Urmia University, 165 Urmia, Iran
**Corresponding author: hasanghahri148@gmail.com*

Abstract

An experiment was conducted to study the effect of savory (*Satureja khuzistanica*) extract on the performance, organ weight, immune response and hepatic enzymes in broiler chickens. 320 day-old Ross chickens were assigned to four distinct treatments in a completely randomized design. Each treatment was administered to four replicates of twenty birds. The variables were heat stress (34 ± 2 ˚C for 8 hours) and savory extract (0.4 ml/L) in drinking water. Feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were measured in successive weeks of the trial. The relative weights of different organs (dressing, breast, thigh, liver, heart, spleen and bursa of Fabricius) determined at 42 days. The serum glucose and blood plasma content of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured by blood sampling at 42 days. Plasma IgG were quantified on days 21, 28, 35 and 42. The savory extract did not affect FCR, or the relative weights of different organs ($P>0.05$). BW and FI increased with savory oil inclusion ($P<0.05$). Further, the savory extract reduced plasma glucose, AST and ALT in heat stressed broilers significantly ($P<0.05$). ALP content also declined, but not significantly ($P>0.05$). Blood IgG in heat stressed broilers, increased in every case of treatment with savory extract ($P<0.05$). In conclusion, in conditions of heat stress, 0.4 ml/L of savory extract improves economic proficiency in broiler flocks due to the accumulation of minute advantages in increased WG, FI, improved IG and reduced hepatic enzymes.

Keywords: heat stressed broilers, hepatic enzymes, immune function, performance, savory extract

Introduction

Heat stress (HS) is one of the most important negative factors in poultry growth and immune function in tropical countries, as well as in the warm seasons in temperate countries that can be very costly. The main problem caused by HS in young birds is depressed weight gain, occurring mainly due to reduced feed intake (FI) and elevated energy use in reducing body temperature (May and Lott 1992; Belay and Teeter 1993). Nutritional manipulation may be the cheapest way to negate the harmful effects of heat stress.

The general health and performance of broilers exposed to heat stress responded positively to diets supplemented by antibiotics (Männer and Wang, 1991; Çabuk et al., 2006). In poultry feed, the extracts and essential oils of some herbs have also attracted considerable interest as unique feed additive alternatives to AGP (Suderman and Solikhah 2011; Zeinali et al. 2011).

Savory (Satureja khuzistanica Jamzad) is a plant identified for its therapeutic effects in traditional medicine (Abdollahi et al., 2003). The upper (aerial) parts of the savory plant collectively include up to 3% of an essential oil spectacularly rich in carvacrol (Khosravinia, 2016). Carvacrol is a phenolic, bitter-tasting and caustic component with good stability demonstrating antioxidant and antimicrobial properties (Khosravinia, 2016). Correspondingly, it has been reported, mainly in experiments conducted under standard directorial practice and normal environmental situations, that Savory essential oils have antioxidant and antibacterial effects (Abdollahi *et al.,* 2003; Radonic & Milos 2003; *Azaz et al.,* 2002). Furthermore, some reports have indicated that savory extract is beneficial in heat stress situations and also helpful in overcoming the harmful effects of this stressor (Khosravinia, 2016). Therefore, the purpose of this experiment was to evaluate the effects of savory extract in drinking water on the performance, organ weight, immune response and hepatic enzymes in broilers under heat stress.

Materials and Methods

Experimental design

The experiment was conducted in the Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Iran. A total of 320, one-day-old, male, Ross 308 broiler chickens were used. During the experiment, feed and water was accessible *ad libitum*. The savory extract was obtained from a local company. Chicks from 21 to 42 days old (800± 100 gr) were used in completely randomized fashion. The 4

treatments included a control treatment with no heat stress (Control+); a control treatment with heat stress (Control-), in which the temperature was about 34 ± 2 ˚C for 8 hours per day; a treatment containing 0.4 ml savory extract mixed with drinking water with no heat stress (Control+ + 0.4 ml savory extract); and a treatment containing 0.4 ml savory extract mixed with drinking water with heat stress (Control- + 0.4 ml savory extract). Each treatment was administered to four replicates of twenty birds. The broilers' diet (Table 1) was formulated in consideration of Ross 308 catalogue guidelines (Ross 308, 2007). During the experimental period, there were periods of 23 hours of light and 1 hour of darkness.

Table 1. The nutrient composition and ingredients of the experimental diets

Ingredients (%)	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Corn	56.12	58.89	62.61
Soybean meal (44%)	37.9	33.80	30.04
Soy oil	1.23	2.83	3.25
$_{L}$-lysine	0.30	0.33	0.19
$_{DL}$-methionine	0.18	0.23	0.17
Dical phosphate	2.13	1.88	1.74
Oyster shell	1.28	1.17	1.13
Salt	0.36	0.37	0.37
Vit premix[1]	0.25	0.25	0.25
Min premix[2]	0.25	0.25	0.25
Composition analysis			
AMEn[3] (kcal/kg)	2845	2990	3060
CP (%)	22.00	20.50	19.00
Lysine (%)	1.38	1.30	1.10
Methionine (%)	0.55	0.58	0.50
Methionine + cysteine (%)	0.92	0.92	0.82
Calcium (%)	1.00	0.9	0.85
Available phosphorus (%)	0.50	0.45	0.42
Sodium (%)	0.16	0.16	0.16
DCAD[4]	222	201	192

[1] Provided per kg of diet: vitamin A: 9000 IU; vitamin D: 2000 IU; vitamin E: 18 IU; vitamin K : 3 mg; vitamin B : 1.78 mg; vitamin B : 6.6 mg; vitamin B 3 1 2 6 : 3 mg; vitamin B12 : 0.015 mg; Niacin: 30 mg; Pantothenic acid: 10 mg; Biotin: 0.15 mg and Choline: 1500 mg; [2] Provided per kg of diet: Cu: 10 mg; I: 0.99 mg; Fe: 50 mg; Mn: 100 mg; Se: 0.08 mg; and Zn: 100 mg. [3] AMEn : apparent metabolizable energy corrected for nitrogen. [4] DCAD: dietary cation anion difference.

Performance parameters

Feed intake (FI) and Body weight (BW), were determined and body weight gain (BWG), and feed conversion ratio (FCR) were computed based on hen day per period.

Organ weights

At 42 days old, two birds from each pen were caught, weighed and killed by decapitation to obtain the Dressing and relative weights of organs. The organs consisted of breast, thigh, heart, liver, spleen and bursa Fabricius (percentage of live body weight).

Biochemical analysis

Blood samples were collected on day 42, in sterile vacutainers (heparinised) for analysis of blood glucose, ALP, ALT, and AST. Blood samples were centrifuged at 1,500×g for 10 minutes, within 30 minutes of blood collection. Plasma was harvested and stored at –20 °C until analysis. All biochemical analyses were assayed on an automated biochemical analyzer.

IGg assay

At days 28, 35 and 42, two chicks from each pen were chosen at random, and blood samples were taken from a wing vein. Before harvesting the serum, blood samples were allowed to coagulate at 4°C, and then centrifuged at 3,000 × g for 10 minutes at 4°C. All serum samples were stored at −20°C until they were analyzed. Serum concentrations of IgG were determined by a sandwich ELISA set, using chicken-specific IgG ELISA quantitation kits and microtiter plates (Jiancheng Biological Engineering Research Institute, Nanjing, China, Cat. No. H106). The ELISA procedure was carried out in consideration of the producer's protocol, and absorbance was determined at 450 nm.

Statistical analysis

The results obtained were subjected to variance analysis procedures suitable for a completely randomized design via the general linear model procedures of SAS (2004). Duncan's multiple-range test was applied to determine the statistical significance of differences between treatments.

Results

Growth performance

The results of growth performance by broiler chickens during the experiment are displayed in Tables 2 and 3. The results available indicate no significant difference (P> 0.05) for BWG and FCR between treatments. However, there were significant differences between control+ vs. control- diet (P=0.009) and 0.4 ml savory extract vs. control+ diet (P=0.101) for BWG.

Also, there was significant difference in feed intake values between different treatments (P< 0.05). As shown in table 3, the feed intakes by broilers for 3 weeks and the whole period of experiment (21 to 42 days) differ significantly (P< 0.05) and the control+ group (basal diet with no heat stress) had the highest feed intake.

Table 2. The effect of different treatments on weight gain (WG) by experimental broilers at different weeks during the experiment (gr).

Treatments	21-28 d	29-35	36-42	21-42 d
Control+[1]	396.25	439.5	458.00	1293.75[a]
Control-[1]	359.00	404.5	375.50	1139.25[b]
Control+ + 0.4 ml savory extract	396.00	459.0	420.25	1275.25[a]
Control- + 0.4 ml savory extract	389.00	433.5	410.75	1233.25[ab]
SEM	3.36	4.9	12.32	16.52
Probability	0.34	0.27	0.95	0.22
Independent comparisons	Probability			
Control+ vs control-	Ns	Ns	Ns	0.009
Control+ vs savory	Ns	Ns	Ns	0.101
Savory vs control-	Ns	Ns	Ns	Ns

[1]Control+ = birds grown in normal temperatures throughout the trial (21-42 days), and Control- = birds grown under heat stress throughout the trial (21-42 days); the means within the same column with different letters, have significant differences (P<0.05). SEM: standard error of the mean.

Table 3. Table 2. Effect of different treatments on feed intake (FI) and feed conversion ratio (FCR) by experimental broilers at different weeks during the experiment.

	Feed intake (gr)				Feed conversion ratio			
Treatments	21-28 d	29-35 d	36-42 d	21-42 d	21-28 d	29-35 d	36-42 d	21-42 d
Control+[1]	723.00[a]	847.25[a]	1101.5[a]	2671.75[a]	1.82	1.93	2.42	2.05
Control-[1]	698.00[b]	815.50[b]	1016.5[b]	2530.00[b]	1.95	2.01	2.71	2.22
Control^{+} + 0.4 ml savory extract	712.25[ab]	847.00[a]	1098.75[a]	2658.00[a]	1.81	1.87	2.63	2.10
Control^{-} + 0.4 ml savory extract	714.00[ab]	829.75[ab]	1072.0[ab]	2615.75[a]	1.84	1.93	2.66	2.14
SEM	4.15	6.53	15.39	21.05	0.03	0.04	0.08	0.03
Probability	0.63	0.4	0.19	0.07	0.39	0.36	0.60	0.78
Independent comparisons	Probability							
Control+ vs control-	Ns	0.008	0.01	0.003	Ns	Ns	Ns	Ns
Control+ vs savory	Ns	0.37	0.15	0.02	Ns	Ns	Ns	Ns
Savory vs control-	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

[1]Control+ = birds grown in normal temperatures throughout the trial (21-42 days), and Control- = birds grown under heat stress hroughout the trial (21-42 days); the means within the same column with different letters, have significant differences ($P<0.05$). SEM: standard error of the mean.

Organ weights

No significant differences in relative weights were indicated for dressing, breast, thigh, liver, heart, bursa of Fabricius and spleen in experimental broilers at 42 days old ($P>0.05$; Table 4). Adding 0.4 ml savory extract gave an increase in relative weight of thigh and breast at 42 days old, as compared with other treatments, but not significantly so ($P>0.05$). Further, heart and liver weights increased in birds that received treated water, but the differences were not significant ($P>0.05$). Overall, the use of savory extract for 21 days had a positive effect on lymphoid organs (bursa of Fabricius and spleen), although the differences were not significant.

Table 4. Effect of different treatments on relative weights of dressing, breast, thigh, liver, heart, spleen and bursa of Fabricius at 42 days old. (% body weight).

Treatments	Dressing	Breast	Thigh	Liver	Heart	Spleen	Bursa
Control+[1]	65.46	25.16	20.92	2.33	0.46	0.11	0.17
Control-[1]	64.59	24.76	19.33	2.37	0.44	0.13	0.18
Control+ + 0.4 ml savory extract	64.59	26.07	19.42	2.41	0.43	0.13	0.20
Control- + 0.4 ml savory extract	63.57	31.62	24.79	2.95	0.58	0.14	0.16
SEM	0.08	1.68	1.5	0.15	0.03	0.01	0.01
Probability	0.61	0.27	0.58	0.26	0.47	0.46	0.95
Independent comparisons	Probability						
Control+ vs control-	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Control+ vs savory	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Savory vs control-	Ns	Ns	Ns	Ns	Ns	Ns	Ns

[1]Control+ = birds grown in normal temperatures throughout the trial (21-42 d), and Control- = birds grown under heat stress throughout the trial (21-42 d); the means within the same column with different letters, have significant differences ($P<0.05$). SEM: standard error of the mean.

Biochemical results

The effects of different treatments on blood plasma glucose content and liver function, which were monitored by determining the activities of alanine amino-transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are shown in table 5. The results obtained indicate that the glucose, AST and ALT contents of blood plasma in broilers in group 4 (Control- + 0.4 ml savory extract) reduced significantly compared with the control- group ($P< 0.05$). Additionally, there was no significant difference between the different treatments in the ALP content of blood plasma in the experimental broilers ($P> 0.05$).

Table 5. The effects of different treatments on blood plasma activity in the experimental broilers for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and blood plasma glucose content at 42 days old.

Treatments	Glucose	AST (U/L)	ALT (U/L)	ALP (U/L)
Control+[1]	188.5[b]	234.25[b]	13.00[a]	1400.50
Control-[1]	264.75[a]	273.75[a]	20.00[c]	1871.75
Control+ + 0.4 ml savory extract	189.00[b]	227.25[b]	13.75[bc]	1672.75
Control- + 0.4 ml savory extract	232.5[ab]	228.75[b]	16.25[b]	1580.50
SEM	10.64	6.12	0.83	78.11
Probability	0.3	0.007	0.14	0.94
Independent comparisons	Probability			
Control+ vs control-	0.001	0.02	0.0004	Ns
Control+ vs savory	0.29	0.03	0.03	Ns
Savory vs control-	Ns	0.017	Ns	Ns

[1]Control+ = birds grown in normal temperatures throughout the trial (21-42 days), and Control- = birds grown under heat stress throughout the trial (21-42 days); the means within the same column with different letters, have significant differences ($P<0.05$). SEM: standard error of the mean.

IgG

IgG measurements are shown in Table 6. As is clear, the IgG contents of chickens' blood were significantly affected by different treatments in different weeks ($P<0.05$). IgG rose with the inclusion of savory extract in broilers' drinking water, and in the 42-day treatment 3, (Control+ + 0.4 ml savory extract), had the highest IgG content ($P<0.05$). Also, at 42 day the IgG extent of treatment 4 (Control- + 0.4 ml savory extract) improved significantly in compare with control- group ($P<0.05$).

Table 6. Table 4. Effect of different treatments on IgG of experimental broilers at 42 day of age. (mg/ml).

Treatments	21d	28d	35d	42 d
Control+[1]	2.35	1.93[ab]	1.81[b]	2.03[a]
Control-[1]	1.79	1.38[c]	1.55[c]	1.57[c]
Control+ + 0.4 ml savory extract	2.46	2.17[a]	1.98[a]	2.20[a]

Control- + 0.4 ml savory extract	1.91	1.65^{bc}	1.90^{ab}	1.82^{b}
SEM	0.11	0.09	0.05	0.07
Probability	0.15	0.04	0.0003	0.005
Independent comparisons	Probability			
Control+ vs control-	2.40^{a}	2.05^{a}	Ns	2.12^{a}
Control+ vs savory	1.85^{b}	1.52^{b}	Ns	1.70^{b}
Savory vs control-	Ns	1.91^{a}	1.94^{a}	2.01^{a}

[1]Control+ = birds grown in normal temperature throughout the trial (21-42 days), and Control- = the birds grown under heat stress throughout the trial (21-42 days); the means within the same column with different letters, have significant differences (P<0.05). SEM: standard error of the mean.

