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ANTIOXIDANT PROTECTIVE EFFECT OF VITAMIN E IN PENICILLIN AND STREPTOMYCIN-INDUCED HEPATOTOXICITY IN GUINEA PIG

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ABSTRACT

This study aims to evaluate the protective effect of vitamin E as antioxidant against penicillin and streptomycin-induced hepatotoxicity in guinea pigs. A total of sixty adult male guinea pigs weighing 800-900g were divided into six groups of ten guinea pigs each, and the experiment lasted 30 days. Group I served as control, group 2 were administrated orally with Vitamin E 100 mg/kg, group 3 were (i.p.) injected with penicillin 50000 IU/kg, group 4 in addition penicillin were orally administrated with Vitamin E 100 mg/kg, group 5 were (i.p.) injected with streptomycin 50 mg/kg, and group 6 in addition penicillin were orally administrated with Vitamin E 100 mg/kg. The result showed a significant increase in the levels of AST, ALT and ALP. And a significant decrease in the levels of total protein and albumin in penicillin and streptomycin treated groups, as well as there were histopathological changes in the liver of these groups when compared to the control. Moreover, groups exposed to penicillin and streptomycin with vitamin E showed significant reduction in AST, ALT and ALP with an increase in total protein and albumin levels relative to penicillin and streptomycin treated groups . Histopathologically, administration of vitamin E improve the degenerative changes in liver, the structure of liver and hepatocytes appearance were more or less similar to control group as well its function. The present results indicate that Vitamin E may play an important role as cytoprotective and pave the way for further studies on the possible use of Vitamin E.

Keywords: hepatoprotictive, vitamin E, penicillin, streptomycin, biochemical and histological study.

1. INTRODUCTION

Bacterial infections are one of the leading infectious diseases confronting public health, and the antibacterial therapy remains relevant in treatment and control of such infections especially in developing countries [1].

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 causes of drug induced many side effects [2].

Penicillin and streptomycin have long been used in antibacterial therapy [3]. The side effects which associate with the therapy by penicillin and streptomycin are mainly due the generation of an excessive amount of reactive oxygen species (ROS), resulting in the detrimental effects of the cellular antioxidant defense system, as well as, enhancement of the lipid peroxidation (LPO) process [4-8].

Reactive oxygen species (ROS) are an inevitable byproduct of cellular respiration causing oxidation of lipids, nucleic acids, and proteins. The (ROS) damage is an underlying cause of disease, including cancer, inflammatory, and neurodegenerative diseases [9-11], hepatotoxicity [12,8].

Antioxidants protect key cell components from damage by neutralizing the free radicals [13]. Antioxidants that occur naturally in the body or are consumed through the diet may block damage to cells [14].

Therefore, supplementation of antioxidants can be considered as the alternative method to reduce such alterations. In fact, several studies demonstrated that the cellular antioxidant an activity is reinforced by the presence of dietary antioxidants [15]. Accordingly, interest has recently grown in the role of natural antioxidants used as a strategy to prevent oxidative damage as a factor in the pathophysiology of various health disorders [16].

Among antioxidants, the vitamin E is the primary liposoluble antioxidant, which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes, thus maintaining its permeability [17,18]. Vitamin E may also affect oxidative changes which occur in other cell organelles [19]. Moreover, it is known that antioxidants, such as vitamin E, coenzyme Q, vitamin C (Vit C), glutathione (GSH) and selenium may act synergically, preventing lipid peroxidation and cell destruction [18,20 -22].

Many studies of the different indicate to protective effects of vitamin E against many alteration caused by organophosphate insecticides and some medicines that induced hepatotoxicity [23-26]. We have not found in the previous literatures any study on protective effect of vitamin E against penicillin and streptomycin-induced hepatotoxicity. The goal of this study aims to evaluate the protective effect of vitamin E as

antioxidant against penicillin and streptomycin-induced hepatotoxicity in guinea pigs.

2. MATERIALS AND METHODS

2.1. Chemicals

Vitamin E (DL-a- tocopherolacetate; purity 99%), white crystal powder, was supplied by Merck (Germany), Penicillin, Streptomycin was purchased from (Ave Group-USA-Colombia-Mexico), Diagnostic kits for the aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and albumin. All other chemicals and reagents were of highest purity commercially available.

