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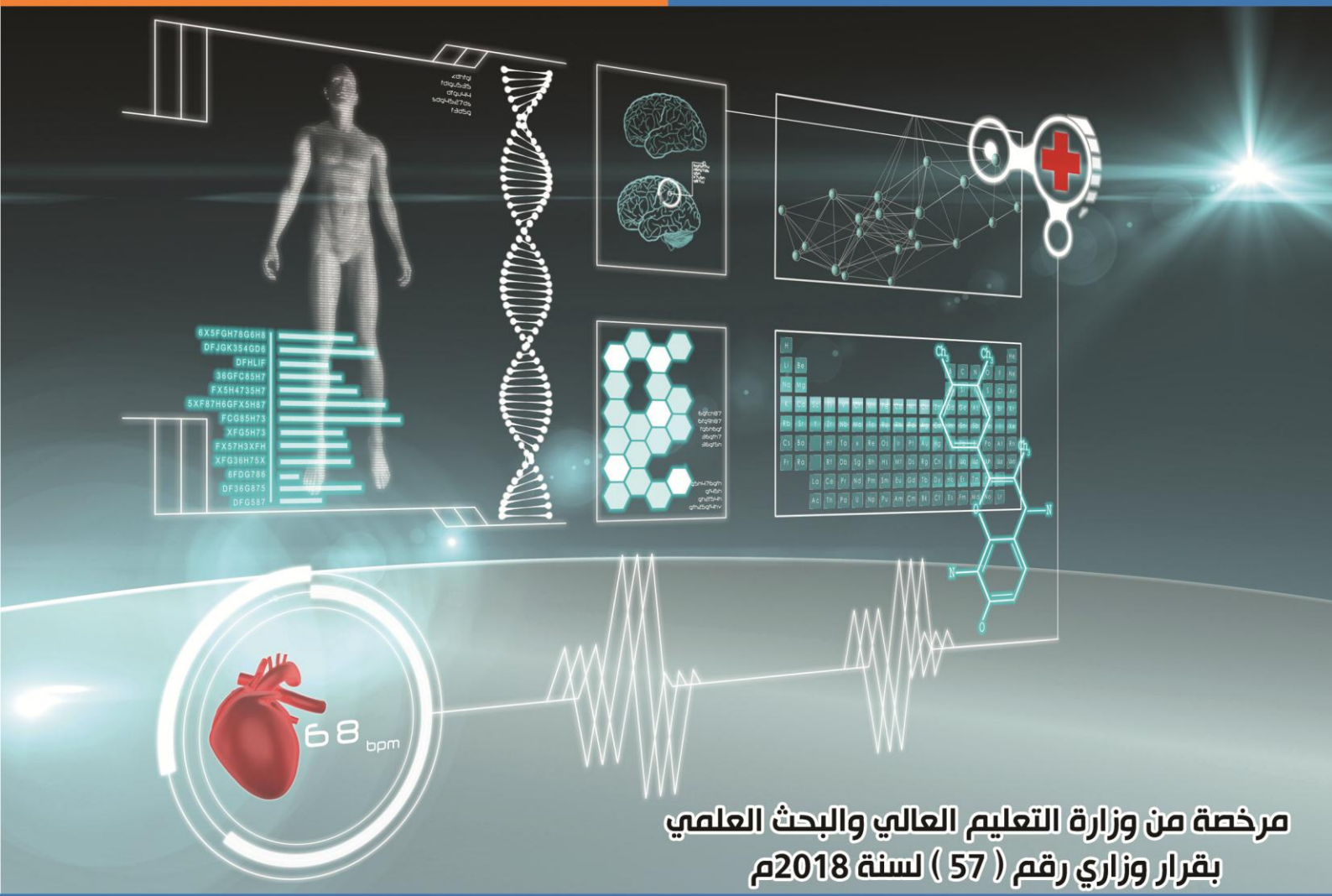

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Evaluation of Lipid Profile Level in Penicillin-Induced Guinea Pigs Treated with Antioxidant Sider Honey and Vitamin A, C and E

