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The Toxicological Effect on the Liver Function caused by Fecal Coliform Bacteria

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Abstract

This study was carried out on 80 male Swiss Webster Albino mice (MFI Strain) treated for 90 days with drinking well water contaminated with fecal coliform bacteria at four doses, control (0 colony/L), low dose (490 colonies/100 L), medium dose (1100 colonies/100 L) and high dose (2400 colonies/100 L) to test the bacterial effect on the liver functions. The animal blood plasma was tested for determination of the concentrations of the substances total proteins, glucose, creatine, bilirubin, cholesterol, triacylglycerol, high density lipoprotein, low density lipoprotein and the enzymes aspirate aminotransferase, alanine aminotransferase and alkaline phosphatase. The results indicated significant increase in the concentrations of the total proteins, lipoprotein, cholesterol, creatine and bilirubin and significant decrease in the concentration of the enzymes alanine aminotransferase, aspirate aminotransferase, alkaline phosphatase and in high density lipoprotein, triacylglycerol in the animal's blood. The functions of the liver were affected by the toxicity resulting from the fecal coliform bacteria concentrations in the well drinking water particularly at the medium and high doses.

Keywords: Swiss Webster albino mice; Fecal coliform bacteria; Blood physiology

Introduction

Fecal coliform bacteria (FCB) are the most common microbiological contaminants of natural waters. Fecal coliform live in the digestive tracks of warm-blooded animals, including humans, and are excreted in the feces. Although most of these bacteria are not harmful and are part of the normal digestive system, some are pathogenic to humans. Those that are pathogenic can cause disease such as gastroenteritis, ear infections, typhoid, dysentery, hepatitis A, and cholera. The biological analysis of mice blood samples is used to investigate the significant variations in the levels of some important compounds in the blood which act as a laboratory indication to the presence of defects in the physiological activities of the liver which normally accompany administration of pathogenic substances to the animal. The liver normal function is to metabolize, synthesize and/or degrade both absorbed and circulating products, and this function places it in potentially direct contact with gut-driven bacteria [1]. Scientists in their toxicity tests normally depend on the biochemical analysis of blood characters for observation of any significant variations in concentration of some important compounds which work as clinical indicators of disturbances that take place in the physiological functions of the liver [2]. Studies by Lichtman et al. [3] suggested that intestinal bacterial overgrowth or infection with helicobacter alone contributed to hepatic pathological change including increased serum Alanine aminotransferase (ALT) release and inflammatory cell recruitment. Bacterial effects on chain fatty acids could disrupt the normal metabolic processes, specifically reducing glucose utilization and promoting lipid storage [4]. Evans et al. [5] said that when consumption of energy by far exceeds the combustion of calories, the non-burnt energy is conserved in the form of fat (triacylglycerol TRIGL). The presence of (FCB) in drinking water may be harmful to humans and animals causing changes in the metabolic processes of the liver, so blood plasma is tested for the concentration of a number of enzymes and other substances as indication of liver function. The Food and Agricultural Organization [6] has suggested that drinking water should not contain more than 1000 colonies/100 ml. Environmental Protection Agency (USEPA) [7] review criterion of 400 col/100 ml. Srivastava et al. [8] found significant increase in levels of cholesterol (Chol) and proteins in the blood of Heteropneustes fossilis at all toxicity concentrations caused by malachite green for more than 30 days. The liver weight and the concentration of bilirubin (Bun) in the blood of the male Sprague-Dawley Rats significantly increased when the animals were administered the toxic 1,2–Dichlorobenzene at doses of 37.5, 75, 150 and 300 mg/kg/day (10 day) and 25, 100, and 400 mg/kg/day (90 day) in corn by oral gavage, control animals received corn oil [9]. The enzymes alkaline phosphatase (ALP), aspirate aminotransferase (AST) and (ALT) which are found in blood plasma are considered an important clinical indication of the disorder in the liver physiological functions like that resulting from the taking of hepatotoxic compounds, which are accompanied by rise in the level of liver enzymes in the blood. So in this study the mice were given drinking water containing different concentrations of FCB (control, 490, 1100 and 2400 colonies/L) for 90 days to see their effect on the liver function through analysis of the following enzymes and substances in the mice blood are ALT, AST, ALP, TP, (Chol), (TRIG) (Glu), LDLP and HDLP.