2.2. Experimental animals

60 healthy adult male guinea pigs (weighing 800 - 900 g), were obtained from the zoo, Sana'a- Yemen. Animals were housed in the animal house- Department of Biology- Faculty of Science- Sana'a University, under standard conditions in room temperature. Animals were allowed to acclimatize to the laboratory environment for 30 days. The animals were feeding fresh grass hay, alfalfais, legume, cabbage, carrot, celery and spinach as recommended by HCDGP [27], GPCS [28] and tap water ad libitum. Subsequently the animals were randomly divided to 6 groups as follows:

Group1: 10 animals were orally given 0.5 ml. normal corn oil once a day period of 30 days.

Group2: 10 animals were orally given vitamin E in a daily single dose 100 mg/kg b.w. period of 30 days. Vitamin E was dissolved in corn oil.

Group3: 10 animals were intraperitoneally (i.p.) injected with penicillin in a daily single dose 50000 IU/kg b.w. period of 30 days. Penicillin was dissolved in distilled water.

Group4: 10 animals were i.p. injected with penicillin in a daily single dose 50000 IU/kg b.w. and orally treated with a vitamin E in a daily single dose 100 mg/kg b.w. period of 30 days.

Group5: 10 animals were i.p. injected with Streptomycin in a daily single dose 50 mg/kg b.w. period of 30 days. Streptomycin was dissolved in distilled water.

Group6: 10 animals were i.p. injected with streptomycin in a daily single dose 50 mg/kg b.w. and orally treated with vitamin E in a daily single dose 100 mg/kg b.w. period of 30 days.

The selected dose of Penicillin was based according to [24, 25], the selected dose of Streptomycin was based according to [25, 26], the selected dose of Vitamin E was based according to [27, 28].

After 30 day of treatment, guinea pig in all group were fasted overnight for 12h, and sacrificed and dissected under ether anaesthesia, the blood was immediately collected and centrifuged, and serum was discarded and kept at - 21ºC for the biochemical tests. The liver tissues were removed as small pieces and then were washed with normal saline to remove residual blood and then were fixed by using a 10% neutral formalin fixation for 24 hours, then washed by the running tap water and stored in 70% ethyl alcohol at room temperature, until further processing.

2.3. Estimation of liver function

2.3.1. Alanine-aminotransferase (ALT) and asparatate-aminotransferase (AST) assay

The estimation was carried out according to the method originally developed by Reitman and Frankel [29].

2.3.2. Alkaline phosphatase assay

ALP was determined using a colorimetric method as described by Kind and King [30].

2.3.3. Total protein assay

The total protein was determined by Biuret method explained by Tietz [31].

2.3.4. Albumin assay

Serum albumin was determined according to the method of Doumas *et al*., [32].

2.4. Histological studies

The liver of each guinea pig were removed. After the organs were removed, they were fixed by using a 10% neutral formalin fixation for 24 hours. The fixed tissues were dehydrated in series of alcohol concentrations 70%, 80%, 90% and 100%. The dehydrated tissues were then cleared by using xylain as clearing agents. Then the cleared tissues were embedded in paraffin wax at 60°C. Blocks were cut at 5mm thick and stained with hematoxylin and eosin [33].

2.5. Statistical analysis

The statistical analysis was performed by SPSS; continuous data are expressed as mean ±S.E. Data were compared using one - way ANOVA. P value <0.01 was considered to be statistically significant. Post hoc analysis of grope differences was performed by LSD test. The treated groups were compared both with each other and with untreated control groups.

Table-1. Statistical analysis of result of liver function tests after 30 days of penicillin and vitamin E administration in dose 50000 IU/kg and 100 mg/kg, respectively.

Parameter / Groups	AST U/L		ALT U/L		ALP U/L		Total protein g/dl		Albumin g/dl	
	M±SD	Change	M±SD	Change	M±SD	Change	M±SD	Change	M±SD	Change
Control	21.95±1.7	-----	27.41±1.8	------	52.91±2.2	------	7.52±0.28	------	3.78±0.15	------
Vitamin E	22.23±1.5[a]	1.3%	27.99±1.6[a]	2.1%	52.15±1.6[a]	2.3%	7.67±0.13[a]	2%	3.86±0.13[a]	2.1%
Penicillin	46.88±4.9[C]	113.6%	63.06±5.6[C]	130.1%	78.06±4.3[C]	47.5%	6.03±0.45[C]	19.8%	2.96±0.18[C]	21.7%
P+Vitamin E	24.94±2.9[a]	13.6%	31.44±2.2[b]	14.7%	54.59±3.2[a]	3.2%	7.35±0.36[a]	2.3%	3.64±0.12[a]	3.7%
ANOVA F-Value (df=34)	146.78 P<0.01 Sig		251.23 P<0.01 Sig		164.72 P<0.01 Sig		43.87 P<0.01 Sig		79.64 P<0.01 Sig	

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.