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Abstract

Background*: Penicillin have long been used in antibacterial therapy. The effects of penicillin on physiological parameters are well studied. It has been reported that the effects of penicillin mainly due to the generation of an excessive amount of reactive oxygen species (ROS) Antioxidants protect key cell components from damage by neutralizing the free radicals. .* ***Aim****: To investigated the effect of supplementation of Sider honey and vitamin A, C and E on penicillin-induced guinea pigs by lipid profile level.* ***Methods****: A total of Ninety-five adult male guinea pigs weighting 800-900g were divided into twelve groups of five-ten guinea pigs each, and the experiment lasted for 30 consecutive days. Animals in group I served as control, animals in groups 2 were intraperitoneal (i.p.) injected with penicillin 50000 IU/kg, animals in group 3-7 were administrated orally with Sider honey 600 mg/kg, vitamin A 10000 IU/kg, vitamin C 100 mg/kg, vitamin E 100 mg/kg and vitamins A,C and E respectively, animals in group 8-12 in addition penicillin were orally administrated with Sider honey, vitamin A, vitamin C, vitamin E and vitamin A,C and E respectively.* ***Results****: The result showed a significant increase in total cholesterol, triacylglycerol, LDLC and total lipid. And a significant decrease in HDLC in penicillin treated guinea pigs when compared to the control guinea pigs, but penicillin exposed and antioxidant vitamins A, C, E and Sider honey treated groups produced significant (P<O.O1) reduction in total cholesterol, triacylglycerol, LDLC and total lipid and with an increase in HDL levels relative to penicillin only treated groups were observed.* ***Conclusion****: These results suggest adverse effect of penicillin and ameliorating role of Sider honey, vitamins A, C and E on guinea pigs.*

Keywords: Penicillin, Honey, Vitamin A, C and E, Lipid profile

Introduction

Antibiotics constitute a family of drug, which taken as a group, represents one of the most frequently prescribed around the world. Thus, not surprisingly antibiotics, along with Non-steroidal anti-inflammatory drugs (NSAIDs), list on the top of causes of drug induced many side effects[1]. The incidence of side effects induced by antibiotics reported in clinical and experimental studies is fairly uniform ranging from 0.29/100000 (95% confidence interval "CI": 0.17-0.51) to 9/100000 (95% CI: 6-15). However compared with these results a higher risk of liver related hospitalization was reported (3-23 per 100000 patients)[1,2]. Penicillin is a group of antibiotics derived from Penicillium fungi[3]. They include penicillin G, procaine penicillin, benzathine penicillin, and

penicillin V. Penicillin antibiotics are historically significant because they are the first drugs that were effective against many previously serious diseases, such as syphilis, and infections caused by staphylococci and streptococci[4].

All penicillins are β-lactam antibiotics and are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms[5]. Penicillins are still widely used today, though many studies that confirm the side effects on liver[6,7], Kedney[8,9], reproductive tissues[10,11] and hematological parameters[12,13].

They are substances that protect other chemicals of the body from damaging oxidative reactions by reacting with free radicals and other reactive oxygen species within the body, hence, hindering oxidation. Although oxidative reactions are crucial to life, they can also be destructive; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and E as well as enzymes such as catalase, superoxide dismutase and various peroxidases[14]. As oxidative stress might initiate many human diseases and the use of antioxidants in pharmacology is presently gaining acceptance, particularly as potential treatments for atheriosclerosis, cancer and neurodegenerative diseases[15]. Antioxidants are now used as dietary supplements in maintaining health and preventing diseases such as cancer and coronary heart disease. A dietary antioxidant is a substance that significantly decreases the harmful effects of reactive oxygen species and nitrogen molecules, which disrupt normal physiological functions at the cellular level in animals and humans. Examples of dietary antioxidants include vitamins C and E, selenium and carotenoids. A number of other nutrients, including minerals such as copper, manganese, and zinc, phytochemicals such as flavonoids in grape seed extract and phenols found in green tea, and coenzymes also possess antioxidant properties[16] (Stacy and Childs, 2000). The primary function of vitamin C is for production of collagen, which forms the basis for connective tissues in bones, teeth and cartilage[17].

Aim of the study

The aim of this study was to investigate the effect of supplementation of Sider honey and vitamin A, C and E on penicillin-induced guinea pigs by lipid profile level.

Subject and methods

A Randomized clinical trial study was administered on ninety-five adult male guinea pigs (5-6 months old) weighing between 800 - 900 g were obtained from the Zoo, Sana'a- Yemen. The animals were housed in plastic cages in the animal house of the department of biology- faculty of education- Amran University, under standard conditions in room temperature, fed a standard laboratory diet and water *ad libitum*. Animals were allowed to acclimatize to the laboratory environment for 30 days.

Materials: Sider honey (*Ziziphus spina- christi* honeybee) was obtained from beekeeper, Mabian-Hajjah-Yemen, Penicillin (Procaine G penicillin) was obtained from Ave Group-USA-Colombia-Mexico.