Discussion

In the current study, savory extract exhibited hopeful effects on WG in broiler chickens from 21–42 days old when the birds suffered extreme heat stress. The savory was identified as a natural product rich in essential oils and carvacrol; practically all its attributes are characterised by the carvacrol (Khosravinia, 2016). It has been shown that supplementing drinking water with 200, 300, 400 or 500 mg/L of savory essential oils over 1–28 days, may effectively influence and increase weight gain from 29–42 days old (Khosravinia *et al.*, 2013). Our findings are also consistent with the results in Lee *et al.* (2003) which found a 2% improvement in average daily weight gain in broiler chickens with the inclusion of 0.2 g/kg carvacrol in the diet. Although, some reports recorded no positive effects from savory on weight gain in broiler chickens (Khosravinia, 2016). Nevertheless, the differences in observations may be due to the physiological status of broilers; thus, in the current study chicks were raised in conditions of heat stress. The improved FI in treated chickens in this study, corresponded with results observed by Basmacioglu *et al.* (2004), who found that an addition of 0.2 g/kg carvacrol and 0.15 g/kg oregano extract produced +2% and −6% difference in the FI of treated birds, compared with control groups. The results for FI in this study were not in line with the findings of Lee *et al.* (2003) or (Khosravinia, 2016). Furthermore, the improvement in FI in this study may be due to the phytogenic properties of savory; there are suggestions that a dietary inclusion of phytogenic foodstuffs may improve digestion processes in avian species (Mellor, 2000). The data on FCR in this study differed from the results of almost all other reports. In this study, Savory-treated water caused no change in FCR. These findings disagree to some extent with

reports in Basmacioglu *et al.* (2004) and Lee *et al.* (2003), who observed a decline in FCR in chickens treated with carvacrol.

Furthermore, the use of savory oil up to 21 days had a positive effect on organs, although this influence was not significant. Mohammad et al. (2013) reported that the inclusion of savory essential oil for 21 days (days 21-42) in the diet of heat stressed broilers had no significant effect on the lymphoid organs (spleen, bursa of Fabricius and thymus,) and weight, and our observations confirm that. Besides, in some studies conducted in fields, an influence by medicinal plants on increases in the relative weights of different organs has been reported (Schuberth *et al.*, 2002; Rivera *et al.*, 2003). Rahimi *et al.* (2011) reported that thyme extract (0.1%), dissolved in drinking water, affected the relative weight of the bursa of fabricius in broilers significantly. Moreover, broilers treated with dietary polysavone (alfalfa extract) saw increases in the relative weights of the thymus, bursa and spleen (Dong *et al.*, 2007).

In the current study, blood glucose in experimental birds was influenced by savory essence and decreased significantly; while these observations are dissimilar to results obtained by Ghazi *et al.* (2015) and Saadat *et al.* (2004), who reported no effect by savory on blood glucose in broilers. A disturbance of glucose metabolism in the liver was suggested to be a result of an anti-diabetic effect of savory oil, which might be related to savory's antioxidative property (Souri, 2015). Thus, any medication to alter hepatic gluconeogenesis or glycogenolysis might affect glucose homeostasis (Souri, 2015). Further, reductions in fasting blood glucose and triglyceride were reported when savory essential oil was given to diabetic and hyperlipidemic rats (Abdollahi *et al.*, 2003).

The present results showed statistically significant reductions in activity by aspartate aminotransferase (AST) and alanine aminotransferase (ALT) following the addition of savory. Alkaline phosphatase (ALP) also declined in activity with the addition of savory to drinking water, albeit not significantly ($P > 0.05$). It is clear that the highly active AST, ALP and ALT in the blood are bioindicators of liver damage (Rosa *et al.*, 2001; Safameher *et al.*, 2008; Mohamed & Mohamed, 2009; Valchev *et al.*, 2014) so a reduction of these hepatic enzymes by adding savory, indicated a positive effect by savory essence on reparation of the liver.

The IgG measurements from experimental broilers revealed a significant improvement in IgG content in the blood of those treated with savory. Observations in Souri *et al.* (2015) confirm our findings. It is vital for the poultry industry to improve immunity and avoid infectious diseases. A range of factors including vaccination failure, infection by immunosuppressive diseases and abuse

of antibiotics can induce immunodeficiency. The use of immunity stimulants is one way of increasing immunity in animals and reducing their vulnerability to infectious disease (Chen *et al.,* 2003). Plants with a high content of flavonoids, such as T. vulgaris, expand the activity of vitamin C, act as antioxidants and may therefore enhance functions of immunity (Cook & Samman, 1996; Manach *et al.,* 1996). The addition of 0.3% savory to the broilers' diet elevated chickens' Newcastle disease titers because the high volumes of vitamin A and vitamin E in the herb assist antibody production, improving serum antibody levels and the phagocytic activity of immune cells (Tampieri *et al.,* 2005). Flavonoids and polyphenolic complexes exhibit several pharmacological properties, including antioxidant activity, the inhibition of histamine release from mast cells and of arachidonic acid metabolism (Amresh *et al.,* 2007). Essential oil extracted from savory reversed oxidative damage to rat lymphocytes induced by hydrogen peroxide (Hajhashemi *et al.,* 2011).

Conclusion

In conclusion, the present study has shown that the inclusion of savory extract at 4 ml/L in drinking water to broilers under heat stress may improve economic efficiency in broiler flocks. Advantageous effects: greater WG and FI, improved IG over 21–42 days of age, as well as reduced hepatic enzymes, are due to the accumulation of minute quantities of savory extract.

References

Abdollahi, M., Salehnia A., Mortazavi, S.H., Ebrahimi, M., Shafiee, A., Fouladian, F., Keshavarz, K, Sorouri, S., Khorasani, R., & Kazemi, A. (2003). Antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory properties and safety of essential oil of *Satureja khuzistanica* in rat in vivo: a toxicopharmacological study. Med. Sci. Monit. 9:331-335.

Amresh, G., Reddy, G., Rao, C.V., & Singh, P. (2007). Evaluation of anti-inflammatory activity of Cissampelos pareira root in rats. J. Ethnopharmacol. 110, 526-531.

Azaz, D., Demirci, F., Satil, F., Kurkcuoglu, M., & Baser, K.H. (2002). Antimicrobial activity of some Satureja essential oils. Z Naturforsch C. 57:817–821.

Basmacioglu, H., Tokusoglu, O., & Ergul, M. (2004). The effects of oregano and rosemary essential oils or alpha-tocopheryl acetate on performance and lipid oxidation of meat enriched with n-3 PUFAs in broilers. South Afr J Anim Sci. 34:197–210.

Belay, T., &Teeter, R.G. (1993). Broiler water balance and thermo-balance during thermoneutral and high ambient temperature exposure. Poult Sci. 72:116–124.

Çabuk, M., Bozkurt, M., Alçiçek, A., Çatlı, A.U., & Başer, K.H.C. (2006). The effect of a mixture of herbal essential oils, a mannan oligosaccharide or an antibiotic on performance of laying hens under hot climatic conditions. S. Afr. J. Anim. Sci. 36:135–141.

Chen, H., Li, D., Chang, B.Y., Gong, L., Dai, J., & Yi, G. (2003). Effects of Chinese herbal polysaccharides on the immunity and growth performance of young broilers. Poult. sci. 82, 364-370.

Cook, N. & Samman, S. (1996). Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J. Nutr. Biochem. 7, 66-76.

Dong, X.F., Gao, W.W., Tong, J.M., Jia, H.Q., Sa, R.N., & Zhang, Q. (2007). Effect of polysavone (Alfalfa extract) on abdominal fat deposition and immunity in broiler chickens. J. Poult. Sci. 86:1955-1959.

Ghazi, S., Mahdavi, R., Naseri, A., & Habibian, M. (2015). Effects of supplementation of Satureja khuzistanica Jamzad essential oils in the drinking water on growth performance, serum metabolites and differential counts of white blood cells in broiler chickens. Journal of Livestock Science and Technologies,3(1), 1-10.

Hajhashemi, V., Sajjadi, S.E., & Zomorodkia, M. (2011). Antinociceptive and anti-inflammatory activities of Bunium persicum essential oil, hydroalcoholic and polyphenolic extracts in animal models. Pharmacol. Biol. 49, 146-151.

Khosravinia, H. (2016). Mortality, production performance, water intake and organ weight of the heat stressed broiler chicken given savory (Satureja khuzistanica) essential oils through drinking water, Journal of Applied Animal Research, 44:1, 273-280.

Khosravinia, H., Ghasemei, S., & Rafiei Alavi, E. (2013). The effect of savory (Satureja khuzistanica) essential oils on performance, liver and kidney functions in broiler chickens. J Anim Feed Sci. 22:50–55.

Lee, K.W., Everts, H., Kappert, H.J., Frehner, M., Losa, R., & Beynen, A.C. (2003). Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. Br Poult Sci. 44:450–457.

Manach, C., Regerat, F., Texier, O., Agullo, G., Demigne, C., & Remesy, C. (1996). Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutr. Res. 16, 517-544.

Männer, K., & Wang, K. (1991). Effectiveness of zinc bacitracin on production traits and energy metabolism of heat-stressed hens compared with hens kept under moderate temperature. Poult. Sci. 70:2139–2147.

May, J.D., & Lott, B.D. (1992). Feed and water intake patterns of broilers at high environmental temperatures. Poult Sci. 71:331–336.

Mellor, S. (2000). Nutraceuticals – alternatives to antibiotics. World Poult. 16:14–15.

Mohamed, M.A., & Mohamed, M.H. (2009). Haemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt. Veterinaria Italiana, 45, 323–337.

Mohammad, M., Vahid, A., & Masoud, P. (2013). Supplementation effect of Satureja essential oils in drinking water on immune conduct of broiler chickens reared under stress of heat. Global Journal of Animal and Environmental Biology. Vol. 1 (1), pp. 001-004.

Radonic, A., Milos, M. (2003). Chemical composition and in vitro evaluation of antioxidant effect of free volatile compounds from Satureja montana L. Free Radical Res. 37:673–679.

Rahimi, S., Teymouri Zadeh, Z., Karimi Torshizi, M.A., Omidbaigi, R., & Rokni, H. (2011). Effect of three herbal extracts on growth performance, immune system,

blood factors and intestinal selected bacterial population in broiler chickens. J. Agri. Sci. Tech. 13:527-539.

Rivera, E., & Hu, S. (2003). Ginseng and aluminium hydroxide act synergistically as vaccine adjuvants. Vaccine, 21:1149-1157.

Rosa, C.A.R., Miazzo R., Magnoli, C., Salvano, M., Chiacchiera, S.M., Ferrero, S., Saenz, M., Carvalho, E.C.Q., & Dalcero, A. (2001). Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poultry Science, 80, 139–144.

Ross 308 broiler. 2007. Nutrition specification. Available in: www.aviagen.com.

Saadat, M., Pournourmohammadi, S., Donyavi, M., Khorasani, R., Amin, G., Salehnia, A.N., & Abdollahi, M. (2004). Alteration of rat hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase activities by Satureja khuzestanica Jamzad essential oil. J. Pharm. Pharmacol. Sci. 7, 310-314.

Safameher, A. (2008). Effects of clinoptilolite on performance, biochemical parameters and hepatic lesions in broiler chickens during aflatoxicosis. Journal of the American Veterinary Medical Association, 7, 381–388.

Schuberth, H.J., Riedel, & Caspari, G. (2002). Flow cytometric testing of immunological effects of a phytomedicinal combination (EquiMun) and its compounds on bovine leucocytes. J. Vet. Med. 49(6):291-298.

Souri, H., Khatibjoo, A., Taherpoor, K., Hassan Abadi, A., Fattahnia, F., & Askari, M. (2015). Effect of Thymus vulgaris and Satureja khuzestanica ethanolic extracts on broiler chickens' performance and immune response. Iranian Journal of Applied Animal Science 5, 437-446.

Suderman, A., & Solikhah, S.H. (2011). Performance and meat cholesterol content of broiler chickens fed Pluchea indica L. leaf meal reared under stress condition. Media Peternakan. 43:64–68.

Tampieri, M.P., Galuppi, R., Macchioni, F., Carelle, M.S., Falcioni, L., Cioni, P.L., & Morelli, I. (2005). The inhibition of Candida albicans by selected essential oils and their major components. Mycopathologia. 159, 339-345.

Valchev, I., Kanakov, D., Hristov, T.S., Lazarov, L., Binev, R., Grozeva, N., & Nikolov, Y. (2014). Investigations on the liver function of broiler chickens with experimental aflatoxicosis. Bulgarian Journal of Veterinary Medicine, 17(4).

Zeinali, A., Kermanshahi, H., Riasi, A., Farhangfar, H., Sarir, H., & Ziaie, H. (2011). Effects of sodium selenite and turmeric powder on thyroid hormones and plasma lipids of broiler chickens reared under heat stress condition. Global Veterinaria. 6: 237–240.

Khazar Journal of Science and Technology Volume 3 №2 2019, 41-55

DOI: 10.5782/2520-6133.2019.3.2.41

Changes to Some Hormones and Glucose in the Blood of Rabbits Subjected to Physical Exertion and Regimes of Darkness and Light, and Subfetal Hypoxia of Ontogenesis

Konul Gasim Hasanova
Baku State University,
k.tallinny@gmail.com

Abstract

As the main purpose of this article, we set the task of studying the mechanism of changes in the parameters of melatonin, insulin, adrenaline and glucose in the blood of rabbits that had undergone prefetal hypoxia of prenatal ontogenesis and been subjected to physical exertion and light conditions in the postnatal period of development. The article presents our research and its results. The purpose is to study the effect of long-term "on" and "off" functions of the epiphysis on various stressors, such as hypoxia and physical activity, and on physiological changes in the performance of the pancreas and adrenal glands in rabbits. Based on the results of the experiments, it was found that in physiologically epiphysectomized animals of different ages contained in the light and dark regimes, melatonin levels fall with increased insulin and rise with reduced insulin, and adrenaline levels fall from the norm under the action of physical exercise and photoperiodic action, but rise as a stress hormone during exercise with a reduction of melatonin. As numerous experiments show, the results of our research confirm an inverse relationship between melatonin and insulin, as well as between melatonin and adrenaline. However, the results obtained during the light mode differ from those taken during the dark mode, an increase in the level of melatonin is observed during physical activity in the dark mode, and a decrease in the light mode. It was found that with exercise and the photoperiodic factor in 30-day-old animals, in the circadian rhythm during exercise and under illumination hormone levels increase at first and then decrease in accordance with artificially created stress reactions.

Keywords: hypoxia; melatonin; insulin; adrenaline; glucose; ELISA

Introduction

Melatonin, one of the oldest evolutionary biochemical substances, was discovered by American dermatologist A. Lerner and isolated from bovine epiphyses in 1958. Melatonin from L-tryptophan is formed from serotonin by the agency of arylalkylamine-N acetyltransferase (AA-NAT; a key regulator). This substance is already present in single-celled organisms and in plants, and therefore in ordinary plant food, but in insignificant, 'homeopathic' concentrations that have no effect on the mammalian organism. In vertebrate animals, the main source of melatonin is the pineal gland. Neurologist O. Marburg suggested that the epiphysis - the upper appendage of the brain, releases a substance that inhibits the functions of the hypothalamus (the most important structure at the base of the brain that controls the lower appendage, the pituitary gland) and, as a result, the development of the reproductive system. At about the same time, it was established that the epiphysis contains a substance that causes depigmentation (blanching) of the skin in tadpoles. 40 years later, this fact played a decisive role in the discovery of melatonin. Interestingly, Lerner, the discoverer of melatonin, first described its sedative (sedative) effect when administered to humans (Anisimov *et al.,* 2002; Anisimov *et al.,* 2003; Anisimov *et al.,* 2006; Anisimov *et al.,* 2000; Anisimov *et al.,* 2000).

Melatonin is involved in regulating the functions of the central and autonomic nervous systems, the endocrine organs, the immune system and their daily rhythmic activity. Hypoxia causes a complex restructuring of the functioning of various body systems to ensure the delivery of the required amount of oxygen to tissue. Adaptation to hypoxia has a significant effect on the central nervous system, central haemodynamics, microcirculation in various organs, oxygen metabolism, free radical lipid oxidation, the main enzymes in detoxification systems and immunity. The mechanisms that influence adaptation to hypoxia in the brain are considered. It has been established that improving cerebral circulation is one of the important protective effects of adaptation to hypoxia (Arushanyan, 2005; Arushanyan, 2005; Aliyev & Mammadova, 2017; Aliyev *et al.,* 2018; Aliyev & Mammadova, 2016; Aliyeva & Aliyev, 2017; Aliyev *et al.,* 2017; Aliyev *et al.,* 2009; Aliyeva & Aliyev, 2015; Aliyev *et al.,* 2003).