3. RESULTS

3.1. Biochemical results

Results in Table-1 show that the (i.p) administration of Penicillin in a single dose 50000 IU/kg b.w. per day period of 30 day (Group-3). resulted in high significant P<0.01 increase in the level of AST, ALT and ALP, as compared to control (Group-1), Penicillin i.p. administration resulted also in high significant P<0.01 decrease in the level of albumin and total protein, as compared to the control (Group-1).

The administration of Vitamin E in a single dose 100 ml/kg b.w. per day period of 30 day (Group-2). Resulted in non significant P<0.01 change in the level of AST, ALT, ALP, albumin and total protein, as compared to the control (Group-1).

Results 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. per day (Group-4) beside Penicillin, resulted in non significant P<0.01 change in the level of AST, ALT, ALP, albumin and total protein, as compared to the control.

Table-2. Statistical analysis of result of liver function tests after 30 days of streptomycin and vitamin E administration in dose 50 mg/kg and 600 mg/kg, respectively.

Parameter / Groups	AST U/L		ALT U/L		ALP U/L		Total protein g/dl		Albumin g/dl	
	M±SD	Change	M±SD	Change	M±SD	Change	M±SD	Change	M±SD	Change
Control	21.95±1.7	-----	27.41±1.8	------	52.91±2.2	------	7.52±0.28	------	3.78±0.15	------
Vitamin E	22.23±1.5[a]	1.3%	27.99±1.6[a]	2.1%	52.15±1.6[a]	2.3%	7.67±0.13[a]	2%	3.86±0.13[a]	2.1%
Streptomycin	60.35±6.8[C]	174.9%	79.36±3.5[C]	189.5%	88.46±3.4[C]	67.2%	5.44±0.35[C]	27.7%	2.29±0.12[C]	39.4%
S+Vitamin E	27.39±3.7[b]	24.48%	34.37±4.3[b]	25.4%	56.93±4.0[a]	7.6%	7.27±0.37[a]	3.3%	3.20±0.17[a]	15.3%
ANOVA F-Value (df=34)	198.43 P<0.01 Sig		276.08 P<0.01 Sig		301.03 P<0.01 Sig		126.67 P<0.01 Sig		232.68 P<0.01 Sig	

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.

Results in Table-2 show that the (i.p) administration of Streptomycin in a single dose 50 mg/kg b.w. per day period of 30 day (Group-5). resulted in high significant P<0.01 increase in the level of AST, ALT and ALP, as compared to control (Group-1), Penicillin i.p. administration resulted also in high significant P<0.01 decrease in the level of albumin and total protein, as compared to the control (Group-1).

Results showed that Vitamin E significantly (P<0.01) reduced the toxicity of Streptomycin, where administration of Vitamin E in dose 100 mg/kg b.w. per day (Group-6) beside Streptomycin, resulted in non significant P<0.01 change in the level of AST, ALT, ALP, albumin and total protein, as compared to the control.

3.2. Histological results

The control livers show normal lobular architecture with central vein and radiating cords of hepatocytes, separated by blood sinusoids. Hepatocytes are large and polyhedral in shape with slightly acidophilic

granular cytoplasm. They have large, rounded, vesicular nuclei with prominent nucleoli (Figures 1, 2).

The liver cells of group 3, 5 animals showed obvious histological changes, in the form of distortion in the hepatic organization, dilatation and congestion of the blood sinusoids and central vein, infeltration, heamorrhage, congestion, inflammation, metaplasia, hyperplasia, hypertrophy, necrosis, vasodilutation, thickening in the central vein, some hepatocytes showed signs of degeneration in the form of hypertrophy with highly vacuolated cytoplasm and deeply stained nuclei. Other hepatocytes exhibited hyalinized cytoplasm with pale nuclei and prominent nucleoli (Figures 3, 5).

The liver cells of group 4,6 appeared more or less similar to those of the control apart from few hepatocytes appeared with vacuolated cytoplasm and pyknotic nuclei (Figures 4, 6).