Vitamin A (Retinol Assay: 99 Appearance: Slightly yellow solid Formula: C20H30O Molecular Weight: 286.50), was supplied by Look for chemical (Hangzhou, China). Vitamin C (L-) ascorbic acid Assay: 99%-100% Appearance: White crystalline powder Formula: C6H8O6

Molecular Weight: 176.14), was supplied by Carlo Erbo (Milano, Italy). Vitamin E (DL-alpha- tocopherol acetate Assay: 96% Appearance: low yellow powder Formula: C29H50O2 Molecular Weight: 430.71) was supplied by Merck (Germany).

Animals: Ninety-five adult male guinea pigs were divided randomly into 12 groups. Penicillin, Sider honey and vitamin C were dissolved in distilled water, while vitamin A and vitamin E were dissolved in corn oil. Treatments were carried out over a period of 30 days. The divided groups were randomly assigned into the following:

Group 1: 10 animals were served as controls and received orally with distilled water (3ml/kg) and were orally received 0.5 corn oil.

Treatment groups were as follows:

Group 2: 5 animals were administered orally with Sider honey (600 mg/kg body weight), dissolved in distilled water, daily for 30 days.

Group 3: 5 animals were administered orally with vitamin A (10000 IU/kg b.w), daily for 30 days.

Group 4: 5 animals were administered orally with vitamin C (100 mg/kg b.w), daily for 30 days.

Group 5: 5 animals were administered orally with vitamin E (100 mg/kg b.w), daily for 30 days.

Group 6: 5 animals were administered orally with vitamins A, C & E in combination (10000 IU, 100 mg & 100 mg/kg b.w), daily for 30 days.

Group 7: 10 animals were intraperitoneally (i.p.) injected with penicillin (50000 IU/kg b.w), daily for 30 days.

Group 8: 10 animals were i.p. injected with penicillin (50000 IU/kg b.w), concomitant with orally treated of Sider honey (600 mg/kg b.w), daily for 30 days.

Group 9: 10 animals were i.p. injected with penicillin (50000 IU/kg b.w), concomitant with orally treated of vitamin A (10000 IU/kg b.w), daily for 30 days.

Group 10: 10 animals were i.p. injected with penicillin (50000 IU/kg b.w), concomitant with orally treated of vitamin C (100 mg/kg b.w), daily for 30 days.

Group 11: 10 animals were i.p. injected with penicillin (50000 IU/kg b.w), concomitant with orally treated of vitamin E (100 mg/kg body weight), daily for 30 days.

Group 12: 10 animals were i.p. injected with penicillin (50000 IU/kg b.w), concomitant with orally treated of vitamins A,C & E in combination (10000 IU, 100 mg & 100 mg/kg b.w), daily for 30 days.

Collection the blood and tissue: 24 h after last administration, animals of each group were autopsied, blood samples were taken from the heart and collected into sterile tubes centrifuged at rpm for 20 min, and serum was separated for biochemical tests. Estimation lipid profile was done as a follows: the estimation of Total lipids: Serum total lipid was assayed according to the method of Kaplan et al (1984).

Estimation of Total Cholesterol: Serum total cholesterol was assayed according to the method of Naito (1984). Estimation of high density lipoprotein-cholesterol (HDL-C): Serum high density lipoprotein-cholesterol (HDL-C) was assayed according to the method of Grove (1979). Estimation of low density lipoprotein-cholesterol (LDL-C):

Serum low density lipoprotein-cholesterol (LDL-C) was assayed according to the method of Okada et al (1998). Estimation of Triglycerides: Serum triglycerides was assayed according to the method of Buccolo et al (1973).

The data were analysed using SPSS 16.0 for windows. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Fisher's Protected Least Significant Difference (PLSD) test as a post hoc test for comparison between groups. All values were expressed as means ± SD and percentages. Differences were considered significant if $p< 0.05$. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH, 1978), and were approved by the Animal Experiments Local Ethics Committee at the Zoo, Sana'a- Yemen.

Results

Effect of the Penicillin, Sider honey, vitamin A, vitamin C, vitamin E, vitamin A, C, E on the lipid profile:

Results in table1 shows that the i.p. administration of penicillin in dose 50000 IU/kg b.w. per day period of 30 day (Group 2), resulted in high significant $P<0.01$ increase in the level of total lipids, cholesterol, LDL-C and triglycerides as compared to the control (Group1), penicillin i.p. administration resulted also in high significant $P<0.01$ decrease in the level of HDL-C as compared to the control (Group1).