Stress such as hypoxia, various physical activities and exposure to light are one of the most pressing problems of modern medicine and biology (Anisimov *et al.,* 2002; Anisimov *et al.,* 2003; Anisimov *et al.,* 2006; Anisimov *et al.,* 2000). It is known that acute or chronic effects of stressors on the mammalian organism often lead to various forms of metabolic disorder (Aliyev *et al.,* 2017; Aliyev *et al.,* 2009; Aliyeva & Aliyev, 2015; Aliyev *et al.,* 2003). In particular, stress (hypoxia, physical exercise and photoperiodism) is accompanied by changes in the

production of insulin and glucagon, the synthesis of glucose and melatonin, and as a result, change in adrenaline (Anisimov *et al.,* 2006; Arushanyan, 2005). Scientific references on stress, hypoxia and related diseases revealed pronounced differences in subjects' individual sensitivities to the development of pathological effects of stress loads (Anisimov *et al.,* 2006; Anisimov *et al.,* 2000). A reliable prognostic criterion for resistance to stress by rats is their behavioural activity in the open field test. It has been established, in particular, that active animals are prognostically more resistant to similar effects of stress than passive individuals (Anisimov *et al.,* 2006; Aliyev & Mammadova, 2017).

To protect the body from the negative effects of emotional stress, the leading task is to increase individual resistance to stress. Scientific data, as well as the results of our previous experiments, indicate that the epiphyseal neurohormone melatonin is one of the natural anti-stress substances produced in mammals (Anisimov *et al.,* 2006; Broedel *et al.,* 2003; Lazarev *et al.,* 1976). In experimental studies on rats, it was demonstrated that under acute stress loads, melatonin prevents changes in organs that mark stress (the thymus and adrenal glands) and prevents a reduction in the content of glycosaminoglycans, the main components of the skin's connective tissue. It was found that melatonin's involvement in the body's response to emotional stressors is associated with changes in the biochemical and neurochemical processes in brain structures. Melatonin's specific participation in the maintenance of physiological functions in animals of different individually typological characteristics was established (Kvetnaya & Knyazkin, 2003; Komarov *et al.,* 2004; Levin *et al.,* 2005; Malinovskaya & Komarov, 2006; Musina *et al.,* 2005).

In recent years, along with other neurotransmitter systems, the melatonergic system (Pishchak, 2004; Tunez *et al.,* 2003; Vinogradov & Pogorelov, 1987; Zamorsky & Pischak, 2000; Zamorsky, 2003), which is an integral component of the body's chronoperiodic system (Rom-Bugoslavskaya *et al.,* 1993; Chazov & Isachenkov, 1974; Pishchak, 2004; Tunez *et al.,* 2003; Vinogradov & Pogorelov, 1987; Zamorsky & Pischak, 2000; Zamorsky, 2003), has been isolated. Characteristic features of the melatonergic system are thought to be (Chazov & Isachenkov; Magner, 1990; Sapronov & Fedotova, 2002; Fedotova & Sapronov, 2005; Klieverik *et al.,* 2005; Pishchak, 2004): 1) photosensitivity, 2) daily (or circadian) rhythm (with the highest levels of melatonin production at night, in the dark), 3) an age-related chronic progressive weakening of its activity. An involvement of the melatonergic system in the pathogenesis of certain diseases is considered possible (Musina, 2005; Sapronov & Fedotova, 2002). It may be assumed that the components of the melatoninergic system can be used by the body to adapt to the action of not only photoperiodically-dependent adverse environmental effects, but

also to non-periodic hazardous effects, for example, during hypoxia and exposure to the photoperiodic factor (Broedel *et al.,* 2003; Kvetnaya & Knyazkin, 2003; Komarov *et al.,* 2004; Lazarev *et al.,* Lazarev; Levin *et al.,* 2005; Vinogradov & Pogorelov, 1987; Zamorsky, 2003). Living organisms can exist in changing environmental conditions only due to the presence of innate programmes of adaptation to rhythmic changes in the environment and the corrective mechanisms of those programmes in accordance with external periodicals (Broedel *et al.,* 2003; Rodriges *et al.,* 2004; Rokitsky, 1961; Rowe & Kennaway, 2002). The role of the correcting factor in the body's chronoperiodic system is performed by the photoperiod, or the length of daily illumination. In the photoperiodic system of the brain (Simonneaux & Ribelayga, 2003; Shorokhova *et al.,* 2015), the length of the photoperiod becomes a change in the circulating level of melatonin - the main pineal hormone (Tunez *et al.,* 2003; Vinogradov & Pogorelov, 1987; Zamorsky, 2003). At the same time, melatonin synchronizes the rhythms of peripheral tissues, provides anti-stress and antioxidant protection of the body, modulates the activity of the brain's neurotransmitter systems and the whole neuroendocrine system. This ensures adaptation of the organism to dangerous effects of the external environment, particularly under conditions of total illumination and darkness (Malinovskaya *et al.,* 2006; Fedotova & Sapronov, 2005; Klieverik *et al.,* 2005; Raykhlin & Kvetnoy, 1992).

Materials and methods

1-month-old rabbits of the *Chinchilla* breed were used in the study; they were obtained from adult rabbits that had undergone conditions of several hypoxia at different stages of the pregnancy germinal 1-10, pre-pubertal 10-20 and fetal 20-30 periods of development. In this paper, we have shown results from the pre-pubertal 10-20 hypoxia.

The hypoxia model nitrogen (N2) 93% and oxygen (O2) 7%, proposed by B. P. Khvatov and Ye.M. Khvatova (Rokitsky, 1961; Shorokhova *et al.,* 2015), was chosen for the hypoxia of pregnant individuals. B.P. Khvatov is an outstanding embryologist who gradually studied the development of the embryo. Using his methodology, our department has been working with prenatal hypoxia for a long time, exposing pregnant rabbits to embryonic, prefetal and fetal hypoxia of prenatal development of embryogenesis. The animals were kept in a pressure chamber with mixtures of these gases (mix of oxygen and nitrogen) for 20 minutes. After normal prenatal hypoxia, which normally grew and grew, blood was taken from the marginal ear vein and centrifuged for 20 minutes at a speed of 1500 rpm to obtain

plasma. An EDTA preservative was added to the plasma samples to maintain the molecular integrity of the hormones melatonin and insulin. Their quantities were determined by enzyme immunoassay using a Cusabio reagent-kit according to the manufacturer's protocol (ELISA - enzyme linked immune sorbent assay). Quantitative measurement was performed on a StatFax + 303 analyzer manufactured in the USA (Awareness Technologies Inc.) using light filters with a wavelength of 450 or 630 nm for the hormones. Further, the rabbits were subjected to 5-, 20- and 40-minutes of physical exertion - running on a treadmill, after which blood was taken and the hormone content determined: melatonin, insulin and adrenaline. Illumination conditions were arranged as follows: a group of 30-day-old rabbits was divided into 2 groups: one group in conditions of complete illumination, and one in conditions of complete darkness, for 10 days each.

The data obtained were processed by statistical methods, using the standard Excel and Student data packages. All the data obtained were processed by methods of variation statistics with an assessment of reliability using the bases of variation statistics for biologists, Rokitsky P.F. (Rokitsky, 1961; Shorokhova *et al.*, 2015).

The studies were carried out on the Chinchilla breed of rabbit and were planned in 4 series:

The first series of experiments is the study of changes in some hormones and glucose in the blood of 30-day-old rabbits subjected to physical exertion and a photoperiodic factor in normal conditions.

The second series of the experiment is the study of changes in certain hormones and blood glucose in 30-day-old rabbits, subjected to physical exertion and a photoperiodic factor in conditions of germinal hypoxia.

The third series of the experiment is the study of changes in some hormones and blood glucose in 30-day-old rabbits, subjected to physical exertion and a photoperiodic factor in conditions of pre-feminine pre-fetal hypoxia.

The fourth series of experiment is the study of changes in some hormones and blood glucose in 30-day-old rabbits, subjected to physical exertion and a photoperiodic factor in conditions of fetal hypoxia.

Animals were divided into two groups: control and experimental. For the study, we took for each series 72 control and 216 experimental rabbits, with each series consisting of a norm (no PE, no FF), a norm (5 min PE, no FF), a norm (20 min PE, no FF), norm (40min PE, no FF), norms (no PE, light FF), norms (5min PE, light FF), norms (20min PE, light FF), norms (40min PE, light FF), norms (no PE,

darkness FF), norms (5min PE, darkness FF), norms (20 min PE, darkness FF), norms (40min PE, darkness FF).

For the experiment, blood is taken from the outer marginal vein of the rabbit's ear to a tube with the anticoagulant K_2EDTA (potassium ethylenediaminetetraacetate). The tube of blood is then centrifuged to obtain plasma. Next, the plasma's hormone level is determined by the ELISA method using the Cusabio kit. The experiment is conducted according to the manufacturer's protocol. The hormone results (ELISA - enzyme linked immunosorbent assay), are quantified on a StatFax + 303 US analyzer (Awareness Technologies Inc) using light filters with a wavelength of 450 or 630 nm to quantify hormones in plasma.

Enzyme-linked immunosorbent assay (abbreviated as ELISA) is a laboratory immunological method for the qualitative or quantitative determination of various low-molecular compounds, macromolecules, viruses, etc. based on a specific antigen-antibody reaction. Identification of the resulting complex is carried out by using the enzyme as a label to register a signal. The theoretical basis of ELISA lies in modern immunochemistry and chemical enzymology, knowledge of the physicochemical laws of the antigen-antibody reaction, and in the basic principles of analytical chemistry.

ELISA is one of the most rapidly developing areas of chemical enzymology. This is because in ELISA a unique specificity of the immunochemical reaction (that is, antibodies bind exclusively to certain antigens, and to no other) is combined with highly sensitive detection of the enzyme label (up to 10−21 mol in the sample). The high stability of the reagents, the simplicity of registration methods, the possibility of creating cascade systems to amplify various chemical signals, the relatively low price and many other advantages of the ELISA method have contributed to its widespread adoption in various fields of medicine, agriculture, the microbiological and food industries, environmental protection and scientific studies (Anisimov *et al.*, 2003; Anisimov *et al.*, 2006; Anisimov *et al.*, 2000a; Anisimov *et al.*, 2000).

Results and discussion

The data we obtained are shown in table 1, table 2 and table 3. As can be seen from table 1 (with prefetal hypoxia), indicators for melatonin, insulin, adrenaline and glucose in the blood plasma of 30-day-old rabbits were 26.55 ± 3.87 ** U / ml, 26.95 ± 1.2 ** U / ml, 275.38 ± 24.27 ** U / ml and 90.5 ± 0.97 **, respectively. These parameters after a short 5-minute physical activity were: for melatonin -

36.31 ± 2.26 ** U / ml, for insulin - 15.58 ± 2.34 * U / ml and adrenaline 428 ± 50.09 ** * U / ml, glucose 97.3 ± 2.58 *. These parameters, after a short 20-minute exercise, were: for melatonin - 77.86 ± 3.23 *** U / ml, for insulin - 19.48 ± 1.2 *** U / ml and adrenaline 475.6 ± 24.27 *** U / ml, glucose 151.83 ± 3.84 ***. After 40 minutes of long-term physical exercise, these parameters were: for melatonin - 5.76 ± 0.85 *** U / ml, for insulin - 65.01 ± 2.02 *** U / ml and adrenaline 780.43 ± 50.09 *** U / ml, glucose 68.16 ± 2.58 ***.

The amounts of melatonin, insulin, adrenaline and glucose in the blood were also determined in 30-day-old rabbits subjected to a photoperiodic factor. The photoperiodic factor in our experiment is characterized by conditions of light and darkness.

During prefetal hypoxia of embryogenesis, in 30-day-old rabbits, the concentrations of melatonin, insulin, adrenaline and glucose in the blood plasma of the month-old rabbits, which had a 10-day coverage, were expressed as: 46.36 ± 2.46*** U / ml, 135.43 ± 6.79 *** U / ml, 314.55 ± 16.94 *** U / ml and 89.16 ± 2.34***, respectively. When the content was of rabbits in dark conditions, these parameters were: for melatonin - 119.13 ± 4.36 *** U / ml, for insulin - 96.88 ± 2.2** U / ml, adrenaline 372.58 ± 15, 86 *** U / ml and glucose 66.33 ± 4.55 ***, respectively. During prefetal hypoxia and hypoxia of embryogenesis, 30-day-old rabbits undergoing 10-day lighting were subjected to short-term, 5-minute exercise and the concentrations of melatonin, insulin, adrenaline and glucose in the blood plasma of monthly month old rabbits were expressed: 31.28 ± 1.87 * ** U / ml, 143.16 ± 3.56 *** U / ml, 421.05 ± 6.27 *** U / ml and 48.5 ± 6.33 ***, respectively. During embryonic hypoxia of embryogenesis, 30-day-old rabbits undergoing 10-day coverage were subjected to a short-term, 20-minute exercise and the concentrations of melatonin, insulin and adrenaline in the blood plasma of monthly rabbits were expressed: 24.28 ± 1.7 ** U / ml, 151.58 ± 2.08 ** U / ml, 543.76 ± 35.4 * U / ml and 93.83 ± 4.82 ***, respectively. During prefetal hypoxia of embryogenesis, 30-day-old rabbits undergoing 10-day coverage lighting were subjected to long-term 40-minute exercise and the concentration of melatonin, insulin, adrenaline and glucose in the blood plasma of monthly rabbits were expressed: 12.91 ± 2.13 ** * U / ml, 163.25 ± 3.67 **** U / ml, 743 ± 35.83 *** U / ml and 70.33 ± 1.69 ***, respectively.

During prefetal hypoxia of embryogenesis, in 30-day-old rabbits, undergoing a 10-day condition of darkness were subjected to short-term 5-minute exercise and the concentration of melatonin, insulin, adrenaline and glucose in the blood plasma of monthly rabbits were expressed: 69.56 ± 2.89 * ** U / ml, 119.6 ± 2.48 * U / ml, 458.41 ± 1.85 *** U / ml and 88.66 ± 4.18 *****, respectively. During prefetal

hypoxia of embryogenesis, in 30-day old rabbits, undergoing a 10-day condition of darkness were subjected to short-term 20-minute exercise and the concentration of melatonin, insulin, adrenaline and glucose in the blood plasma of monthly rabbits were expressed: 59.63 ± 3,93 $^{* **}$ U / ml, 111.06 ± 1.95 ** U / ml, 632.17 ± 23.11 *** U / ml and 147.8 ± 6.78 ***, respectively. During embryonic hypoxia of embryogenesis, in 30-day-old rabbits, undergoing a 10-day condition of darkness were subjected to long-term 40 minute exercise and the concentrations of melatonin, insulin, adrenaline and glucose in the blood plasma of monthly rabbits were expressed: 94.43 ± 4.01 $^{* **}$ U / ml, 138.1 ± 3.35 ***** U / ml, 825.23 ± 23.97 *** U / ml and 70.16 ± 3.87 ***, respectively.

Table 1. The concentration of melatonin, insulin, adrenaline and glucose in the blood plasma of one-month-old rabbits after physical exercise (PE). Parameters to be determined: subfetal hypoxia + PE

Determine hormones	Subfetal hypoxia+PE			
	NORM	5 min	20 min	40 min
Melatonin	26.55±3.87**	36.31±2.26**	77.86±3.23***	5.76±0.85***
Insulin	26.95±1.2**	15.58±2.34***	19.48±1.2***	65.01±2.02****
Adrenaline	275.38±24,27**	428±50.09***	475.6±21.24***	780.43±50.09***
Glucose	90.5±0.97**	97.3±2.58*	151.83±384***	68.16±2.58***

P> 0.01* ; P <0.05** ; P <0.001*** ; P<0.02**** ; P> 0.5*****.

Table 2: Concentration of melatonin, insulin, adrenaline and glucose in the blood plasma of 1 month old baby rabbits after the influence of a photoperiodic factor (light) + physical exercise (PE).