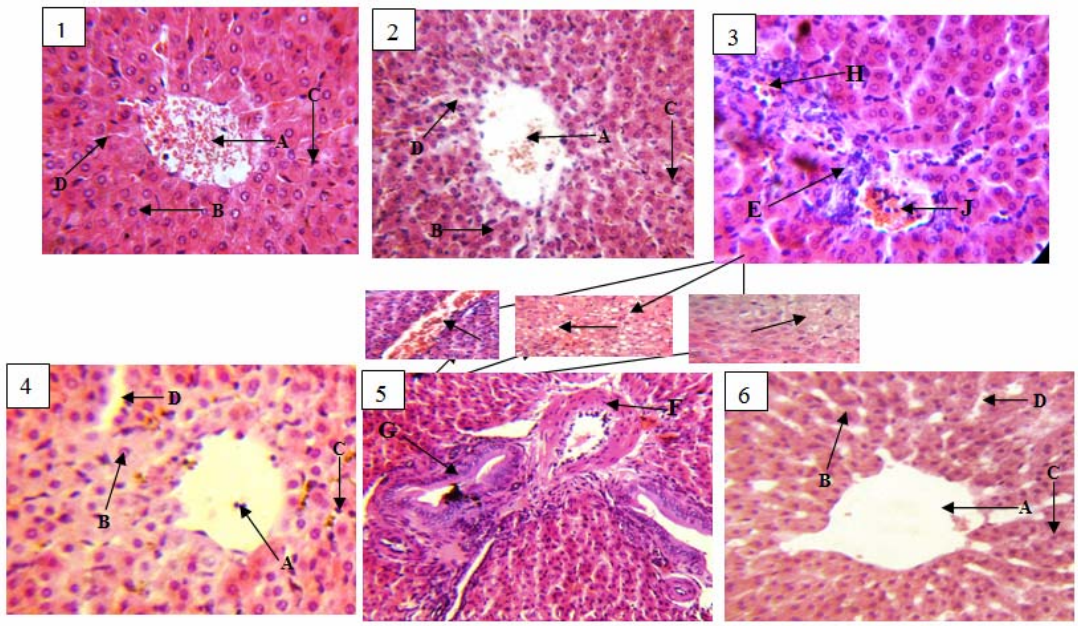

Figure-1-6. Hepatoprotective effect of Vitamin E against Penicillin and Streptomycin-Induced Hepatotoxicity in Guinea pig. Liver sections were stained H and E. (1) Control; (2) Vitamin E (100mg/kg b.w); (3) Penicillin- treated animals (50000 IU/kg b.w); (4) Penicillin and Vitamin E (50000 IU/kg b.w and 100 mg/kg b.w); (5) Streptomycin- treated animals (50 mg/kg b.w); (6) Streptomycin and Vitamin E (50mg/kg b.w and 100 mg/kg b.w); (A)Central vein; (B) Hepatocytes; (C) Kupver cells; (D) Sinusoids; (E) Infeltration; (F) Thickning in the central vein; (G) Metaplasia; (H) Heamorrhage; (I) Vasodilutation; (J) Congestion; (Q) Hypertrophy; (N) Necrosis; (M) Odema.

4. DISCUSSIONS

ALT,AST, ALP, total proten and albumin are the most sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity [39-41]. In Our study. Administering penicillin and streptomycin to guinea pigs resulted in a statistically highly significant increase the enzymes AST, ALT and ALP in the serum of the guinea pigs injected of penicillin or streptomycin only compared with the control group. These results may indicate to degenerative changes and hypofunction of liver [42-44] as well as hepatic cell necrosis [45] which increase the releasing of these enzymes in the blood stream [46]. Elevated levels of these enzymes in the serum are presumptive markers of drug-induced necrotic lesions in the hepatocytes [45]. The enhanced susceptibility of hepatocyte cell membrane to drug-induced peroxidative damage might have resulted in an increase releasing of these diagnostic marker enzymes into the systemic circulation. An increase in the AST and ALT levels indicates a reversible change of the cell membrane permeability [47]. Our observations are highly supported by the other studies which suggest effect penicillin and streptomycin on liver function tests [29,31,48-51].

In this study also. Administering penicillin and streptomycin to guinea pigs resulted in a statistically highly significant decrease the level total protein and albumin in the serum of the guinea pigs injected of penicillin or streptomycin only compared with the control group. The reduction of total protein and albumin levels indicates that the administration of drugs has caused an impairment of liver function, e.g. its capacity to synthesize albumin from the hepatic parenchyma. [52] Khan et al. [52] reported that there was a differential binding of penicillin with serum albumin, while [53] Shen et al. [53] observed that albumin secretion of gel entrapped hepatocytes was reduced by penicillin. The decrement of

alpha 1-globulin in the serum of streptomycin-administrated animals could be due to liver dysfunction which affects the synthesis of alpha protein fractions in the liver. The increment of gamma-globulin level in the serum of tetracycline-treated animals may be due to hyperplasia of the reticulo-plasmic tissue of the bone marrow induced by penicillin administration [54]. Our results are in agreement with [29,49,49,55,56].