The administration of Sider honey in a single dose 600 ml/kg b.w. per day period of 30 day (Group3), resulted in non-significant $P<0.01$ change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides as compared to the control (Group1).

The administration of vitamin A in a single dose 10000 IU/kg b.w. per day period of 30 day (Group3), resulted in non-significant $P<0.01$ change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides as compared to the control (Group1).

The administration of vitamin C in a single dose 100 mg/kg b.w. per day period of 30 day (Group3), resulted in non-significant $P<0.01$ change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides as compared to the control (Group1). The administration of vitamin A in a single dose 10000 IU/kg + vitamin C in a single dose 100 mg/kg + vitamin E in a single dose 100 mg/kg b.w. per day period of 30 day (Group3), resulted in non-significant, $P<0.01$ change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides as compared to the control (Group1).

Table. 1: Effect of the Penicillin, Sider honey, vitamin A, vitamin C, vitamin E, vitamin A, C, E on the lipid profile.

Effect of supplementation with Sider honey and vitamin A, C and E on lipidic profile in normal and penicillin-induced guinea Pigs:

Parameter / Groups	Total lipids Mg/dl		Cholesterol Mg/dl		HDL-C Mg/dl		LDL-C Mg/dl		Triglycerides Mg/dl	
	M±SD	Change	M±SD	Change	M±SD	Change	M±SD	Change	M±SD	Change
Control	296.8±17.8	------	40.0±3.4	------	21.51±2.6	------	24.98±3.4	------	60.19±4.0	------
Penicillin	321.5±12.7^{C}	8.3%	48.2±3.3^{C}	20.5%	15.21±2.1^{C}	29.3%	33.19±3.3^{C}	32.9%	68.66±4.2^{C}	14.1%
Honey	290.0±7.3^{a}	2.3%	35.7±4.3^{a}	10.8%	22.19±1.2^{a}	3.2%	20.68±5.1^{a}	17.2%	56.16±5.1^{a}	6.9%
Vitamin A	294.6±5.2^{a}	0.1%	38.8±5.1^{a}	3%	21.82±2.2^{a}	1.4%	23.76±3.4^{a}	4.9%	58.37±5.9^{a}	3%
Vitamin C	294.6±6.0^{a}	0.1%	37.6±4.1^{a}	6%	22.12±1.3^{a}	2.8%	21.90±4.1^{a}	12.3%	59.44±4.6^{a}	1.2%
Vitamin E	291.0±7.1^{a}	2%	36.9±3.4^{a}	7.8%	22.10±1.7^{a}	2.7%	22.55±4.5^{a}	9.7%	60.04±3.1^{a}	0.2%
Vit A,C,E	291.2±7.5^{a}	1.9%	37.3±4.5^{a}	6.8%	22.04±3.01^{a}	4.1%	22.28±4.1^{a}	10.8%	57.48±4.5^{a}	4.5%
ANOVA; P-Value	***P<0.01***		***P<0.01***		***P<0.01***		***P<0.01***		***P<0.01***	

The values are given as Mean± Standard Deviation (M±SD), degrees of freedom (df), (in each group).-^{a}Non significance,-^{b}Low significance,-^{C}High significance at (P<0.01) vs. control.

Table 2 shows that the i.p. administration of penicillin in dose 50000 IU/kg b.w. per day period of 30 day (Group 2), resulted in high significant P<0.01 increase in the level of total lipids, cholesterol, LDL-C and triglycerides as compared to the control (Group1), Penicillin i.p. administration resulted also in high significant *P*<0.01 decrease in the level of HDL-C as compared to the control (Group1). Results showed that honey significantly (*P*<0.01) reduced the toxicity of Penicillin, where administration of Sider honey in dose 600 mg/kg b.w. (Group8) beside penicillin, resulted in non-significant P<0.01 change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides as compared to the control (Group1). Results showed that vitamin A significantly (*P*<0.01) reduced the toxicity of penicillin, where administration of vitamin A in dose 10000 IU/kg b.w. (Group9) beside penicillin, resulted in non-significant *P*<0.01 change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides as compared to the control (Group1). Results showed that vitamin C significantly (P<0.01) reduced the toxicity of penicillin, where administration of vitamin C in dose 100 mg/kg b.w. per day (Group10) beside penicillin, resulted in non-significant P<0.01 change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides as compared to the control (Group1). The findings of the study showed that vitamin E significantly (*P*<0.01) reduced the toxicity of penicillin, where administration of vitamin E in dose 100 mg/kg b.w. (Group 11) beside penicillin, resulted in non-significant *P*<0.01 change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides as compared to the control (Group1). Results showed that vitamin A, C and E significantly (P<0.01) reduced the toxicity of penicillin, where administration of vitamin A in dose 10000 IU/kg + vitamin C in dose 100 mg/kg + vitamin E in dose 100 mg/kg b.w. (Group 12) beside penicillin, resulted in non-significant *P*<0.01 change in the level of total lipids, cholesterol, LDL-C, HDL-C and triglycerides, as compared to the control (Group1).