Materials and Methods

Materials

In this study mice were administered (FCB) with different concentrations to investigate its effect on the mice blood physiology. Eighty (80) male Swiss Webster Albino mice (MFI Strain) were used.

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Similar in weight (28-30 g) and age (8-10 weeks). Fed on Pillsbury diet and kept in cages at 18-25°C [10] and light was controlled by a timer (12 D/12 L).

Methods

The animals were grouped into 4 groups each 20 (N=20) mice were supplied with the treated water for 90 days. Group I is given pure distilled water, group II is given drinking water with low dose (LD) of FCB 490 colonies/100 L, group III is given medium dose (MD) of FCB of artesian well water containing 1100 colonies/L and group IV is given high dose (HD) of FCB of artesian well water containing 2400 colonies/L.

The biochemical tests for blood samples

The following tests were carried out. Plasma total protein (TP) was analysed according to Henry et al. [11] method. Glucose (Glu) was analysed using Trinder [12] method, and adsorption was carried out using spectrophotometer. Bilirubin (Bun) was analysed according to Jendrassik et al. [13] and adsorption was used by spectrophotometer. Cholesterol (Chol) was analysed by Stadtman [14] method. Triacylglycerol (TRIG), the method of Rautela et al. [15] was used to determine TRIG. High density lipoprotein (HDLP), the method of Burtis et al. [16] was used. Low density lipoprotein (LDLP), the same (HDLP) method was used (AST), the method of Saris [17] was used (ALT), the method of Bergmeyer et al. [18] was used to determine the enzyme. Alkaline phosphatase (ALP), the method of Principato et al. [19] was used to determine this enzyme.

Statistical analysis

Significant differences were determined by analysis of variance ANOVA. The differences between means were analyzed at the 5% and 1% probability level (p values of less than 0.05 and 0.01 were considered as statistically significant).

Results

The statistical analysis (Table 1) of the concentrations of the different enzymes and substances tested in the blood samples of mice after treatment of these samples with different FCB concentrations illustrated significant differences as regard level of the enzymes (AST) ($P \geq 0.01$), (TP) ($P \geq 0.05$), (LDLP) ($P \geq 0.01$), creatine (Crea) ($P \geq 0.01$), (ALT) ($P \geq 0.05$) and (TRIG) ($P \geq 0.05$), while the other enzymes the (HDLP), (ALP), (Chol), (Bun) and (Glu) have no significant differences with the control.

The average values (Table 2) show reduction in AST concentration in the mice blood from 128 in the mice blood treated with pure distilled water (control) to 111.8 and 113 in the low and high FCB toxic doses with no significant differences between them, but the AST reduction up to 85.2 in the medium FCB dose treatment reached a significant difference with the control. The (TP) concentration in the mice blood increased from 52.3 in the control to up to 53.2, 54.2 and 58.6 with the FCB low, medium and high concentrations respectively. The (LDLP) concentration in the mice blood decreased from 0.21 in the control to 0.19 in the low FCB concentration, then increased none significantly to 0.27 in the medium FCB concentration, and then increased significantly to up to 0.32 at the high FCB concentration. Blood concentration of (Crea) increased non-significantly from 20.2 in the control treatment to 21.7 at the low FCB concentration treatment, and then increased significantly to 24.9 and 25.1 in the medium and high FCB concentration treatment respectively. Glucose concentration in mice blood decreased significantly from 3.23 in the control treatment to 2.15, 2.53, and 4.41 in the low, medium and high FCB concentration treatment respectively. ALT concentration in mice blood decreased from 50.4 in the control treatment to 38.9, 44.0 and 43.3 in the low, medium and high FCB concentration treatment respectively. And also the mice blood concentration of the (TRIG) was decreased from 2.34 in the control treatment to 1.96, 1.2 and 2.01 in the low, medium and high FCB concentration treatment respectively. The concentration of the other enzymes and substances in mice blood showed no significant differences between the control treatments and the FCB concentration treatments.