Determine hormones	Subfetal hypoxia+FF(light)+ PE			
	NORM (light)	5 min	20 min	40 min
Melatonin	46.36±2.46***	31.28±1.87***	24.28±1.7***	12.91±2.13***
Insulin	135.43±6.79***	143.16±3.56***	151.58±2.08**	163.25±3.67****
Adrenaline	314.55±16.94***	421.05±6.29***	543.76±35.83***	743±35.83***
Glucose	89.16±2.34***	118.5±6.33***	93.83±4.82***	70.33±1.63***

P> 0.01* ; P <0.05** ; P <0.001*** ; P<0.02**** ; P> 0.5*****.

Table 3: Concentration of melatonin, insulin, adrenaline and glucose in the blood plasma of 1 month old baby rabbits after the influence of a photoperiodic factor (dark) + physical exercise (PE).

Determine hormones	Subfetal hypoxia+FF(dark)+PE			
	NORM (dark)	5 min	20 min	40 min
Melatonin	119.13±4.36***	69.56±2.89***	59.63±03.93***	94.43±4.01***
Insulin	96.88±2.2**	119.6±2.48*	111.06±1.95**	138,1±3.35****
Adrenaline	372.58±15.86***	458.41±1.85***	632.17±23.11***	825.23±23.97***
Glucose	66.33±4.55***	88.66±4.18*****	147.8±6.78***	70.16±3.87***

P> 0.01* ; P <0.05** ; P <0.001*** ; P<0.02**** ; P> 0.5*****.

As a result of our experiments, it was found that the absence of photoperiodism with constant illumination has a modulating effect on the study of indicators, depending on what period of ontogenesis the effect began - in our case it lasted for 10 days, starting on the 30th day of the rabbit's life. Also, the effect of prenatal hypoxia for 10 days in the embryonic period of development, at the time of formation of the internal organs and body systems, inhibits the synthetic function of the epiphysis from the moment of birth. And physical activity leads to a unidirectional change in the activity of antioxidant enzymes, although their expression is different and we see changes in the results. The suppression of the synthesis of melatonin, an antioxidant that can directly interact with other hormones, is also reflected in changes in insulin and adrenaline. According to the literature, exposure to light is a desynchronizing factor that causes significant changes in various body systems, including the immune system (Arushanyan, 2005; Aliyev & Mammadova, 2017; Aliyev *et al.,* 2018; Aliyev & Mammadova, 2016).

Suppressing the function of the pineal gland, achieved by epiphysectomy or having content under constant light (physiological epiphysectomy) leads to synchronization of the circadian rhythms of many physiological functions: accelerated aging of a number of functional systems, development of a number of age-related pathological processes, including malignant neoplasms and, ultimately, shortening the life of the individual (Brizzi *et al.,* 1997; Broedel *et al.,* 2003; Kvetnaya & Knyazkin, 2003; Komarov *et al.,* 2004; Lazarev *et al.,* 1976; Reiter, 1995; Reppert *et al.,* 1989; Rodriges *et al.,* 2004). Light deprivation, which stimulates the function of the pineal gland, has the opposite effect, a geroprotective effect (Lazarev *et al.,* 1976; Rokitsky, 1961). The presence of animals during prenatal development in a condition of short-term hypoxia and with constant

illumination and darkness from the moment of birth for 10 days, affects change in the indices of melatonin, insulin and adrenaline.

The use of the epiphyseal hormone melatonin inhibits the carcinogenesis of animals in both normal lighting mode and with constant light, it prevents the development of pathology associated with age and increases animals' life expectancy. Using melatonin to prevent cancer can be very effective, especially in northern regions, where there are "white nights" in the summer, and electric light shines on for a long polar night (Aliyev *et al.,* 2017; Aliyev *et al.,* 2009; Aliyev *et al.,* 2015; Aliyev *et al.,* 2003; Brizzi *et al.,* 1997; Broedel *et al.,* 2003; Kvetnaya & Knyazkin, 2003; Komarov *et al.,* 2004; Lazarev *et al.,* 2005; Levin *et al.,* 2005; Malinovskaya *et al.,* 2006).

So from plentiful data in the literature (Anisimov *et al.,* 2003; Anisimov *et al.,* 2006; Aliyev & Mammadova, 2017; Aliyev *et al.,* 2018; Aliyev & Mammadova, 2016; Aliyeva & Aliyev, 2017; Aliyev *et al.,* 2017; Aliyev *et al.,* 2009) it is known that the pineal gland hormone - melatonin - is mainly synthesized in the dark phase, while in the light phase this is insignificant. Therefore, disturbances in the normal rhythm of light and darkness in 30-day-old rabbits, laboratory rats and quails cause changes in the circadian rhythm and disturbances in metabolic processes (Anisimov *et al.,* 2006; Anisimov *et al.,* 2000, Aliyeva & Agayev, 2015; Brizzi *et al.,* 1997). From the data obtained it can be noted that, depending on the age of the animals, before and after exercise and the photoperiodic factor, neuro-hormonal regulation of the blood changes during the day. The effect of constant darkness, in contrast to constant illumination, enhances the work of the epiphysis and leads to an increase in melatonin synthesis by this gland (Reiter, 1995; Simonneaux & Ribelayga, 2003; Tunez *et al.,* 2003). This is what we observed in our series of experiments on the dark phase of the photoperiodic factor.

Thus, the levels of melatonin, insulin and adrenaline in intact animals, depending on the circadian rhythm after exercise, vary by decreasing, then increasing, i.e. with a decrease in melatonin, insulin and adrenaline increase , and with an increase in melatonin, insulin and adrenaline decrease. And the amount of adrenaline as a stress hormone increases accordingly. The reason for the decrease or increase of hormones in the blood of intact and animals kept in dark and light conditions and both before and after exercise, is associated with the activation or inhibition of function in the epithalamic-hypothalamic-pituitary-adrenal systems (Anisimov *et al.,* 2006; Anisimov *et al.,* 2000a; Anisimov *et al.,* 2000; Arushanyan, 2005a; Arushanyan, 2005; Aliyev & Mammadova, 2017). The epiphysis acts as an endogenous synchronizer of circadian and seasonal rhythms. The revelation that melatonin does not stand out under bright light has served to revive phototherapy

and now light therapy in the west is widely used by chronobiologists for the treatment of desynchronosis (Anisimov & Zabezhinsky, 2000a; Anisimov *et al.*, 2005; Aliyev & Mammadova, 2016). The data we obtained in the light and dark regimes can also be used by chronophysiologists for the prevention and treatment of diabetes. Based on the above, we can conclude that circadian rhythms affect changes in the level of hormones in the blood of healthy people and mammals; The level of melatonin in the blood of a little rabbit falls in daylight and rises in the dark (Arushanyan, 2005; Aliyeva & Aliyev, 2017). The levels of insulin and adrenaline, on the contrary, rise in daylight, and fall in the dark. This is reflected in the dynamics of all processes and functions that are affected by the epiphysis and depending on a light or dark phase, thereby connecting the body to the general chronometric and biorhythmic regulation embedded in phylogenesis and ontogenesis during exercise and lighting conditions (Anisimov *et al.*, 2003; Anisimov *et al.*, 2000a; Arushanyan, 2005; Aliyev *et al.*, 2018; Aliyev *et al.*, 2009; Zamorsky, 2003).

The effects of constant illumination and constant darkness on the physiological parameters revealed here may be associated with changes in melatonin synthesis by the pineal gland. This hormone plays a significant role in the regulation of puberty, reproductive cycles, stress response and immune response (Reiter, 1995; Reppert *et al.*, 1989; Rodriges *et al.*, 2004; Rowe & Kennaway, 2002; Simonneaux & Ribelayga, 2003; Tunez *et al.*, 2003).

Conclusion

It is interesting to note that the secretion of certain hormones with distinct circadian fluctuations is regulated primarily by circadian clocks (for example, cortisol, melatonin), while the secretion of other hormones is largely dependent on sleep (for example, prolactin, growth hormone), and the secretion of a third group of hormones is simultaneously related to endogenous circadian factors and to sleep (for example, thyroid stimulating hormone, insulin, adrenaline). Being independent of sleep and relatively independent of external masking effects, melatonin secretion is an ideal indicator for the study of circadian factors that can be associated with effective disorders. In recent years, fundamentally new data on the role of melatonin in the regulation of insulin secretion and the pathophysiology of carbohydrate metabolism disorders have been obtained, and the prospects for melatonin use in treatment are discussed. Perhaps this is due to the fact that hormones of the pineal gland, pancreas and adrenal glands in rabbits have a

significant protective effect on the substance of the brain under conditions of ischaemia, hypoxia and stress reaction (physical activity and photoperiodic factor).

The melatoninergic system is significant in the system of antihypoxic protection of the body, carrying this out dependent on the duration of the photoperiod. In an organism adapted to hypoxia, a restructuring of the sympathoadrenal system occurs, and is characterized by hypertrophy of sympathetic neurons, an increase in catecholamine synthesis and catecholamine reserves in the adrenal glands, as well as an increase in cardiac adrenoreactivity. Thus, there is an increase in the reserve capacity of the sympathetic nervous system. It was shown that a course of interval hypoxic training also led to an increase in the power of the mechanisms of autonomic regulation of heart functions at rest due to an increase in the activity of the parasympathetic autonomic nervous system; it also caused optimizing effects on the degree of shifts in heart rate variability (HRV) during simulated acute hypoxia. Hypoxic preconditioning contributed to an increase in the body's resistance to conditions of simulated acute hypoxia, which was manifested in a less pronounced degree of haemoglobin desaturation and a smaller increase in heart rate. It has been established that the training effects of a course of interval hypoxic training are more pronounced in groups of individuals who initially have low resistance to the hypoxic factor relative to subjects who are resistant to acute hypoxia (48, 102). Thus, adaptation to hypoxia has a significant effect on the central nervous system, central haemodynamics, blood microcirculation in various organs, oxygen metabolism, free radical lipid oxidation, the main enzymes of detoxification systems and immunity.

References

Anisimov, V.N., Baturin, D.A. & Aylamazyan, E.K. (2002). Epiphysis, light and breast cancer. Vopr. oncol. (48), 524-535.

Anisimov, V.N., Aylamazyan, E.K., Baturin, D.A., Zabezhinsky, M.A., Alimova, I.N., Popovich, I.G., Beniashvili, D.S., Menton, K.R., Provincali, M. & Francesca, K. (2003). Light regime, anovulation and the risk of malignant neoplasms of the female reproductive system: communication mechanisms and prevention. J. gynaecology and female diseases. (52):2, 47-58.

Anisimov, V.N. & Vinogradova, I.A. (2006). Light Mode, Melatonin, and Cancer Risk. Vopr. Onkol., (53):5, 491 -498.

Anisimov, V.N. & Zabezhinsky, M.A. (2000). Popovich I.G. Melatonin inhibits colon carcinogenesis induced by 1,2-dimethylhydrazine in rats: effects and possible mechanisms. Vopr. oncol. (46): 2, 136-148.

Anisimov, V.N., Kvetnoy, I.M., Komarov, F.I., Malinovskaya, N.K. & Rapoport, S.I. (2000). Melatonin in the physiology and pathology of the gastrointestinal tract. - M.: "Soviet Sport", 184 p.

Arushanyan, E.B. (2005). Chronopharmacology at the turn of the century. Stavropol: Ed. SGMA, 576 p.

Arushanyan, E.B. (2005). The hormone of the epiphysis melatonin and its therapeutic capabilities. Breast Cancer. (13):26, 1755-1760.

Aliyev, A.H. & Mammadova, S.J. (2017). Physical loading effect of blood components dynamic changes in the 30 days hypoximal baby rabbits in the prenatal ontogenesis. The Journal of Medicine and Philosophy, 6(2), 42, 1312-1317.

Aliyev, A.H., Aliyeva, F.A., Mammadova, S.J. & Hamidova, J.E. (2018). The influence of physical loading to erythroform indicators in rabbits puppies suffered hypoxia. International Journal of Biology Research. (3):1, 138-140.

Aliyev, A.H. & Mammadova, S.J. (2016). Physical loading effect of blood components dynamic change in hypoxianal baby rabbits in the prefetus period of prenatalontogenesis. "Jomard Publishing" Advances in Biology and Earth Sciences. (1): 1, 81-85.

Aliyeva, F.A. & Aliyev, A.H. (2017). Changing the quantity of insulin and melatonin blood sugar load in rabbits undergoing hypoxia in prenatal ontogenesis. BioScience, 12(2): vol.67, 1436-1445.

Aliyev, A.H., Aliyeva, F.A. & Mamedova, K.Q. (2017). Dynamics of some hormones and glucose of blood in rabbits after prenatal hypoxia and subjected to postnatal physical exertion. The Journal of Medicine and Phylosofy. 6 (2): vol.42, 1299-1305.

Aliyev, A.H., Farhadi, N., Rostami, H., Arasteh A., Madatova, V.M. & Aliyeva F.A. (2009). The effect of maternal hypoxia, pineal gland, physical activity and circadian rhythm on serum levels of cholesterol, insulin and glucose and on thrombin time. J. NEWS of Baku State University.,Vol.3, 130-137.

Aliyeva, F.A. & Agayev T.M. (2015). The effect of sugar load on the dynamics of melatonin and glucose in the blood of rabbits undergoing prenatal hypoxia of ontogenesis. J. Problems of physiology & biochemistry. Vol. 33, 33-41.

Aliyev, A.H., Khalilov, R.I., Neymanzadeh, N.K., Mikailova, U.T., Aliyeva, F.A. & Aliyev, A.V. (2003). The role of the changes in the state of brain for regulation of some lipid and carbohydrate of metabolic processes. NATO sciences series I: Life and behavioral sciences. Vol. 342, 183-190.

Brizzi, G., Carella, C., Foglia, M.C. & Frigino, M. (1997). Thyroid hormone plasmatic levels in rats treated with serotonin in acute and chronic way. J. Physiol. Paris. 91 (6): 307–310.

Broedel, O., Eravci, M., Fuxius, S., Smolarz, T., Jeitner, A., Grau, H., Stoltenburg-Didinger, G., Plueckhan, H., Meinhold, H. & Baumgartner, A. (2003). Effects of hyper- and hypothyroidism on thyroid hormone concentrations in regions of the rat brain. Am. J. Physiol. Endocrinol. Metab. 285 (3): 470–480.

Kvetnaya, T.V. & Knyazkin I.V. (2003). Melatonin: the role and importance in age-related pathology. SPb. VmedA, 93 p.

Komarov, F.I., Rapoport, S.I., Malinovskaya, N.K. & Anisimov, V.N. (2004). Melatonin is normal and pathological. Publishing House Medpraktika-M, 308 p.

Lazarev, N.I., Ird, E.A. & Smirnova I.O. (1976). Experimental models of endocrine gynecological diseases. Medicine. 175 p.

Levin, Y.I., Malinovskaya, N.K., Komarov, F.I., Rapoport, S.I. & Raikhlin, N.T. (2005). Melatonin (Melaxen®) in the treatment of insomnia. Breast Cancer. 13(7): vol. 24, 498-500.

Malinovskaya, N.K., Komarov, F.I., Rapoport. S.I., Raikhlin, N.T. & et al. (2006). Melatonin in the treatment of duodenal ulcer. Clinical medicine. No.1, 5-11.

Musina, N.Z., Alyautdin, R.N., Romanov, B.K. & Rodionov, O.N. (2005). Correction of biorhythms with melatonin in flight personnel.Ross. Honey. Journal, No.6, 37-39.

Rom-Bugoslavskaya, E.S., Bondarenko, L.A., Somova, E.V. & Komarova, I.V. (1993). The role of the pineal gland in the development of atherosclerosis. The effect of round-the-clock lighting on some aspects of the pathogenesis of atherosclerosis. Probl. aging and longevity. No.2, 91-97.

Chazov, E.I. & Isachenkov, V.A. (1974). Epiphysis: the place and role in the system of neuroendocrine regulation. Medicine. 238 p.

Magner, J.A. (1990). Thyroid-stimulating hormone: biosynthesis, cell biology and bioactivity. Endocr. Rev. 11 (2): 354–385.

Sapronov, N.S. & Fedotova, Y.O. (2002). Hormones of the hypothalamic – pituitary – thyroid system and the brain. S.Pb . Lan. 184 p.