Table. 2: Effect of supplementation with Sider honey and vitamin A, C and E on lipidic profile in normal and penicillin- induced guinea Pigs

	M±SD	Change	M±SD	Change	M±SD	Change	M±SD	Change	M±SD	Change
Control	296.8±17.8	------	40.0±3.4	------	21.51±2.6	------	24.98±3.4	------	60.19±4.0	------
S	321.5±12.7[C]	8.3%	48.2±3.3[C]	20.5%	15.21±2.1[C]	29.3%	33.19±3.3[C]	32.9%	68.66±4.2[C]	14.1%
S + honey	298.1±5.1[a]	0.4%	41.8±3.1[a]	4.5%	19.55±1.9[a]	9.1%	26.80±3.1[a]	7.2%	62.03±3.6[a]	3.1%
S +Vit A	304.7±11.6[a]	2.7%	44.5±2.2[b]	11.3%	17.52±1.1[b]	18.5%	29.48±3.2[b]	18%	64.77±2.3[a]	7.6%
S +Vit C	302.8±6.4[a]	2%	43.5±3.4[a]	8.9%	19.00±2.1[a]	11.7%	28.47±3.3[a]	14%	63.09±3.5[a]	4.8%
S +Vit E	301.9±8.8[a]	1.7%	43.9±2.8[a]	9.8%	18.69±2.1[a]	13.1%	28.87±3.8[a]	15.6%	62.52±3.6[a]	3.9%
S +VitA ,C ,E	299.0±12.2[a]	0.7%	42.3±2.8[a]	5.8%	19.30±1.5[a]	10.2%	27.25±2.8[a]	9.1%	61.72±3.3[a]	2.5%
ANOVA; *P*-Value	**P<0.01**		**P<0.01**		**P<0.01**		**P<0.01**		**P<0.01**	

The values are given as Mean±Standard Deviation (M±SD), degrees of freedom (df), (in each group).-[a]Non significance,- [b]Low significance,- [C]High significance at (P<0.01) vs. control.

Discussion

The total cholesterol, triglycerides, LDL-C, and total lipid concentration have been proved to be indicate are in the diagnosis of some clinical conditions such as hepatitis, chronic obstructive jaundice and coronary heart disease precipitated by atherosclerosis with attendant hyperglycemia[23]. Among the serum lipid fraction, total cholesterol is the most implicated and predominant constituent of atherogenic plaque[24].

Results of the present indicate to significant increase in total lipid, LDL and triglycerides in guinea pigs treated with penicillin alone. A significant increase was also noted in total cholesterol and decrease HDL values in the group treated with penicillin only when compared to the control group.

These results are in agreement with those reported by Vijayalekshmi Amma and Leelamma[25] who found that the levels of the total cholesterol, triglycerides and LDL-cholesterol in the serum of rats were significantly increased after treatment of penicillin for 7 days, while a significant decrease in HDL-C levels was noticed. Tasduq et al.[26] demonstrated a significant increase in triglycerides and cholesterol levels of rats after administration of rifampicin for 30 days. Santhosh et al.[27] detected significant increases in triglycerides, cholesterol and free fatty acids in the serum of rats after receiving anti-TB drugs (rifampicin, isoniazid) for 30 days.

The increase of cholesterol levels might be due to the increase synthesis of cholesterol in liver and cholesterol ester hydrolase following to the increase levels of triglycerides and LDL[28]. The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and cell membranes[29].

Machado *et al.*[30] observed that the organelles which changed in the presence of penicillin were mainly mitochondria in which, the beta oxidation enzyme is inhibited, resulting in an accumulation of triglycerides inside the cytoplasm and this stated that the hypertriglyceridemia may be due to increased release of lipoproteins into the circulation. Vijayalekshmi and Leelamma[25] reported that the uptake of penicillin rich lipoprotein from the circulation is also decreased which is evident from the decreased activity of lipoprotein lipase of the extrahepatic tissue and this indicate to that the high density lipoproteins are believed to be involved in the transport of cholesterol

from the tissue to the liver for its catabolism.