Discussion

This study showed that there is significant effects on the mice blood plasma concentrations of some enzymes and substances after the subjection of the Albino mice to drinking water infected with low (420 colonies/100 L), moderate (1100 colonies/100 L) and high (4200 colonies/100 L) doses of (FCB) for 90 days. The mice blood concentrations of (TP), (LDLP), (Glu) and (Crea) were significantly increased and the enzymes ALT, AST and LDLP were significantly decreased while ALP, HDLP, Chol and Bun were not significantly different when compared to the control treatment. The increase in concentration of (TP) in mice blood is an indication of a bacterial injury of the liver, which induces liver to secrete more protein in the blood as a result of the effect on the mechanism of permeability in the permeable cell tissue. This is in agreement with Shakoori et al. [20] and also with the results of Srivastava et al. [8] who found significant increase in levels of cholesterol and proteins in the blood of the catfish *Heteropneustes fossilis* at all toxicity concentrations caused by malachite green for more than 30 days, but not with findings of Lukowicz et al.

	Degree of freedom	AST	ALP	ALT	TP	HDL	LDS	Chol	TG	Crea	Bun	Glu
FCB conc.	3	3167.6	380	225	*78.02	0.22	**0.034	0.12	2.3	*60.36	1.66	9.9
Error	36	932.64	293	122	18.38	0.32	0.006	0.34	1.2	19.96	2.79	0.4
FCB = fecal coliform bacteria, LSD = least significant difference, NS = not significant at P ≥ 0.05, ** significant at P ≥ 0.01												

Table 1: Analysis of variance enzyme and other substances concentration in mice blood treated with FCB concentrations in well water.

Conc.	AST	ALP	ALT	TP	HDLP	LDLP	Chol	TG	Crea	Bun	Glu
	(IU/L)	(IU/L)	(IU/L)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
Control	128 A	58.9 A	50.4 A	52.3 B	3.19 A	0.21 bc	3.19 A	2.34 A2.0	20.2 b	7.59 A	3.23 b
Low	111.8 Ab	63.6 A	38.9 b	53.2 B	3.14 A	0. 19 c	3.1 A	1.96 Ab	21.7 Ab	8.1 A	2.15 c
Medium	85.2 b	60.1 A	44 Ab	54.2 b	3.07 A	0.27 Ab	3.22 A	1.2 b	24.9 A	8.53 A	2.53 c
High	113 A	49.2 A	43.3Ab	58.6 A	2.85 A	0.32 A	3.36 A	1 Ab	25.1 A	7.8 A	4.41 A
LSD	27.69	15.53	10.02	3.88	0.51	0.07	0.53	0.98	4.05	1.51	0.54

Table 2: Averages of enzyme concentrations in mice blood treated with different FCB concentrations in well water.

[21] and Seki et al. [22] who did not find an increase in of TP in the blood of the treated mice infected with bacteria , and considered this is due to the infection of the liver because one of its jobs is excrete protein, and also disagree with the findings of El-Demerdash et al. [23], who found decrease in TP in the plasma of male rates due to toxicity from cadmium. The concentration and thus the activities of the enzymes ALT and AST in the mice blood were significantly affected due to the bacterial treatment, and this is in agreement with Raja et al. [24] and the importance of ALT and AST is their role in energy release. Liver enzymes are proteins measured in the blood plasma to test how liver is functioning. The (LDLP) concentration in mice blood was significantly increased due to the bacterial infection, but no significant differences were observed in the (HDLP) concentration in mice blood as a result of the bacterial infection. Animals need to store lipids in their body to get energy during harsh environmental conditions, but liver injury may affect the changes that happen to these lipids as mentioned by Malaguarnera et al. [25]. The findings in this study agree with the results of AL-Shaikh [26] who found increase in the concentration of LDLP and a decrease in HDLP in blood serum of animals subjected to toxicity. Also these findings agree with that of Mohamed [27] and Yousif et al. [28] where they found increases in the LDLP and HDLP concentrations in the mice blood subjected to toxicity and Kwong [29] mentioned that subjection of body organs to toxicity affect their metabolic activities and this is reflected in the biochemical structure of the blood . This increase in LDLP concentration in mice blood may be due to the role played by the trans-locating proteins in distributions of the toxic materials to the cells and this affects the liver which plays an important role in the metabolism process of the lipoproteins, and thus the liver might be injured by the bacteria causing disturbance in the lipoprotein synthesis as was suggested by Zielinski et al. [30].

