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EVALUATION OF LIPID PROFILE LEVEL IN PENICILLIN-INDUCED GUINEA PIGS TREATED WITH ANTIOXIDANT SIDR HONEY AND VITAMIN A, C AND E

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ABSTRACT

Aim: The present study investigated the effect of supplementation of Sidr 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 weighted 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 intraperitoneally (i.p.) injected with penicillin 50000IU/kg, animals in group 3-7 were administrated orally with Sidr honey 600mg/kg, vitamin A 10000IU/kg, vitamin C 100mg/kg, vitamin E 100mg/kg and vitamins A, C and E respectively, animals in group 8-12 in addition penicillin were orally administrated with Sidr 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 Sidr 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 Sidr honey, vitamins A, C and E on guinea pigs.

Key word: Penicillin, Sidr Honey, Vitamin A, C and E, Lipid profile

1. 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 Nonsteroidal anti-inflammatory drugs (NSAIDs), list on the top of the 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 the liver [6-7], Kedney [8-9], reproductive tissues [10-11], 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 atherosclerosis, 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]. The primary function of vitamin C is for the production of collagen, which forms the basis for connective tissues in bones, teeth and cartilage [17].

This study was conducted to investigate the effect of supplementation of Sidr honey and vitamin A, C and E on penicillin-induced guinea pigs by lipid profile level.

MATERIALS AND METHODS

Animals:

For this experiment, eighty 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 Science- Sana'a University, under standard conditions at room temperature, fed a standard laboratory diet and water *ad libitum*. Animals were allowed to acclimatize to the laboratory environment for 30 days. 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 [18], and were approved by the Animal Experiments Local Ethics Committee at the Zoo, Sana'a- Yemen.

Materials:-

Sidr honey (*Ziziphus spina-christi* honey) was obtained from a 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).

Experimental Animals:-

Ninety-five adult male guinea pigs were divided randomly into 9 groups. Penicillin, Sidr 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. Treatment groups were as follows:

Group 1: 10 animals were served as controls and received orally with distilled

water (3ml/kg) and were orally received 0.5 corn oil.

Group 2: 5 animals were administered orally with Sidr honey (600mg/kg body weight), dissolved in distilled water, daily for 30 days [19-20].

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

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

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

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

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

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

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

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

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

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

Collection the blood and tissue

After 24 h of the 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

Estimation of Total lipids:-

Serum total lipid was assayed according to the method of Kaplan [21].

Estimation of Total Cholesterol:-

Serum total cholesterol was assayed according to the method of Naito [22].

Estimation of high density lipoprotein-cholesterol (HDL-C):-

Serum high density lipoprotein-cholesterol (HDL-C) was assayed according to the method of Grove [23].

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 [24].

Estimation of Triglycerides:-

Serum triglycerides was assayed according to the method of Buccolo et al [25].

Statistical analysis:

The data were analyzed 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. Differences were considered significant if $p< 0.01$.

2 RESULTS

Results in table1 show that the (i.p.) Administration of penicillin in dose (50000 IU/kg B.w.) / Day for the period of 30 days. (Group2), resulted in the 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 Sidr honey in a single dose (600ml/kg B.w.) / Day period of 30 days (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 (10000IU/kg B.w.) / Day period of 30 days (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 (100mg/kg B.w.) / Day period of 30 days (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 (10000IU/kg) + vitamin C in a single dose 100mg/kg + vitamin E in a single dose (100mg/kg B.w.) / Day period of 30 days (Group6), 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 in table 2 show that the (i.p.) Administration of penicillin in dose (50000IU/kg B.w.) / Day period of 30 days (Group7), resulted in highly significant at $P<0.01$ 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 Sidr honey in dose (600mg/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 (10000IU/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 doses (100mg/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). Results showed that vitamin E significantly (P<0.01) reduced the toxicity of penicillin, where administration of vitamin E in dose (100mg/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 (10000IU/kg) + vitamin C in dose (100mg/kg) + vitamin E in dose (100mg/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. 1: Effect of the Penicillin, Sidr honey, vitamin A, vitamin C, vitamin E, vitamin A,C,E on the lipid profile

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 F-Value (df=54)	15.08 P<0.01 Sig		21.69 P<0.01 Sig		31.63 P<0.01 Sig		21.69 P<0.01 Sig		21.76 P<0.01 Sig	

The values are given as a 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: Effect of supplementation with Sidr honey and vitamin A, C and E on lipid 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%
p + 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%
p +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%
p +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%
p +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%
p +Vit A,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 F-Value (df=54)	15.08 P<0.01 Sig		21.69 P<0.01 Sig		31.63 P<0.01 Sig		21.69 P<0.01 Sig		21.76 P<0.01 Sig	

The values are given as a 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
P= penicillin

DISCUSSION:

The total cholesterol, triglycerides, LDL-C, and total lipid concentration have been proved to be indicateors in the diagnosis of some clinical conditions such as hepatitis, chronic obstructive jaundice and coronary heart disease precipitated by atherosclerosis with attendant hyperglycemia [26]. Among the serum lipid fraction, total cholesterol is the most implicated and predominant constituent of atherogenic plaque [27].

The results of the present inadequate 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 and Leelamma [28], 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 were noticed. Tasduq *et al.* (2007) demonstrated a significant increase in triglycerides and cholesterol levels of rats after administration of rifampicin for 30 days. Santhosh et al. [30] 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 the liver and cholesterol ester hydrolase following to the increase levels of triglycerides and LDL [31]. The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and cell membranes [32].

Machado *et al.* [33] 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 [28] 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. The 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 the cell membrane into the blood stream [34].

Antioxidants reductive effect on total serum cholesterol and/or in decreasing atherosclerosis and preventing cardiovascular diseases have been demonstrated in human and animal models [35-36]. Antioxidants have been shown to reduce or delay the progression of atherosclerosis, thus preventing the cardiovascular diseases [37-39].

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 difference when compared with the control. Our results are in agreement with many studies [40-43] reported that honey exhibited a decrease in serum total cholesterol, LDL-C and triglycerides concentrations and increase in HDL-C, and with [44] who reported that vitamin A, C and E individually and in combination exhibited a decrease in serum total cholesterol, LDL-C and triglyceride concentrations and increase in HDL-C. McRae [36] who reported that vitamin C supplementation resulted in a significant reduction in both LDL-C and triglycerides. Also our result in agreement with Ukpanukpong et al. [34], who showed the 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 [45]. 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 [46-47]. Moreover, a combination of vitamin C, vitamin E has been shown to prevent the LDL oxidation, which is thought to lead towards atherosclerosis [48]. 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 (Odeh *et al.,* 1995). HDL also inhibits 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 [48].

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