The increment of total cholesterol level in the serum of penicillin-administrated guinea pigs may be attributed to liver dysfunction that has been observed in the histological study as a result of degeneration and necrosis of the hepatocytes caused by the toxic action of penicillin. Liver plays a pivotal role in the metabolism of lipids, also synthesizes many important substances needed by the body, such as cholesterol which is packaged in the cell membrane of hepatocytes as an end product of metabolism, and then distributed to the body to be used. So, the distortions in the architectural and functional integrity of the liver (necrosis and damage in hepatocytes) might cause the release of the cholesterol from the hepatocytes through cell membrane into the blood stream[31.]

Antioxidants reductive effect on total serum cholesterol and/or in decreasing atherosclerosis and preventing cardiovascular diseases have been demonstrated in human and animal models[32,33]. Antioxidants have been shown to reduce or delay the progression of atherosclerosis, thus preventing the cardiovascular diseases[34,35,36,37].

Our results revealed that administration of antioxidant vitamins A, C and E in separated and in combination beside penicillin had a significant decrease in total lipid, total cholesterol, triglycerides and LDL-C and increase HDL, when compared with penicillin treated but no significant different when compared with the control. Our results are in agreement with Münstedt et al.[38] and Mushtaq et al.[39] who reported that honey exhibited a decrease in serum total cholesterol, LDL-C and triglycerides concentrations and increase in HDL-C, and with Nawazish *et al.*[40] who reported that vitamin A, C and E individually and in combination exhibited a decrease in serum total cholesterol, LDL-C and triglycerides concentrations and increase in HDL-C. Thus McRae[33] who reported that vitamin C supplementation resulted in a significant reduction in both LDL-C and triglycerides, and with Al-Jowari *et al.*[41] who showed a significant decrease in serum cholesterol, triglycerides, LDL,VLDL concentrations and atherosclerosis index except HDL which increased significantly in the treated group with acetaminophen and vitamin E compared with the group treated with the group treated with acetaminophen, and with Ukpanukpong *et al.*[42] who showed ameliorating role of vitamins C and E in normalized cholesterol, triglycerides, LDL and VLDL levels elevated by pefloxacin in rats.

Our results may suggest a possible role of vitamin A, C and E alone or in combination in lowering lipid parameters in hypercholesterolemic guinea pigs, and can be explained due to their inhibitory effect on β-hydroxy–β-methylglutaryl (HMG) CoA reductase activity, thus inhibiting cholesterol biosynthesis. Antiatherogenic role of the vitamins may therefore be due to drastic reduction in the filtration of fatty acids from the blood into the artery and consequent reduction in cholesterol and other fatty acids[43]. The obtained results are in agreement with the reports that insured the role of vitamin C and E in improving hyperlipidemia and cardiac functions in rats[44]. Moreover, a combination of vitamin C, vitamin E has been shown to prevent the LDL oxidation, which is thought to lead towards atherosclerosis[34]. The observed improvement in our study could be attributed to the vitamins A, C and E ability to intercept reactive oxygen species, thereby significantly

reducing plasma lipid peroxide levels and inhibiting oxidative modification of LDLs and it also protects HDL-C from lipid oxidation and making it available for reverse cholesterol transport[45]. HDL also inhibit LDL oxidation and this free radical scavenging effect occurs via an antioxidant enzyme called HDL-associated paraoxonase and the loss of this enzyme during oxidative stress is prevented by vitamin C[34.]

Conclusion

The results of the present work for the first time reveal that the Sider honey and vitamins A, C & E play an important role as a cytoprotective compounds on lipid profile level in guinea pigs.

Recommendations

Further studies on the possible uses of honey and vitamins as protective compounds during treatment with antibiotics are needed.