Glucose level was significantly increased in the mice blood under the high bacterial dose (2499 colonies/100 L) and this agrees with what have been found by AL-Shaikh [26], who found the same increase of glucose in mice blood suffered from toxicity and also with the findings of El-Demerdash et al. [23], who found increase in concentration of glucose in male rats plasma treated with cadmium. This might be due to the incapability of the liver in practicing its function in organizing glucose sugar in the blood and transforming it into glycogen, a fact which affects the organization process and leads to an increase of glucose in the blood plasma. Also the pancreas may be affected by the bacteria becoming unable to excrete the insulin hormone, as was mentioned by Luskova et al. [31].

The study also showed an increase but not significant in the concentration levels of Chol and Bun in the mice blood due to the infection by bacteria (FCB). This might indicates that the injury of the liver might not be so bad as regards these substances concentrations in mice blood, as the liver plays an important role in the metabolism of Chol and any disturbance in liver function will lead to Chol increase in blood plasma as said by Saleh et al. [32] and by El-Demerdash et al. [23] who found increase in (Glu), (Crea) and (Bun) in male rats due to toxicity. In the case of (Bun) concentration its increase or reduction in blood is due to destruction of the haemoglobin by breaking down of the erythrocytes as a result of cell death or clotting of the bile ducts, a process that leads to the return of Bun to the liver and from it to the blood. This means an increase of Bun concentration in the blood plasma as was also said by El-Demerdash et al. [23] and AL-Shaikh [26]. Bilirubin is a chemical released into blood indicating bile production and metabolism and its normal level is 0.2–1.2 mg/dL, and its increase in plasma may be a sign of liver problem. Increase of Bun in the mice blood agrees with the result of Robinson et al. [9] who found significantly increased in Bun levels in blood when male Sprague-Dawley Rats were administered the toxic 1,2–Dichlorobenzene at doses of 37.5, 75, 150 and 300 mg/kg/day (10 day) and 25, 100, and 400 mg/kg/day (90 day). Also an increase in TRIG concentration in the blood. These findings in this study agree with that given by some researchers who found an increase in Chol concentration in the blood plasma and attributed this to the injury of the liver and also agree with that of Zielinski et al. [30] who found significant increase in TRIG concentration in the animal blood plasma due to liver injury.

Conclusion

The blood samples tested of the white Swiss Albino mice treated with well drinking water contaminated with (FCB) at moderate (1100 colonies/100 L) and high (2400 colonies/100 L) doses for 90 days showed significant increases in concentration of (TP), (LDLP), (Chol), (Crea) and (Bu) and significant decrease in the enzymes (ALT), (ALP), (AST), (HDLP) and (TRIG) compared to the control with pure drinking water free from bacteria. The increases of TP and LDLP concentrations in the blood plasma of mice is an indication of an effect on the mechanism of permeability in the permeable cell tissue of the liver and also an indication of an effect on the metabolism process thus causing disturbances in the lipoprotein synthesis and enhancing more protein secretion by the liver in the blood.

The increase in mice blood concentration of (Glu) at the higher FCB dose (2400 colonies/100 L) might be due to the incapability of the liver as an organizer of the glucose sugar in the blood and transforming it into glycogen, and also the pancreas might have been affected becoming unable to excrete insulin. The significant decrease in concentration of the enzymes ALT and AST in the mice blood plasma is because of the effect of bacteria in the drinking water particularly at the higher dose (2400 colonies/100 L). Cholesterol and Bun concentrations in the mice blood plasma increased but not significantly. The bacteria in drinking water have caused disturbances in the functions of the liver which plays an important role in Chol metabolism thus leading to increase in Chol in the mice blood plasma. (Bun) increase in the blood plasma of the mice is mostly due to destruction of the hemoglobin as a result of breaking down of the erythrocytes due to cell death or clotting of the bile duct.

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