Fedotova, Y.O. & Sapronov, N.S. (2005). Effects of thyroid hormones in the central nervous system. Fundamentals of neuroendocrinology. Ed. V.G. Shalyapin, P.D. Shabanova. S.Pb .: Elbi-SPb. 204–249.

Klieverik, L., Kalsbeek, A. & Fliers, E. (2005). Autonomic innervation and its functional implications. Hot Thyroidology. (1): 3–4.

Pishchak, V.P. (2004). The photoperiod is the main time integrator of physiological systems. J. Integrative Anthropology. 2 (4): 74-79.

Raykhlin, N.T. & Kvetnoy, I.M. (1992). Diffuse endocrine system (APUD-system). Medicine.

Reiter, R.J. (1995). The pineal gland and melatonin in relation to aging: A summary of the theories and the data. Exp. Gerontol. 30, 199–212.

Reppert, S.M., Weaver, D.R. & Rivkees, S.A. (1989). Prenatal function and entrainment of a circadian clock// Res. Perinat. Med. Vol. 9, 25-44.

Rodriges, C., Mayo, V.C., Sainz, R.M., Antolin, I., Herrera, F., Martin, V. & Reiter, J.R. (2004). Regualtion of antioxidant enzymes: a significant role for melatonin. J. Pineal Res. Vol. 36, 1-9.

Rokitsky, P.F. (1961). Basics of variation statistics for biologists. Minsk.

Rowe, A.S. & Kennaway, D.J. (2002). Melatonin in rat milk and the likelihood of its role in postnatal maternal entrainment of rhythms. Am. J. Physiol. Regul. Integrat. Comp. Physiol. Vol. 282, 797-804.

Simonneaux, V. & Ribelayga, C. (2003). Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides and other pineal transmitters//Pharmacological Reviews,

Vol. 55 (2): 325-395.

Shorokhova, I.S., Kislyak, N.V. & Mariev, O.S. (2015). Statistical methods of analysis. Ekaterinburg.

Tunez, I., Munoz, M.C., Feijoo, M. & Valdelvira, M.E., Munoz – Castaneda, J.R. & Montilla, P. (2003). Melatonin effect on renal oxidative stress under constant light exposure. Cell Biochem. Funct. Vol.21, 35- 40.

Vinogradov, S.Y. & Pogorelov, Y.V. (1987). Thyroid neurotransmitter bioamines and structural and functional aspects of its homeostasis. Archive Anat. gistol. embryol. Vol. 1, 12–22.

Zamorsky, I.I. & Pischak, V.P. (2000). The role of nonadrenergic regulation in the reaction of the pineal gland of rats to acute hypoxia and administration of epithalamin. G. Vopr. Medical Chemistry. 46(1): 28-35.

Zamorsky, I.I. (2003). Functional organization of the photoperiodic system of the brain. Successes of the physiological sciences. 34(4):37-53.

Khazar Journal of Science and Technology Volume 3 №2 2019, 56-62
 DOI: 10.5782/2520-6133.2019.3.2.56

Bioecological Features of Some Feed, Poisonous and Noxious Plants on the Winter Pastures of the Mil Steppe in Azerbaijan

Kamala Asadova
Baku State University, Baku, Azerbaijan
asadovakamala@yahoo.com

Abstract

The winter pastures of the Mil steppe are used as a source of forage in animal husbandry by legal and physical persons. The bioecological features of main feed and poisonous and noxious plants were investigated during field research. As well as considering the bioecological features of dominant and subdominant species, recommendations for rational usage and measures of improvement were prepared.

Introduction

In the region studied there are some valuable feed plants, including *Salsola dendroides, Petrosimonia brachiata, Suaeda dendroides, Salsola nodulosa, S. ericoides, Alhagi pseudoalhagi, Hordeum leporinum, Eremopyrum orientale, Lolium rigidum, Anisantha rubens* and ephemers like *Hordeum leporinum, Eremopyrum orientale, Lolium rigidum, Anisantha rubens,* from the ephemeroids *Poa bulbosa,* as well as *Artemisia lerchiana,* in the species content of formations (Imeskenova,2014; Hasanov, 2016; Vatan, 2004).

Results of the research conducted determined that there are 41 species of vegetation in the geobotanical content of formations found in desert and semidesert feed, including poisonous and noxious plants. Of them 30 species (73,2%) are main feed plants and of medicinal importance, 11 species (26,8%) are poisonous and noxious.

Based on this information, the bioecological features of some fodder, poisonous and noxious plant species distributed on the region's winter pastures were studied.

Materials

A list of the plants found in the species content of the formations studied was prepared in preparation for studies of their bioecological features (Table 1).

Table 1. List of main feed, poisonous and noxious plants distributed in the desert and semidesert vegetation of winter pastures

№	Name of species
Feed and medicinal plants	
1	Salsola dendroides Pall.
2	Alhagi pseudoalhagi (Bieb.) Desv.
3	Halostachys belangeriana (Mog.) Botsch.
4	Lolium rigidum Gaudin.
5	Eremopyrum triticeum (Gaertk.) Nevski.
6	Petrosimoniabrachiata (Pall) Bunge.
7	HordeumleporinumLink.
8	ArtemisialerchianaWeb.
9	Climacopteracrassa (Bieb.) Botsch.
10	Kalidiumcaspicum (L.) Ung.-Sternb.
11	AegilopscylindricaHost.
12	BromusjaponicusThunb.
13	Medicagominima (L.) Bartalini
14	Suaedadendroides (C.A.M.) Moq.
15	SalsolaericoidesBieb.
16	Salsolanodulosa (Moq.) İljin.
17	Anisantharubens (L.) Nevski
18	PoabulbosaL. (Link.) Schul.
19	TragopogongraminifoliusDC.
20	Gamantuspilosus (Pall.) Bunge.
21	Eremopyrumorientale (L.) Yaub. EtSpach.
22	PlantagocoronopusL.
23	Cynodon dactylon (L.) Pers.
24	Aeluropus reflexiaristata (Nevski) Nevski
25	Aeluropus littoralis (Gouan.) Parl.
26	Phleum puloides (L.) Karst.
27	Caragana arberoscens Lam

28	Limonium meyeri (Boiss.) O.Kuntze
29	Glycyrrhiza glabra L.
30	Plumbago lanceolata L.
Poisonous and noxious plants	
31	Cirsiumvulgare (Savi.) Ten.
32	Taraxacummarianum (L.) Gaertn.
33	Adonis binertii Butk.
34	Tamarix ramosissima Lebed.
35	Euphorbia ispanica Boiss.
36	Anabasis aphylla L.
37	Allium rubellum Bieb.
38	Capparis herbacea Willd.
39	Erodium cicutarium (L.) L'Her.
40	Psylliostachys spicata (Willd.) Nevski
41	Xanthium spinosum L.

Results

Feed plants. Shrubs.

These plants belong to the *Chenopodiaceae Vent*. family.

Suaeda dendroides(C.A.Mey.) Moq.

60-80 cm in height, salinity tolerant. Branches are covered with brittle, grey and brittle hairs. Sometimes young branches are hairless. Leaves are small, juicy, oval, blue and 5-10 mm in length. Flowers are 2-3 in number, in locules. Seeds are smooth, round and bright black (Parachin *et al.*, 2015; Toleubayeva & Kartayeva, 2015). Vegetation begins in May; flowering in August-September and semination in November-December. *Suaeda dendroides* is eaten by livestock after frosty days in the autumn-winter period.

Subshrubs

Salsola dendroides Pall.

50-70 cm in height. The stem is ligneous, multi-branched. Length of leaves, 2-5 mm. Vegetation is in April-May, flowering and semination in October-November. *Salsola dendroides* is the main fodder plant on the pastures and is eaten as a feed plant by livestock in the winter season (Toleubayeva & Kartayeva, 2015).

Prilipko (1970) noted that the leaves and young branches of *Salsola* are eaten by sheep in January after frost. Salsola is the dominant species of the Artemisieto-Salsoletum formation on the region's winter pastures. As a subshrub plant, *Salsola dendroides* is formed on grey-brown soils and is considered an indicator of ground water.

Undershrubs

Kalidium caspicum (L.) Ung. - Sternb.

10-30 cm in height. The leaves and biennial branches are succulent. This species is found on saltish, grey-brown and saline soils.

Flowering and seeding is in April-October and November. It is found in the region's saline deserts. *Kalidium caspicum* is considered a feed source on the winter pastures. *Kalidium caspicum* is eaten by livestock on freezing winter days, because ephemers and ephemeroids pass dormant period.

Salsola nodulosa(Moq.) İljin

30-40 cm in height. Irregularly branched, the stem is covered by light grey bark. Young branches are small and hairy. Leaves are arranged in alternate directions and are 5 mm in length. Reproduction is by seed. Vegetation begins in June-July, flowering in July-August and semination in October-November. *Salsola nodulosa* is distributed on winter pastures and slopes, as well as on saline, grey-brown and saltish soils. It is a halophyte undershrub. Caucasian endemic (Imeskenova & Komendanova 2018; Gurbanov, 2004; Grossheim, 1967; Toleubayeva & Kartayeva, 2015).

Salsola nodulosa is dominant in desert and semidesert phytocenosises, as well as in Artemisetum-Salsoletum formations. As with *Artemisia fragrans, Salsola nodulosa* is considered a main fodder plant of winter pastures in these formations. R.A. Aliyev noted that the edible part of a Salsola shrub amounts to 50-120 q.

Herbs. Annuals

Petrosimonia brachiata (Pall.) Bunge.

Petrosimonia brachiata belongs to the *Chenopodiaceae Vent.* family and is a characteristic annual halophyte. 5-20 cm in height. The leaves are 4 cm in length.

The stem is branched, flowers are covered with a double-layered perianth, 3-5 divided, greenish or grey coloured.

Vegetation of *Petrosimonia brachiata* begins from April, flowering in October-November and semination in December.

This halophyte plant is considered an indicator of saline and brackish, grey-brown soils. Thus, *Petrosimonia brachiata* is dominant in a Suaedata- Salsoleta-Petrosimonum formation and is subdominant in Artemisieta-Salsoletum and Salsoleta-Artemisetum formations.

Petrosimonia brachiata is low in fodder quality. In the frosty winter period its dried branches are eaten with pleasure by livestock.

Ephemers

Hordeum leporinum Link.

Hordeum leporinum belongs to the *Poaceae Barnhart* family. Its height is 15-20 cm. The width of its leaves reaches 3-4 mm. Its spikes consist of close spikelets 4-5 cm in length and the awns are very coarse. Flowering and semination is from April to June.

This species is found in the species content of formations distributed in desert and semi-desert vegetation types. *Hordeum leporinum* is distributed on grey-brown and saline soils.

Before flowering it is considered a high-quality fodder plant in the early period of vegetation. Then plant's stem hardens and cannot be eaten.

Eremopyrum orientale (L.)Yaub. et Spach.

10-30 cm in height. Spikes are flattened, two sided and cylindrical in form. Leaves are in two ranges and hairy. Reproduction is by seed. In autumn and winter it gives seedlings. In early spring vegetation accelerates. Flowering is in April and semination is in May. It is a typical ephemer. This plant is found on saline, clay and grey-brown, as well as on salty and sandy soils. *Eremopyrum orientale* is dominant in Artemisia-Ephemeretum, Artemisia-Salsoletum and Suaedata-Salsoleta-Petrosimonum formations. It is a feed plant. In the pasture areas *Eremopyrum orientale* is eaten by liverstock until spikes are formed (Prilipko, 1970; Clemants & Cherepanov, 1995).

Lolium rigidum L.

15-30 cm in height. An ephemer, reproduction is by seed. The leaves are flattened, the spikes are narrow, straight or slightly bent. Flowering is in April-May, semination in May-June. *Lolium rigidum L.* is dominant in Artemisia-Ephemeretum formations. It is also found as ephemer content in other formations. It is considered one of the better feed plants on the winter pastures. It is eaten by livestock as a high quality feed plant in the early period of vegetation, as well as during flowering and semination. *Lolium rigidum* is considered a promising plant for improvement of the region's winter pastures.

Anisantha rubensL.Nevski

10-25 cm in height. The width of leaves reaches 2-4 mm. The panicle is straight and yellow. Flowering is observed until April. *Anisantha rubens* is found in Suaedata-Salsoletum-Petrosimonium formations of desert vegetation. As a typical ephemer it is found on grey-brown and saline soils. At the beginning of vegetation it is eaten by livestock as a feed plant. Then the plant's vegetative organs become rough and cannot be eaten. Seed germination capacity is very high; 80-90% of 100 seeds sown may germinate.

Besides the above-mentioned plants *Poa bulbosa L., Medicago minima (L.) Bartalini, Alhagi pseudoalhagi (M. B.) Desv., Artemisia lerchiana Web*. also have a great importance for feed and these plants are suitable for improving soil fertility.

Poisonous and noxious plants

It was determined that there are 11 species of poisonous and noxious plants within the research area. We studied their bioecological features and life forms (ANAS, 1969; Hatamov, 2000).

Allium rubellum Bieb.

This species belongs to the Alliaceae Agardh. family. A perennial herb and geophyte; the bulb is egg-shaped, yellowish, 102 mm in width. Height is 10-20 cm. The leaves are narrow and linear. The stem is covered by a sheath from below. The umbel is semicircular. Densely flowered, the stalk is longer than the flower. The perianth is 4-5 mm, pink. Leaves are long and sharp.

This plant has a strong odour and a bitter taste, thus it is not eaten by livestock. It is poisonous for livestock.

Euphorbia ispanica Boiss., Cirsium vulgare (Savi.) Ten., *Xanthium spinosum L.*are also poisonous plants. To prevent their initial spread, rational usage of winter pastures and the implementation of measures of improvement are important. As well as the protection of feed plants, study of their bioecological features is advisable.

References

ANAS. (1969). Fodder plants for haymaking and the pastures of Azerbaijan. II volume. Publishing house of Academy of Sciences of Azerbaijan SSR. Baku.

Hatamov, V.V. (2000). Pasture ecosystems of Azerbaijan and their protection. Baku Publishing House of Elm, 184 p.

Prilipko, L.I. (1970). Plant cover of Azerbaijan. Science publishing house. Baku, Azerbaijan, 170 p.

Imeskenova, E.G. & Komendanova, T.M. (2018). Contemporary condition of winter pastures. Agricultural sciences. Bulletin of Krasnoyarsk State Agrarian University. No1, p. 25-30.

Imeskenova, E.G. (2014). Ecological evaluation of pasture vegetation of the Tunkin region of the Buryatiya Republic. Bulletin of Novosibirsk State Agrarian University.Vol (1) 30. 25–29.

Gurbanov, E.M. (2004). Flora and vegetation of Atropatena province (within Azerbaijan Republic). Autoreferat of dissertation for the degree of doctor of biological sciences. Baku, Azerbaijan, 55 p.

Hasanov, V.N. (2016). Superficial improvement of winter pastures of Azerbaijan. Bulletin of Kurgan State Agricultural Academy by T.S. Maltsev. No 3, Kurgan, Russia, 12-13.

Clemants, S. & Cherepanov, S.K. (1995). Vascular Plants of Russia and Agrosent states (The former USSR). Brittonia, 48: 284.

Grossheim, A.A. (1967). Flora of the Caucasus. M.-L Vol. I-VII. Publishing House of Branch of the Academy of the USSR in Azerbaijan.

Vatan. (2004). Instruction on index symbols of typological classifications of natural fodder areas of Azerbaijan Republic. Baku.

Parachin, N.B., Kobozev, I.V., Gorbachev, I.V. (2015). Fodder making: Textbook for higher educational institutions. 432 p.