References

1. Maliha S.S. Shahed M.J. Janker M.N. Abasi K.W. Nigm-Rahman A.E. Clinical and Experimental Evidences in Antibiotics Side Effects and Toxicity Associate with Overdose and Long-Term of Use. Pharmacol. Assoc. J. 2009; 6(3): 23-31.
2. Westphal J.F. Vetter D. Brogard J.M. Hepatic side-effects of antibiotics J. Antimicrob. Chemother. 1994; 33 (3): 387-401.
3. Kasten B. Reski. R.. β-lactam antibiotics inhibit chloroplast division in a moss (Physcomitrella patens) but not in tomato (Lycopersicon esculentum)". J. Plant Physio. 1997; 150 (1–2): 137–140.
4. Aharonowitz Y and C. Cohen.. The microbiological production of pharmaceuticals. Sci. Am. 245(3) :11983; 41-152.
5. Balows A. Hausler W. Herrmann K. Isenberg. H and Shadomy.. Manual of clinical microbiology, 5th ed. Washinton. D. C. Am. Soc. Microbial. 1991
6. Evan J. Michelle J. Pat A. and Brenda A. Penicillin - allergic guinea pigs and anaphylactic response. J. Clin. Anesth. 2001; 13(8): 561-564.
7. Akande T, Balogun S.T and Gabriel O. The effects of penicillin streptomycin on liver aminotransferases, alkaline phosphatase and total serum protein in rabbits (Orcytolagus coniculus). J. of Appli. Pharm. Sci. 2012; 2 (1): 32-35
8. Harry Eagle. Fleischman R and Musselman D. Experimental approach to the problem of treatment failure with penicillin in mice. Amer. J. Med. 1992; 13 (4) : 389 – 399.
9. Alves J.R .Fonseca I.P. and Ramalho M.T. Optimisation of penicillin extraction. J .Biochem. 2003; 15(2) :81–86.
10. Landgren S. Backstrom T. and Kalistratov G. The effect of progesterone on the spontaneous in terictal Spike evoked by the application of penicillin to the cat's cerebral cortex .J .Neuro sci. 1998; 36(1): 119-133.
11. Harold A.T .Penicillin in benign late and Visceral Syphilis in rats. Amer. J. Med. 1998; 5(5):702–708.
12. Kaiz F. Dermographia: another side effect of penicillin therapy. J. Aller . 1996; 28(6) 64-68.
13. Joseph Alcalay, Michael David, Arie Ingber and Bilha Hazaz. An untoward side effects of penicillin. J .Amer. Acad. dermatol. 1988; 18(2): 345-349.
14. Vankates K.Z. Siempos I.I. Grammatikos A. Athanassa Z. Korbila I.P. Respiratory fluoroquinolones for the treatment of community acquired pneumonia: ame-analysis of randomized controlled trials" American J. Clin. Medi. 2008; 179 (12): 1269-77.
15. Murray R... Porphyrins and bile pigments. In: R.K. Murray DK, Granner PA. Mayes VW, Rodwell (eds), harpers biochemistry, 26th ed. New York: The McGraw-Hill Companies Inc. 2006; Pp. 270- 285.

16. Stacy J. Childs M.D. Safety of fluoroquinolone antibiotics: focus on molecular structure. Infectious Urology (Usa: FQ Research). 2000; 13 (1): 3-10.
17. Cann H.M. Verhulst H.L. Fatal acute chloroquine poisoning in children. Pediatrics. 2007; 27 (1): 95-102.
18. Kaplan A, et al. Lipids. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton. 1998; 918-919.
19. Naito H.K. 1984. Cholesterol. Kaplan A, et al. Clin Chem The CV. Mosby Co. St Louis. Toronto. Princeton. 1194-11206 and 437.
20. Grove T.H. Effect of reagent pH on Determination of HDL Cholesterol by precipitation with Sodium Phosphotungstate-magnesium. Clin Chem. 1979; 25: 560.
21. Okada M. et al. Low-density lipoprotein cholesterol can be chemically measured J. Lab. Clin. Mad. 1998; 132: 195-201.
22. Buccolo G, et al. Quantitative determination of serum triglycerides by use of enzimes. Clin Chem. 1973; 19 (5): 476-482.
23. Lawn R.M. Lipoprotein in heart disease. Scientific American. 1992; 266: 26-32.
24. Ganong W.F. Energy balance, metabolism and nutrition. In Review of Medical Physiology (13th ed). New York: Lange medical publications: 1987; 229-261
25. Vijayalekshmi K.S. Leelamma A.S. Mechanism of hypercholesterolemia produced by some antibiotics. India J. Clin. Biochem. 1991; 6. 31-38.
26. Tasduq S.A. Peerzada K. Koul S. Bhat R. Johri R.K. Biochemical manifestations of anti-tuberculosis drugs induced hepatotoxicity and the effect of Silymarin. Heptol. Res. 2005 31: 132-135.
27. Santhosh S. Sini T.K. Anandan R. Mathew P.T. Effect of chitosan supplementation on antitubercular drugs-induced hepatotoxicity in rats. Toxicology. 2006; 219: 53-59.
28. Leningher A. L. Nelson D. L. Cox M. M. Principles of Biochemistry. CBS Publishers and distributor. 1992.
29. Brown M.S. Goldstein J.L. A receptor mediated pathway for cholesterol homeostasis. Science, 1986; 232: 34-47.
30. Machado A.L.D. Brandão A.A.H. da Silva C.M.O.M da Rocha R.F. Influence of tetracycline in the hepatic and renal development of rat's offspring. Braz. Arch. Biol. Technol. Int. J. 2003; 46 (1): 47-51.
31. Shabana M.B. Hania M. I. Soheir E.M. KhadreMarwa G. Influence of rifampicin and tetracycline administration on some biochemical and histological parameters in albino rats. The J. Bas. Appl. 2012; Zool. 65 (5). 299-308.
32. Liu, S. L.; Esposti, S. D.; Yao, T.; Diehl, A. M. and Zern, M. A. Vitamin E therapy of acute CCl4- induced hepatic injury in mice is associated with inhibition of nuclear factor kappa B binding. Hepatolo. 1995; 22: 1474-1481.
33. McRae, M. Vitamin C supplementation lowers serum low-density lipoprotein cholesterol and triglycerides: a meta-analysis of 13 randomized controlled trials. J. Chiropractic Med. 2008; 7: 48-54.
34. Brude, I. R.; Drevon, C. A.; Hjermann, I.; Sljeflot, I.; Lund-Katz, S.; Saarem, K.; Sandstad. B.; Solvoll, K.; Halvorsen, B.; Arnesen, H. and Nenseter, M. S. Peroxidation of LDL from combined-hyperlipidemic male smokers supplied with -3 fatty acids and antioxidants. Arteriorscler. Thromb. Vasc. Biol. 1997; 17: 2576-2588.
35. Diaz, M. N.; Frei, B.; Vita, J. A. and Keaney, J. F. Antioxidants and atherosclerotic heart disease. N. Engl. J. Med. 1997; 337: 408-416.
36. Sutken, E.; Inal, M. and Ozdemir, F. Effect of vitamin E and gemfibrozil on lipid profiles, lipid peroxidation and antioxidant status in the elderly and young hyperlipidemic subjects. Saudi Med. J. 2006; 27: 453-459.
37. Meertens, L.; Ruido, T.; Diaz, N.; Naddaf, G.; Rodriguez, A. and Solano, L. Relationship between serum lipids and status of vitamin C and E as antioxidants in Venezuelan elderly

people. Arch. Latinoam. Nutr. 2008; 58: 363-370.
38. Munsted, T., Hoffmann, S., Haueschild, A.; Bulte, M.; Georg, V. and Hackethal, R..Effect of honey on serum cholesterol and lipidvalues. J Med Food. 2009; 12: 6 24-628.
39. Mushtaq,R.; Mushtaq, R. and Khan, Z. T. Effects of Natural Honey on Lipid Profile and Body Weight in Normal Weight and Obese Adults: A Randomized Clinical Trial. Pak J. Zool., vol. 2011; 43 (1): 161-169.
40. Nawazish, I. H.; Shamas, A.; Amir, A.; Maqsood, A.; Farrakhzia, K. and Bashr, A. Comparative study of Antioxidant Vitamins and Simvastatin in Hypercholesterolimic Rabbits. Pak. J. Pharm. Sci. 2011; 24(4): 479-484.
41. Al-Jowari, S A A. Protective effect of vitamin E against acetaminophen induced hyperlipidemia in female rabbits. Iraqi J.Sci. 2011; 52 (3): 300-305.
42. Ukpanukpong, R. U.; Eteng, M. U.; Dasofunjo, K.; Pekene, D. B.; Utu-Baku, B. A. and Onyeama, H. P. Haematological studies of antioxidant vitamins C, E and garlic on Pefloxacin Induced Toxicity in Wistar Rats. J. App. Pharm. Sci. 2013; 3 (1): 103-107.
43. Stephen N.G. Parson A. Schofield. Randomized control trial of vitamin E in patients with coronary disease: Cambridge heart antioxidant study (chads). Lancet. 1995; 347:781-105.
44. Manimegalai, R.; Geetha, A. and Rajalakshmi, K. Effect of vitamin E on high fat diet induced hyperlipidemia in rats. Indian J. Exp. Biol. 1997; 17: 2576-2588.
45. Odeh, R. M. and Cornish, L.A. Natural antioxidants for the prevention of atherosclerosis. Pharmacotherapy. 1995; 15: 648-659.