Toleubayeva, Z. & Kartayeva, T. (2015). Regional features of the winter pastures on the Lower reaches of Syrdarya. Bulletin, 75, Kazakhstan. 157-175

Khazar Journal of Science and Technology Volume 3 №2 2019, 63-72
 DOI: 10.5782/2520-6133.2019.3.2.63

An Endowment (Al-waqf) Concept for the Supply of Medical Care: an Exploratory Study of the Sustainability of Health Insurance at Higher Institutions

Abdul-Rahman Balogun Muhammed-Shittu
School of Humanities, Education and Social Sciences (HESS), Khazar University
Email:abdul.rahman@khazar.org

Abstract

Important here is the term endowment, meaning the devotion of assets or properties, either directly or indirectly, to any charitable or religious institution or cause, or to secure advantage or benefit for a person or persons. The purpose of this study is to explore and represent the importance and utility of an endowment form of sustenance for the infrastructures and/or amenities necessary for beneficiaries at higher institutions. However, the specific aims and objectives of this study are to ensure the availability and sustainability of standardized health services at Khazar University by the application and implementation of a policy of endowment. Khazar University is undoubtedly among the world's fastest growing young universities. Obviously, the university strives endlessly to provide services of global standard to meet the challenges involved in competing with older, well-established universities around the world. The study is exploratory in nature and applies a phenomenological approach in presenting the concept of endowment and how it is practised at many world class universities to provide and sustain the necessary facilities at their various institutions of higher education. Following exploration, this study proposes that stakeholders of Khazar University use endowment to upgrade the university's health and medical services to an advanced level. The study highlights the practical experience of endowment-based universities in different part of the world, including: Al-Azhar University in Egypt; the University of Cordova in Spain; IIU- the Islamic University of Indonesia; universities founded in Turkey; the King AbdulAziz University, and many universities in Malaysia, of which the present study focuses on the International Islamic University, Malaysia.

Keywords: endowment; Al-waqf; sustainability, medical care; health centre; health insurance; IIUM; institutions of higher education.

Introduction

Important here is the term of endowment, meaning the devotion of assets or properties, either directly or indirectly, to any charitable or religious institution or cause, or to secure advantage or benefit for a person or people (Mahamood, 2006). The purpose of this study is to explore and exhibit the importance and utility of an endowment practice of sustenance for the infrastructures and/or amenities necessary for beneficiaries at higher institutions. However, specific aims and objectives of this study are to ensure the availability and sustainability of standardized health services at Khazar University by the application and implementation of a policy of endowment. Khazar University is undoubtedly among the world's fastest growing young universities. Obviously, the university strives endlessly to provide services global standard to meet the challenges involved in competing with older, well-established universities around the world. The study is an exploratory in nature and applies phenomenological approach in presenting the concept of endowment and how it is practiced at many world class universities to provide and sustain necessary facilities at their various institutions of higher education. Following exploratory, this study proposes that stakeholders of Khazar University use endowment to upgrade the university's health and medical services to an advanced level. The study highlights the practical experience of endowment-based universities in different part of the world, including: Al-Azhar university in Egypt; the University of Cordova in Spain; IIU- Islamic university of Indonesia, universities founded in Turkey; the King AbdulAziz University; and many universities in Malaysia, of which the present study focuses on the International Islamic University Malaysia.

The endowment "*Al-Waqf*" system is one of the forms applied through the history of world, and especially Islamic civilization. Finance by endowment was the principal support of many systems of education, health and social care, defense and security institutions, and institutions of philosophy and culture. Endowment is an age-old operation within social history of humanity and was neither external nor strange to the dialect and language of a Muslim in the early stages of Islamic civilization. In Islam, the concept of endowment referred to as "a*l-waqf*". It has been defined by a prominent, contemporary scholar as, "a permanent or temporary dedication of a certain form of wealth with the intention of achieving direct benefit from it or benefiting from what arises from it on a regular basis" (Qahf, 1996).

History cannot dispute the fact that endowment, popularly known as waqf in an Islamic context has played a significant role not only, but especially in Muslim societies (Fayzee, 1974). Endowment has benefited many individuals in various societies through ignificant and beneficial establishments such as shelters, hospitals and medical centers for medical care, schools, bakeries, orphanages and other religious and/or educational investments (Mahamood, 2006). Importance here are endowment's roles and efforts in providing relief and eradicating poverty, which is skyrocketing among both humans and animals (Al-Dardir, 1986; Kahf, 1999; Spuler, 2015; Mahamood, 2006). Thus, such activities of endowment-waqf have been enacted by people regardless of their status, whether highly placed bureaucrats or laymen (Makdisi, 1981; Baer, 1997; Gibb and Bowen, 1957; Tibawi, 1978; Kozlowski, 1985; Mahamood, 2006).

For the provision of humanitarian welfare services, a huge number of social cum educational institutions; mosques, hospitals, schools, universities, libraries, dormitories and many other leisure facilities, have been established by *waqf* or endowment funds (Mahamood, 2006). However, endowment _irrespective of its Arabic or Islamic linguistic meaning (waqf)_ is no strange concept in the Western world, which correspondingly has several universities built by the concept and practice of endowment known as *waqf* in the Muslim world.

It is clear that the universities of Yale and Harvard in the United States of America, and those of Cambridge and Oxford in the United Kingdom are among the eminent examples of endowment-based universities in the West. Surprisingly, the notion of compassionate and philanthropical contributions by endowment to higher institutions has been embraced positively the government of the United Kingdom to the extent of recognizing and considering the practice of endowment as the way forward and as one the best approaches to maintaining and financing higher education, acknowledging that it will make the educational sector less contingent upon a sole source of financial income (Higher Education White Paper, 2003). Apparently, the concept of Oxbridge is an indicator of Cambridge and Oxford as endowment-based colleges. Given this, the heads of colleges and their colleagues are regarded as the agent responsible for the activation of pious activities and efforts to sustain the objective of the assistance by maintaining the intertwined roles of trustee and beneficiary (Acharya and Dimson, 2007).

Having established the significant roles of endowment at various institutions in different sectors, as stipulated in previous chapters, this present study explores the importance of health insurance and the need for a medical centre equipped with modern facilities for the benefit of Khazar University students and provided by endowment.

The Evolutional Stages of Endowment and its Developmental Role

The endowment term *"waqf"* in the language literally means confinement and stop, while the term in jurisprudence is "the imprisonment of the source and the sincerity of its benefit." The imprisonment of the source means that he does not act in it by selling or foreclosure or giving, nor does he transfer the inheritance (Swift and Zadeq, 2002).

Since ancient times, there have been different forms of voluntary financial transactions that are not come in the nature of an endowment, in the form of properties that are restricted to places of worship or to benefit them. This was known to the ancient Egyptians, the Romans, Greeks and others (Friedman, 1970). Endowment began with the emergence of Islam. and expanded its scope. It was not limited to temples and rituals but extended to include many kinds of alms and donations for religious, social, scientific and economic purposes. Endowment were to mosques, their maintenance and function, to schools and their role in education, to libraries, scientists and the students of science, to the poor, the needy, and it extended further to include hospitals and pharmacies schools and communities (Swift and Zadeq, 2002).

With this expansion, endowment greatly advantageous and influential in building Islamic civilization and laying the foundations of integration, solidarity, cooperation and brotherhood. The expansion in the care of endowment led to its major role in social and economic development throughout Islamic history (Gaberman, 2008). It is no secret that the endowment system in Islam brings scientific and charitable benefits, as well as appreciation (Swift and Zadeq, 2002). There are also non-material public interests of significant weight in the legislative. Endowment, particularly in Islam is not limited to places of worship and their means; but it has since the era of the Prophet (peace and blessings of Allah be upon him) to served wider purposes for good in society (Friedman, 1970), thus; expanding the scope of the contained money; and extending the purpose of the moratorium. Examples of this are:

Endowment for Education

This paragraph showcases the impact of endowment on the educational system in consideration of the present study’s objective to focus on maintaining a standard health care service at the university. Endowment is one of the most important instruments that are active in the development of education, whether in mosques, schools, libraries or other charitable institutions (Chapra, 2008). Endowment funds

sponsored the process of education from childhood to specialized higher levels, enabling Muslims to progress from a simple life to a prosperous life, while many other societies remained in recession for long periods (Gaberman, 2008).

These endowments have helped education to provide facilities necessary individuals to qualify contribute to the economic, social and cultural development of their societies. These funds spent on education have supported many different aspects of teaching and learning processes (Swift and Zadeq, 2002), most importantly establishment and equipping of schools, the provision of teachers and others, and the encouragement of science students to engage in the process of education by the provision of facilities to do so (Carroll, 1991).

Endowment in Support of Health Care Services

Endowment systems have also made significant impacts in supporting general health care services, for citizens and residents of different sects. Some researchers have discussed about the types of health centers sponsored by endowment providers and drawn the attention of universities to health care, the development of services, and allocation of funds for the construction of integrated medical neighbourhoods (Swift and Zadeq, 2002). Importantly, Mahamood (2006) said that some higher institutions are surrounded by beautiful individuals who help to execute several projects like building the booths and the multiple houses, all of which ran on endowment funds and catered for sick students and staff under the supervision of doctors, pharmacists and medical students.

Islamic endowments also gave significant support to medical and health care in societies. However, an Islamic medical endowment did not focus merely on providing health care services to society, in addition it provided opportunities to sponsor science students studying medicine at the university (Mahamood, 2006).

Çizakça (1998) notes that medical centres were supported by endowment funds and the health services they provided medical treatment, operations, medicine and food, were free in return to the endowments that Muslims monitored for these humanitarian purposes.

Hassan (2010) and Gaberman (2008). assert that health care in most Islamic countries was close to charity, when there were no Ministries of Public Health as there are in the present era. Endowment has had a beneficial effect on the advance of medical sciences, because the role of hospitals operating by endowments was not limited to providing treatment (Gaberman, 2008). They went further: teaching

medical science and in the halls of large hospitals dedicated to lessons and lectures. Examples of such endowments include contributions by the following individuals.

-Nur al-Din Mahmoud bin Zanki built a health centre, a school and a modern home in Damascus as endowments and donated many medical books.

-Moez Bouya built a hospital in Baghdad as an endowment to the value of five thousand dinars. Fakhruddin Mardini, a medical doctor donated the book he wrote on medicine to the people of Mardin.

King Al-Mansour built a palace in Cairo and decided to dedicate it as an endowment with the worth of forty thousand gold annually. The weight = 4.25 grams and the harvest of this endowment is one hundred and seventy thousand grams of gold.

Endowment is gradually becoming a global trend:- the concept gained recognition as the social and economic changes accompanying the rise of capitalism in Europe contributed much to the development of the voluntary sector, where issues of relief and social welfare became part of the landscape in these countries (Azim, 2009). The process culminated, in early 2005, with the inclusion of volunteerism in the draft European Constitution and then in the European Charter as one of the European Community's strategic social activities. In the last few years, many giant corporations have also begun to donate money or return to philanthropy to care for the poor (Al-Harithi, 2009).

This was done by some non-Islamic giants such as the Bill and Melinda Gates Foundation, established in 2000, and incorporating the Gates Education Foundation and the William H. Gates Health Foundation. The first institution specializes in office technology, while the second is concerned with public health (Al-Harithi, 2009). The Foundation's programmes go beyond a local scope to global scale, offering scholarships to the University of Cambridge for students from all over the world. It also aids non-profit institutions and renders assistance to poor communities in a number of countries and international endowment institutions, including the Foundation for International Community Assistance (FINCA), which seeks to rehabilitate individuals. Additionally, the endowment targets qualified individuals, especially women, and gives them small loans ranging from (350 USD) three hundred and fifty dollars (Islahi, 1996). Perhaps this requires Islamic endowment to look at how endowment is being implemented in the non-Islamic world, despite its late appearance.

Endowment Concept Applied on Health Care in Higher Education

Previous studies (Makdisi, 1981; Baer, 1997; Gibb and Bowen, 1957; Tibawi, 1978; Kozlowski, 1985; Mahamood, 2006) have shown the importance and significance of endowment at higher institutions. According to Fayzee, 1974 and Mahamood, 2006, many institutions ultimately resort to the practice of endowment to sustain the necessities that enable their higher educational activities to move forward (Nor, 2012). Al-Azhar University is a great example of an old, renowned higher institution that depends on endowment funds to survive and is a globally recognised as an endowment-based university (Baskan, 2002). The study concentrates on the International Islamic University Malaysia (IIUM) as popular contemporary higher institution, focusing mainly on both the practice and impact of endowment on university's medical services.

Endowment funds can be attracted from different sources: organizations, companies or philanthropists, for general or specific plans (Acharya and Dimson, 2007), or for specific general purposes (Mahamood, 2011). Acharya and Dimson, 2007 show that in many cases donors are the determinants of specific purposes of their endowments, while some donors give the university authority total autonomy in making appropriate decisions about how and where to execute their endowments (Acharya and Dimson, 2007).

Endowment practice at the International Islamic University Malaysia as mentioned above, is the focus of this study. Thus, it is relevant to reckon between the year of IIUM's establishment and the year when the practice of endowment was utilised. IIUM was established in 1982, while the IIUM Endowment Fund (IEF) was established in 1999 by the university's second rector, Prof. Dr. Abdul Hamid Abu Sulaiman (Datuk). The main purpose of the IIUM Endowment Fund is to render assistance to less privileged students. This is not limited to academic assistance but is to ensure the student's holistic wellbeing, particularly in medical and health related issues (Ahmed, 2007).

Methodology

The study applied a simple methodology in gathering information on how endowment is implemented at the International Islamic University Malaysia. The office of the IIUM Endowment Fund was contacted to assemble the necessary information and responses generated during the interviews were accurately presented.

Conclusion

After establishing the crucial role of endowment in various higher institutions; and critical evaluations and reviews of the strategies applied by those universities; IIUM was selected as the central object of study. The following strategic recommendations were drawn up as the conclusion of this exploratory study, pointing to sustainable health insurance and medical care services at Khazar University.

Khazar university was established in 1991 and, of course aimed to contribute to the development of educational values in Azerbaijan. In ensuring that Khazar University achieves a social dimension including a variety of aspects concerning the impact of the institution's operations on people both inside and outside the organization:- sound business relationships, safety and health Ven van de, and Graafland, 2006) the latter being the area central to his study. There is always a need for individuals and collective efforts to promote sound initiations, overcoming obstacles that may lead to marginalization. It is always necessary to move forward and instigate success and development to ensure actualization and progress. Based on the researcher's involvement and investigation of IIUM practical experience, with over 38,832 students (according to IIUM's estimated total enrollment, with regards to endowment, 2007) of endowment related practice. These two workable strategies to generate endowment funds are outlined:

1- Internally Initiated Programmes

2- External Contributions

The first strategy refers to any forms of internal motivation to generate income for the university. This may be conducted in many ways after viewing several programmes of health and medical care endowments. For example: the university may introduce voluntary fund raising internally for health care endowment, there should be no compulsion to achieve this, strictly voluntary action. Accordingly, the coordinator of the IIUM Endowment Fund (IEF) confirmed that more than 145 IIUM staff expressed interest and voluntarily joined the project through which a significant amount of money was generated periodically.

The second strategy for health care endowment was found to be more productive in most endowment-based universities.

Here, the contribution process is not limited to the staff or members of the university. It can be extended to humanitarians and philanthropists within the university's state or at international level, depending on the strength of the

university's network. Apart from individuals, there are many endowment-based organizations that render humanitarian services and they often target institutions to ensure a spread of advantages and benefits. They provide support either in money or by construction. According to the IEF coordinator, in most cases there are conflicting missions behind the idea of construction. For example, building an auditorium, hall and conventional centres for a higher institution will serve two different purposes: firstly, the institution uses it for its own educational events; while, secondly also generating income by renting it out for externally-organised events.

It is imperative to emphasize the fact that some donors may be interested in reciprocity by requesting specific actions in return. A common scenario is a proposal to have their brands advertised by the university. Some medical practitioners voluntarily give free humanitarian services either at the university hospital, or at their own health centrers/hospitals. To sum up, health insurance at higher institutions is essential, especially for international students. The purpose of the study is not to highlight difficulties experienced by students, but this is recommended for future studies on health-related issues, as it may create awareness and trigger the attention of university managements to the value of the practice of endowment.

Reference

Acharya, S. & Dimson, E. (2007). *Endowment Asset Management (Investment Strategies in Oxford and Cambridge)*, Oxford University Press, New York, NY.

Ahmed, H. (2007). *Waqf-based microfinance: Realizing the social role of Islamic finance*. Paper presented at the International Seminar on Integrating *Awqaf* in the Islamic Financial Sector, Singapore, March 6-7.

Al-Dardir, A. (1986). *Al-Sharhal-Saghir 'Ala 'Aqrab Al-Masalikila Madhhab Al-Imam Malik*, Daral-Ma'arif, Cairo.

Al-Harithi, A. (2009). Culture of Social Responsibility Where to Start? a paper presented to the Second Arab Giving Forum, Abu Dhabi, pp 6-7.

Azim, H. A. (2009). *The Consequences of the Marginalization of the Islamic Waqf*, Research Presented to the Third Conference of Awqaf in Saudi Arabia, Islamic Endowment "Economy, Management and Building of Civilization", 3rd Conference of Endowments in the Arab Kingdom, pp 267-268.

Baer, G. (1997). The *waqf* as a property for the social system (sixteenth-twentieth centuries) *Islamic Law and Society*, Vol. 4 No. 3, pp. 264-297.

Baskan, B. (2002). The *Waqf* system as a redistribution mechanism the in Ottoman Empire. Northwestern University, Department of Political Science.

Carroll, A. B. (1991). The Pyramid of Corporate Social Responsibility: Toward the Moral Management of Organizational Stakeholders, Business Horizons, p1 Available in: http://www.cbe.wwu.edu/dunn/rprnts.pyramidofcsr.pdf.

Çizakça, M. (1998). Awqaf in history and its implications for modern Islamic economies. *Islamic Economic Studies*, Vol. 6(1), 437-463.

Chapra, M. U. (2008). Ibn Khaldun's theory of development: Does it help explain the low performance of the present-day Muslim world? *The Journal of Socio-Economics*, Vol. 37(2), 836-863.

Fayzee, A. A. A., (1974). *Outlines of Muhammadan Law*, 4th ed., Oxford University Press, Delhi.

Friedman, M. (1970). The social responsibility of business is to increase its profits. New York, Times Magazine. September 13. available in: http://www.colorado.edu/studentgroups/libertarians/issues/friedman-soc-respbusiness.html

Gaberman, B. (2008). A Global Overview of Corporate Social Responsibility, The John D. Gerhart Center for Philanthropy and Civic Engagement, American University in Cairo, Spring, pp 6-7.

Gibb, H. A. R. & Bowen, H. (1957). *Islamic Society and the West: A Study of the Impact of Western Civilization on Moslem Culture in the Near East*, Oxford University Press, London.

Islahi, A. A. (1996). Provision of public goods: Role of the voluntary sector (*waqf*) in Islamic history. In M. A. Mannan (Ed.), *Financing development in Islam.* Jeddah: IRTI-IDB, 367-391.

Kahf, M. (1999). Financing development of *awqaf* property. *The American Journal of Islamic Social Sciences*, Vol. 16 No. 4, pp. 39-66.

Kozlowski, G. C. (1985). *Muslim Endowments and Society in British India*, Cambridge University Press, Cambridge.

Mahamood, S. M. (2006). *Waqf* in Malaysia: Legal and Administrative Perspectives, University of Malaya Press, Kuala Lumpur.

Makdisi, G. (1981). *The Rise of Colleges*, Edinburgh University Press, Edinburgh.

Nor, S. M. (2012). Integrating moral in a dynamic model of corporate social responsibility in Islamic economics and finance. *Asian and African Area Studies*, Vol. 11 No. 2, pp. 137-150.

Qahf, M.Y. (1996). Sharh Muntaha al-Iradah, Beirut: alam alkutub, Vol. 2, p. 397.

Spuler, B. (2015). *The Age of the Caliphs*, Markus Wiener Publication, St, Princeton, NJ.

Swift, T. & Zadek, S. (2002). Corporate Responsibility and the Competitive Advantage of Nations, The Copenhagen Centre and institute of social and ethical accountability, pp9-10

The Sutton Trust (2003). Higher education white paper. *Discussion Paper on University Endowments – A UK/US Comparison*, *London*, May.

Tibawi, A. L. (1978). *The Islamic Pious Foundations in Jerusalem*, Islamic Cultural Centre, London.

Khazar Journal of Science and Technology Volume 3 №2 2019, 73-79
© Khazar University Press 2019 DOI: 10.5782/2520-6133.2019.3.2.73

The Importance of Using Database Management Systems in Hospitals

Narmin Mahmudova
Faculty of Engineering and Applied Sciences, Khazar University, Azerbaijan
Email: nermin.mahmudova1996@gmail.com

Abstract

The main function of a Database Management System (DBMS) is to store and secure personal and corporate data. In the modern era every area of business needs a DBMS to manage its data, and so do hospitals. Whether the organisation is a hospital, private health centre, clinic or office, it needs effective health management. Medical records must have all of its patients' medical histories, and even all information about the hospital's departments, doctors and other staff. Hospital database systems not only record and keep track of detailed personal communications and medical histories, they also create current accounts and are used in cost and personnel management, organising appointments, arranging patient admissions and hospitalization. This article mainly tries to explain how necessary a database is for any kind of hospital, the benefits it brings and how it should be used. It also examines the current record of the "N Kay Medical" hospital in Khachmaz, Azerbaijan and the creation of a database system for the hospital to help hospital staff and those who handle the hospital's data to perform their work more effectively and efficiently.

Keywords: Data; Database; Database Management System; Health; Hospital; Hospital DMBS; Doctors; Data Record

Introduction

Hospital Database System: this is an intelligent system developed to improve the quality of health services offered to individuals and to ensure safe and speedy integration of patients, doctors and health institutions (Haggerty**, 2019). But:**

First of all, what are data?

The singular form of the word data (datum) comes from the Latin language. Data refers to any events or ideas that a person finds worthy of formulating or registering.

When data are stored on computer, this is mostly done by use of a database management system.

What is a Database?

The most common definition of database is a collection of data arranged in accordance with its intended use. It is a store of information in which logical and physical data are associated with each other (**Haggerty**, 2019).

The data collected on a specific topic are assembled within a database program. When required, all the information collected, or that which meets the specifications desired, can be displayed and printed; new information can even be generated from the original input and used for various purposes.

But how is a database used?

The answer is, by software known as database management systems (DBMS), by which various complex operations, such as creating a new database, editing, developing and maintaining the database, are performed.

Hospital Database Management Systems

Hospital database systems now provide multifaceted support for the diagnosis, treatment and follow-up of diseases and their management. On a hospital database every process and all data are safely recorded and stored. Examination, medication, surgery and hospitalization reports and all the health institution's records are kept together with the finest detail about patients, and the patient is asked to re-enter each time on his / her application. Personal information (Name / Surname, Birth Place / Date, Blood Group etc.) and contact information are stored safely. Each transaction is stored securely in the database and can be queried separately. With parametric query screens and search forms, search options are offered via multiple options (Name, Gender etc.). The user-friendly, flexible and convenient search interface provides fast and easy access to information (existek.com).

Material and Methods

I live in a town where none of the hospitals have a Database Management System, but we all know that a database is an external part of every area of business nowadays. So, I thought that for this study it would be better to explain why every hospital needs a database management system by actually developing one for a hospital to see how it actually works. I visited one of the hospitals that I have used since childhood and began gathering information and interviewing doctors for my research, which includes departments, patients, doctors and rooms, as well as how appointments, treatments, medical tests etc. are handled. I tried to merge all the information and arrive at a solution. I noted that data were stored in a traditional Microsoft Excel file system; the main problem with a traditional file system is that data definition is part of an application program that only works with that specific application. It is known that files require changes to design and coding whenever a new kind of data appears and a management system is desperately needed to manage such large amounts of data. I was there nearly every day for one week and pointed out that the hospital urgently needed a database system that could manage all the data.

Previously, the hospital used to give a type of ticket to patients, who had to bring the tickets with them every time they attended an appointment. But that was not working at all, because patients were always losing their tickets and had to pay five manats for a new one. All the data were stored on paper in an archive and if hospital staff wanted to check a patient's history they had to search the whole archive just to find that one patient's history. As may be imagined, that took a long time; and there was no data protection. If a patient who had been through some procedure returned for a check-up and that patient's history could not be found, that could cause a lot of problems. Those problems could be avoided with a database.

After gathering all the information necessary, I searched the internet for information on designing a good database and was assisted by Microsoft Support, following its database design guidelines to fulfil all the essentials of a good database design (support.office.com).

In this study, some articles and theses on the internet were read for research (Amaechi *et al*., 2018; Verbeke *et al.,* 2012; Ripan, 2017; prezi.com).

Result and discussion

Based on the existing record, the tables required were established by using Microsoft SQL Server 2014 (microsoft.com).

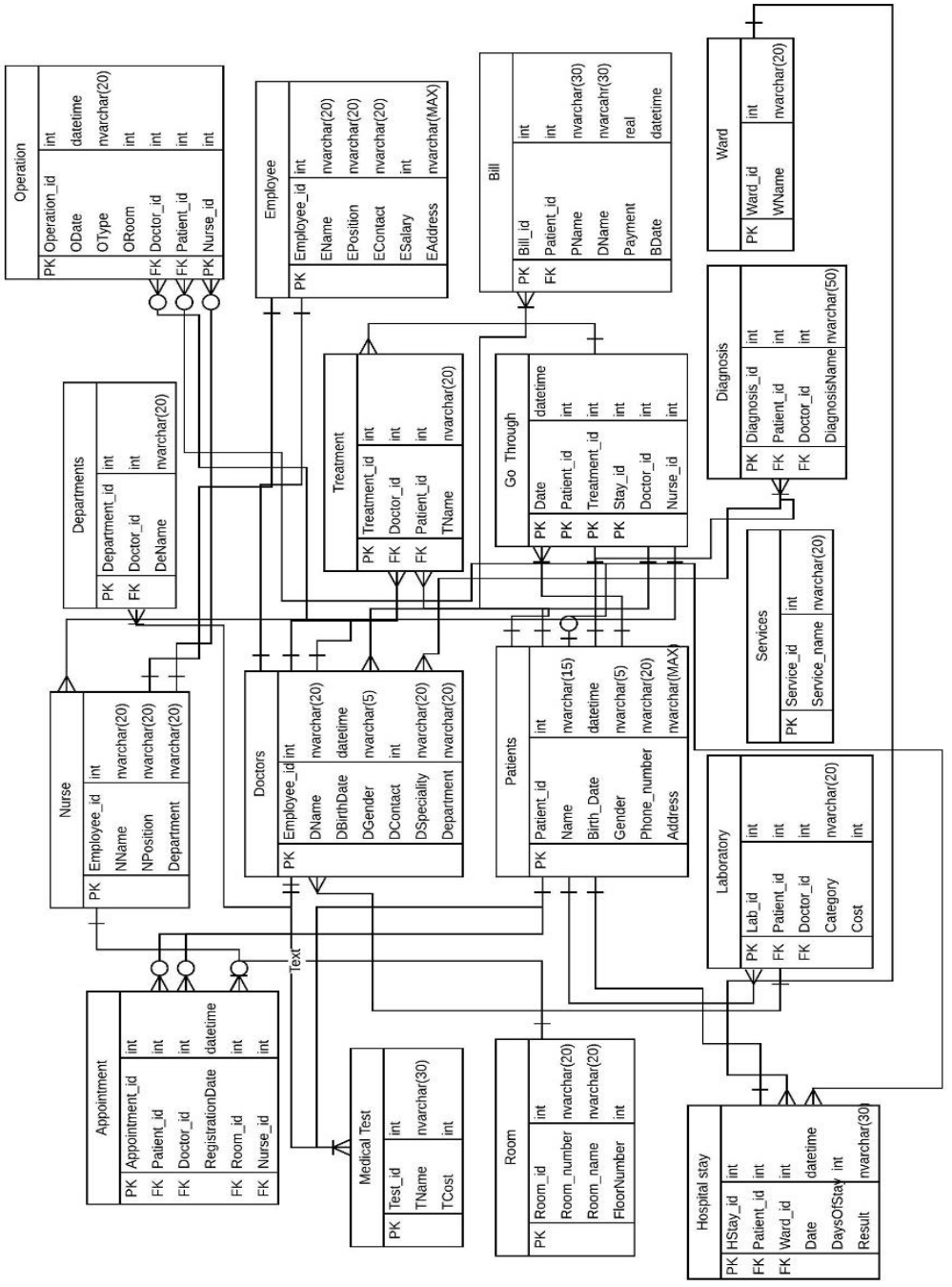

Figure 1. Complete Entity Relationship Diagram.

This included Patients, Doctors, Medical Tests, Nurses, Services, Appointments, Rooms etc. After creating the tables the required relationships were developed between tables with an Entity Relationship Diagram (ERD) (lucidchart.com).

The very next task was to query the database to test its operation by using SQL query language (James *et al.,* 2014).

By implementing a database, any hospital is able to update and check medical records, bill patients, check patients into wards and rooms and match them to the appropriate doctor.

In this thesis we have attempted to demonstrate the pros and cons of having a Hospital Database Management System. In this chapter, we can write much about the ease, success, improvement, benefits etc. that our small hospital will achieve by having a Hospital Database Management System:

- Faster access to more, useful, information,
- Efficiency in decision making,
- Efficiency (More work, lower cost),
- Patient satisfaction,
- Effective use of resources,
- Prevention of time wastage,
- Provision of better quality medical services,
- Facilitation of communication between employees,
- Measurement of employee performance,
- More effective planning of material requirements,
- More effective planning for operating rooms and diagnostic units,
- Introduction of requests and treatment results and many other benefits (techjockey.com).

In addition to the above-mentioned benefits, it is important to mention the problems caused by the system. These disadvantages are;

1. A reduction in patient privacy and subsequent physical, moral and social harm.

2. The data collected may be considered as commodities to be bought and sold and may also be non-objective.

As can be seen, both positive and negative aspects of the developments are of great importance (Healthcare, 2018). Here, the possibilities (benefit-harm) offered by technology should be considered together, both in terms of the patient and the medical profession, and the use of technology should be at the rate that medical ethics overlap with the firsts.

For this reason, it is necessary to make legal and technological arrangements to ensure that the system is regulated on legal grounds, that it is secure and there are control facilities. However, some events revealed that, despite all the measures taken, it was not possible to completely eliminate negative effects.

The most important stage after database creation, the transition to automation in the health institution, will follow to see how it works and what improvements are necessary. As this hospital transforms to a completely new stage, it will definitely face some issues of transition. The transition is gradual and necessary training is provided to staff who will use the program.

Thus, the following should be done in the near feature: ensure security of the data stored in the system, select equipment able to carry the load effectively. Transactions are totally dependent on computers, so maintenance is necessary and backups should be made to maintain functionality 7 /24.

Conclusion

The success of these innovations and implementation of information systems in hospitals depends on strong, supportive leadership and the participation of employees in all departments of the hospital. With this, the hospital information system ensures continuity of care by making data active and up-to-date, while reducing cost by accelerating care services. Many examples can be given of these kinds of database management systems; they include SoftClinic, ProMed, PaleBlue, Hospicare, among others.

Acknowledgment

I would like to thank Assoc. Prof. Ahmed Babanli PhD. (Khazar University) for his expert advice and encouragement for this article.

References

Amaechi J.C., Agbasonu V.C., & Nwawudu S.E. (2018). Design and Implementation of a Hospital Database Management System (HDMS) for Medical Doctors. International Journal of Computer Theory and Engineering. Vol. 10, No. 1, 1190 p.

Haggerty, J. (2019). Why Are Databases Important in Healthcare? https://www.databerry.com/news/why-are-databases-important-in-healthcare/

Gallagher Healthcare. (2018). Industry Insights Blog Top of Form Advantages and Disadvantages of Electronic Health Records

https://existek.com/blog/hospital-managment-system/

https://support.office.com/en-us/article/Database-design-basics-EB2159CF-1E30-401A-8084-BD4F9C9CA1F5)

Ripan, G.K. (2017). Online Hospital Management System Thesis Report School of Engineering and Computer Science BRAC University Conducted, 50 p.

Verbeke, F., Karara, G., Bastelaere, S.V. & Nyssen, M. (2012). Patient Identification and Hospital Information Management Systems in Sub-Saharan Africa: A Prospective Study in Rwanda and Burundi. Rwanda Medical Journal 69(4):7-12.

https://prezi.com/5wozcf8audxy/hospital-database-management-system/

https://www.lucidchart.com

James, R.G., Paul, N.W. & Andrew, J.O. (2009). SQL: The Complete Reference. McGraw-Hill Education; 3 edition. pp.88-93, 95-130

https://www.techjockey.com/blog/best-hospital-management-system

Khazar Journal of Science and Technology Volume 3 №2 2019, 80-86
 DOI: 10.5782/2520-6133.2019.3.2.80

The Importance of Health Information Systems and Implementation of an Electronic Healthcare System

Nigar Mammadova
Faculty of Engineering and Applied Sciences,
Khazar University, Azerbaijan
Email: nigar-4t@live.com

Abstract

As the world's population increases, existing medical systems face many problems in terms of the quality and intensity of medical care. One way to resolve these problems lies in the use of information and communication technologies in the health sector. To date, rich experience has been gained in the use of ICT to ensure a certain level of quality of medical care and accessibility for patients. ICT in healthcare helps to optimize the use of resources in the work of individual medical organizations, as well as in the management of public health systems at national or local level. Modern technologies are currently in use in the healthcare system of the Republic of Azerbaijan. Even now there is rapid development in e-health systems. The processes involved in making an appointment with a doctor are much easier than in previous years. New integrated technologies are also used in the examination of patients. Some hospitals even use a modern hospital management system.

Keywords: Electronic Healthcare System; Health Information System; Electronic Medical Record System; Electronic Health Record System

Introduction

A Health Information System (HIS) is a document management system for medical institutions that combines a medical decision-making support system, electronic medical records on patients, digital medical research data, data from devices monitoring patients' medical conditions, as well as financial and administrative information. HISs usually apply data transfer standard, which describes the procedures and mechanisms for the exchange, management and integration of electronic medical information. A healthcare system based on the application of

information and communication technologies provides users with a wide range of healthcare services. Typically, these services are provided over the Internet (Athina & Konstantinos, 2008).

Do we need a Health Information System?

A health information system (HIS) is a necessary element of IT technology in modern medicine.

The HIS is designed to automate the clinical areas of the work carried out by medical staff. It implements patients' electronic medical records and organizes effective communication between all participants in diagnostic and treatment processes. For example: in working online, no time is wasted in duplicating and entering data into new documents for exchange with other doctors and administration. Patient data, treatment progress, prescriptions and receipts are available online, but only to those who have access to the systems.

HIS reduces, and completely eliminates some routine operations in the preparation of medical documentation, thereby increasing the quality and information content of those documents. In fact, it removes or significantly reduces workloads doctors, nurses and medical registrars. So, more time is available for essential work - communication with patients, providing medical care or management of the medical institution supported by information that is objective and reliable.

The Impact of Health Information Systems on the Work of Medical Institutions

A health information system creates electronic structures for the hospital, its departments and offices, and is able to integrate several medical institutions into a single electronic system. It is convenient for clinics to have an electronic online registry, maintaining hospital-wide patient databases on the cloud, monitoring and planning doctors' work schedules. A unique cloud-based web platform stores, transmits and maintains records of electronic prescriptions and appointments.

A service such as a health information system increases loyalty and inspires trust in patients, because the doctor acquires information quickly and shares it with the patient via the Internet or prints it out during an appointment. Convenient online communication with customers, as well as partner insurance and pharmaceutical companies, also saves time and effort during treatment. At the same time, the electronic medical system improves reliability, the quality of health information and the medical services offered.

How Will Doctors Benefit?

Initially, the doctor acquires a single information space for working on patients' medical records and is able to automatically translate them into English or other languages (if there are multiple languages involved). As the system saves all the patient's past and future medical information in one reliable, secure and always accessible place, patient care is delivered more quickly. Using HIS makes it easy for a doctor to create and use templates of all types of medical documentation.

Quick access to all patient records from inpatient or remote locations contributes to better coordinated and more effective medical care. Notification and reminder functions assist doctors to remember necessary tasks - to communicate and interact with laboratories, colleagues or other participants in the diagnostic and treatment processes. The system can report in real-time and thereby minimize medical error. It is also easier to take care of patients' further treatment by appointing scheduled examinations electronically. Modern telemedical services are also available to users of a health information system and will contribute additional income and competitive advantage to doctors. Using the advantages of HIS saves doctors time on maintaining medical records and increases their specialist effectiveness.

The Patient in The New Medical System

The patient also benefits from use of a health information system, which reduces the likelihood of loss or falsification of medical data, and stores it safely in a cloud environment. If necessary, the patient can independently translate the data to another doctor in one of several languages. With HIS, patients receive advance reminders of visits to the doctor and medication. It is also possible to learn quickly the results of laboratory and instrumental tests, and to follow a doctor's prescription of examination and treatment. All these functions of a health information system work for high quality management of health information and reduce the patient's journey from illness to health.

Information technologies are important and necessary components of the development of modern medicine and can optimize business processes in several directions at once. Health information systems raise the quality of service and the level of patient access to medical care. This, in turn, is a priority for the state, medical workers and citizens.

HIS may be classified according to the medical institution's specialism:

- HIS for hospitals
- HIS for clinics and outpatient clinics
- HIS for dental clinics

- HIS for medical institutions.

Electronic Healthcare Systems in The World

The importance of the healthcare system has been stimulated by e-health in recent years. However, different parts of the world are currently at different stages, and e-healthcare systems are more advanced in European countries. E-healthcare systems were introduced much earlier in those countries and so we have an opportunity to see what problems were met along the way and to what extent we have managed to transfer medical institutions over to the use of electronic documentation.

Figure 1. shows statistics for the transition of healthcare systems to electronic bases in different countries in recent years.

E-healthcare systems in Estonia and Russia are analyzed below.

The Electronic Healthcare System in Estonia

The e-healthcare system in Estonia has its own particular characteristics, thanks to which it is possible to resolve almost all formal medical issues. The country's citizens access their electronic medical records using their ID. With just this, they can view all the information about their health in the system. Access to the database is via the portals eesti.ee or digilugu.ee.

The unified state digital registry of health care includes all patient visits to doctors, diagnoses, appointments, test results, images and chronic diseases. From mobile

applications they may add information, such as from a heart rate monitor or an electronic blood pressure monitor. An adult patient can also see health information about their minor children.

Integrated health information system in Estonia:

- automates all aspects of medical activity in the country;
- records full health information about the patient from birth to death;
- based on reliable architecture, completely safe;
- has passed the test of time nationwide.

It is important that the information exchange platform is protected from unauthorized access. Patient data from the database is available only to the attending physician and to the citizen by ID or mobile ID. A citizen can also see when and which specialist accessed their medical data.

The Electronic Healthcare System in Russia

In Russia, the EMR system has been in use since the 20th century. It offers many services and applications. The Russian Federation is currently developing a unified system for the collection of the electronic health records of all medical facilities.

The general architecture of the "ЕГИЗС" (Unified State Information Health System) consists of one segment of centralized system-wide components and one of applied components.

Firstly, according to the concept, there are subsystems for integrating application systems, maintaining a directory of system users, maintaining a register of normative. These also can be dictionaries of medical terminology, an electronic document register, a certification centre management subsystem, operations management and electronic mail management. The components are operated by the Ministry of Health.

The segment of applied components includes transactional, management and reference (information support for citizens, medical staff, students) subsystems.

Applied information systems are divided by level into federal (created by the Ministry of Health) and regional. Subjects, according to the concept, must create and operate regional application systems, and integrate them with federal application systems and centralized services.

The exchange of data between private healthcare organizations and the unified

state system in 2019 is one issue that concerns the medical community. Decree No. 555 of the Government of the Russian Federation, dated 05/05/2018, established a procedure for the provision of data to the EGISP. Its requirements applied to all non-governmental medical organizations, including private clinics and medical centres, from January 1, 2019.

Decree No. 555 prescribes the transfer to “ЕГИЗС” of a mass of different types of information. First of all, information about the medical organization itself and its doctors. Also, data on cases of medical care, including outpatient, inpatient epicrises. Experts say that not all private organizations are ready to provide all the data required.

Statistics indicate that a doctor receives from 60% to 80% of the information about a patient's condition by laboratory diagnosis. A current medical history in electronic form not only saves the patient’s time but also reduces the number of errors. It significantly improves the efficiency and quality of medical care provision. However, the exchange of data across a city or region is complicated by the fact that medical facilities and laboratories use different information systems. The healthcare market needs a solution that can combine disparate data without significant changes to the processes involved in data exchange.

In 2019, “Netrika” announced an expansion of the functionality of “N3.Health”. This Russian platform is for the integration of health information systems of medical institutions, laboratories, and insurance companies.

The service “N3.Health exchange of laboratory research data” helps medical institutions and laboratories to exchange requests and results of medical research in electronic form. This solution increases the rate of exchange of laboratory data and the availability of research results for doctors. It also reduces the number of unreasonably issued results and errors. The service “Laboratory Test Data Exchange”, based on the integration bus produced by “Netrika”, was launched in St. Petersburg in December 2015. Since then, more than two million directions and research results have been transmitted and received.

Conclusion

Electronic medical records are an effective part of the automatization of health information systems. A health information system creates electronic structures for the hospital, departments and offices. It can integrate several medical institutions into a single electronic system. It increases the integration of information between doctor and patient, making a significant saving of time, and improving quality of service. Fast access to all patient records from inpatient or remote locations contributes to better coordinated and more effective medical care.

Acknowledgment

I want to express special thanks to my supervisor Dr. Muhammad Sharifov for his support and advice.

References

Athina, A.L., & Konstantinos, M.S. (2008). Handbook of Research on Distributed Medical Informatics and E-Health. Medical inforMation science reference, Hershey • New York

Putkina, L.V. & Rezinkina, E.O. (2015). On the experience of implementing electronic medical records in a commercial medical organization. Nauka-Rastudent, No.11(23)

Zingerman, B. (2013). Electronic medical record and principles of its organization. www.osp.ru/medit/blogs/bz/bz_109.html

RIA News. (2014). What is EMIAS and why is it created https://ria.ru/20141224/1040005008.html

Ministry of Health of the Russian Federation (Ministry of Health). (2011). “Rostec” remains the sole contractor for the development of a unified healthcare IT systemhttp://zdrav.expert/index.php/Проект: Единая - государственная - информационная-система - здравоохранения_(ЕГИСЗ)

https://www.academia.edu/34652548/Учебник_по_курсу_ЕМИАС

http://tekhnosfera.com/analiz-i-sintez-meditsinskih-sistem-podderzhkiprinyatiyaresheniy-na-osnove-tehnologiy-statisticheskogo-modelirovaniya#ixzz5yrdVOEfA

http://nauka-rastudent.ru/23/3029/

Information to Contributors

Khazar Journal of Science and Technology focuses on the results of original research projects in Mathematics, Physics, Chemistry, Earth Science, Agriculture and Life Sciences as well as Engineering and Technology.

The journal is published in English.

The Editorial Board does not accept articles published or submitted for publication elsewhere.

Articles may be submitted by email. Please submit your manuscript to the following addresses:

submission-kjsat@khazar.org and submission.kjsat@gmail.com

Preparation of Manuscript

Manuscripts that are not prepared using the template will not be considered for publication.

Manuscript Types

Original research articles, review articles, research notes/short communications, case studies/case reports, and letters to the editor are welcome.

The editor-in-chief can change the manuscript type after manuscript submission.

• **Research Articles**

A research article reports the results of original research and assesses its contribution to the body of knowledge in a given area with the relevant data and findings in an orderly, logical manner.

• **Review Articles**

A review article is written to summarize the recent developments, improvements, discoveries, and ideas in various subjects. Review articles should present an unbiased summary of the current understanding of the topic.

Review articles should cover subjects that fall within the scope of the journal and are of active, current interest. Review articles should be no longer than 50 pages, should have an abstract of 300 words at most, should contain a limit of 120 references, and should have no more than 12 figures and tables combined. Principal sections should be numbered consecutively (1. Introduction, 2. Historical background, etc.), and subsections should be numbered 1.1., 1.2., etc. All reviews should contain an introduction section and a conclusion section, with relevant section headings in between. The introduction should explain the importance of the subject, the text should be comprehensive and detailed, and the references should be exhaustive. Review articles should be written with the support of original published studies of the author(s).

• **Research Notes/Short Communications**

Research notes/short communications are short papers that present original and significant material for rapid dissemination.

A research note/short communication may focus on a particular aspect of a problem or a new finding that is expected to have a significant contribution to science.

Research notes/short communications should be a concise but complete description of a limited investigation that will not be included in a later paper. These should be as completely documented, both by reference to the literature and by description of the experimental procedures employed, as a regular paper. Research notes/short communications should be no

longer than 15 pages, should have an abstract of 300 words at most, and are limited to 30 references and to 5 figures and tables combined.

Research notes/short communications should include all relevant study background and contain all of the sections described below, but without section titles or numbers.

• **Case Studies/Case Reports**

A case study/case report is a detailed report of the symptoms, signs, diagnosis, treatment, and follow-up of the subject of the investigation.

Case studies/case reports should contain introduction, case history, and results and discussion sections. The introduction should indicate the interest of the case for practitioners, the case history should describe the case and the procedures in detail, and the results and discussion section should outline the results with a pertinent discussion and diagnosis. Results and discussion should not be divided into two separate headings. Photographs are desirable. Case studies should be no longer than 8 pages, should have an abstract of 200 words at most, and are limited to 20 references.

Original articles should contain title, authors, abstract, keywords, list of abbreviations, introduction, materials and methods, results and discussion, conclusions, acknowledgements (if there are any), references.

Manuscripts should be 2.5-cm margins on all sides of the page, in Times New Roman font size 12. Every page of the manuscript, including the title page, references, tables, etc., should be numbered. All copies of the manuscript should also have line numbers starting with 1 on each consecutive page. Latin names of plant and animal species should be given according to the International Classification, in Italic.

The text should be organized as follows:

Title

Title of the paper should be brief, accurately identifying the manuscript content, without any abbreviations.

Authors

The forename and surname of each author are to be stated below the title. The institutional affiliations of authors are to be written below the names. If the coauthors are not all at the same institution or department, numbers in suffix should be used to refer the affiliations ([1, 2]). The name and e-mail of corresponding author should be mentioned, marked with asterisk (*).

Abstract and keywords. Abstract reflects shortly and clearly the basic information and scientific impact of the article, with cited the main results obtained. Keywords must be properly selected, giving the essence of the study.

Abbreviations

Abbreviations may be used for other expressions provided that, when first mentioned, they are defined in full, followed by the abbreviation in parenthesis and used consistently thereafter.

Introduction.

Introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the manuscript? What is your hypothesis and aim?

Material and Methods

The objects of research, organization of experiments, chemical analyses, statistical and other methods and conditions applied for the experiments should be described in detail.

Results and Discussion

Results of research can be presented separately from discussion or together in one paragraph. Presentation of results should be precise and without repetitions, and include the evaluation of significant differences and other parameters.

Conclusions

Present the most important consequences for the science and practice resulting from the conducted research. They should be summarized in few sentences.

Illustrations

Illustrations (maps, charts, graphs, drawings, photos, etc.) are to be referred to as 'Figures' and numbered in sequence. They should be as clear and free from text, presented as in format tif, png, xls, eps. Numeration and title of the figures are placed below the figure, with all necessary indications presented. It is recommended the figures to be in black and white. Provide figures and photographs with good dark and light contrast.

Tables and other illustrations should be submitted on separate sheets, and must be cited in the text. The position of each table, graph, figure, etc. in the text must be indicated. Photographs/pictures should be made with high contrast. Each illustration should be accompanied by a caption. Put the captions on a separate list at the end of the manuscript. Explanations should be typewritten under the illustration and given in this list, too.

References

In the text, references should be cited as follows: single author (Morris, 1991); two authors (Barton & Moore, 1994); more than two authors (Levi et al., 1994). When several references are cited simultaneously, they should be ranked by chronological order e.g.: (Petrovic et al., 2011; 2015) and when their publications are in the same year – alphabetically. All published works referred to in the text must be listed in the reference list and vice versa.

Reference list is arranged alphabetically (by the surname of the first author) and chronologically.

The journal follows APA citation style. Data in the reference list should be cited as follows:

a. Journal articles

Author(s) surname and initials, given bold, year, title, journal title , volume, number, pages.