The mechanism of penicillins and aminoglycosides induced hepatotoxicity is found to be mediated through oxidative stress by free radical that cause damage to hepatocytes [57-60], AST,ALT and ALP increases in hepatic damage due to leakage of enzymes from damaged hepatocytes into vascular compartment. Liver damage leads to decrease in synthetic capability leading to fall in serum total protein and albumin levels [60].

Antioxidants can prevent cell damage due to the action of ROS and free radicals [61]. The antioxidant activities are related to a number of different mechanisms, such as free redical- scavenging, hydrogen- donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyle [62].

Vitamin E has a protective role against the side effects of antibiotics (penicillin and streptomycin) in liver as demonstrated by the improvement in the tested biochemical parameters. Administration of vitamin E to guinea pigs beside penicillin or streptomycin highly significant decrease in AST, ALT and ALP and an significantly increase in total protein and albumin levels compared to penicillin or streptomycin groups. This indicates that vitamin E administration prevented liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes through membranes, exhibiting hepatoprotective activity. This might be the main reason for the restoration in the activities of the marker enzymes during administration of honey oxidative damage in a cell or tissue which occurs when the concentration of ROS (O2, H2O2, and OH) generated exceeds the antioxidant capability of free radical scavenger [63]. Similar results by several experimental studies have shown that vitamins E could ameliorate toxicity of the many of chemicals and drugs [33,63-66].

The present investigation clearly demonstrated that the injection of penicillin and streptomycin antibiotics to guinea pigs have induced conspicuous alteration in the histological structure on the liver tissue in treated guinea pigs. These changes included dilatation and marked congestion of the hepatic vascularities (central veins, blood sinusoids and branches of the portal vein), cytoplasmic vacuolation, degeneration, infeltration, congestion, necrosis and karyolysis of hepatic cells as well as hyperplasia of endothelial. In addition, Infeltration, thickning in the central vein, metaplasia, heamorrhage, vasodilutation, hypertrophy and Odema. Our results are in agreement with [48,55,67].

Histopathological changes in liver cells following injection of penicillin and streptomycin were the marked changes occurring in the liver in this study. This feature could be explained according the suggestion both of [48,6568,69] they reported that histopathological changes in liver cells due to free radical generating and free radical scavenging enzymes may be disturbed and leading to disrupt signal transduction pathway and increases the cellular permeability by acting on membrane phospholipids, resulting into a significant hepatic tissue injury.

Dilatation and marker congestion of the hepatic vasculature of liver tissue which were noticed in the present investigation may be due the failure of the heart which produces changes in different organs via two ways. Firstly, excessive blood in venous system increases blood pressure in the veins and capillaries which may exert undue pressure on the neighboring structures. Secondly, this is usually accompanied be a diminished blood supply, thus become subjected to malnutrition, deficient oxygenation and the accumulation of excretory and metabolic products [70].

Interpretation of vacuolar formation following chemical treatments has been subjected to wide speculation by many investigators. (Robbins and Angell [71]. regarded such vacuolation to represent primary morphologic response to many froms of cell injury. They also attributed it to the noxious effects of treatment on the cell membranes, both structurally and functionally, causing market disturbances in its permeability system. This presumably leads to enhanced imbibition of water into the cells. When it sufficiently accumulates in the cells, such intracellular water produced clear cytoplasmic vacuoles indication the occurrence of the pathologic symptoms commonly referred to as hydropic degeneration or fatty degeneration caused by lipid abundance in such instance.

Other authors are of the opinion that cytoplasmic vacuolation is most probably brought about by the increase of lysosome elementsm [72]. The lysosomes contain hydrolytic enzymes, when these organelles are disrupted under cetain pathological conditions: they liberate their powerful enzymes, which bring about considerable autolysis of various cellular parts [73].

Necrosis and degeneration of the hepatic cells following injection of penicillin and streptomycin were the marked changes occurring in the liver in this study. This feature could be explained according the suggestion of Curran [74] who reported that liver cells necrosis may be either due to progressive degenerative action of intracellular enzymes of the injured cells or to a metabolic disturbance and inhibition of synthesis needed of DNA and hence protein synthesis for the growth and maturation of the liver.

The present histological study showed that of viamin E reduced the cellular changes induced by penicillin or streptomycin, indicating that vitamin E contributed to the protection against penicillin or streptomycin induced liver toxicity. Our observations are highly supported by the other studies which suggest that vitamin C exert their protective effects against some

antibiotics (rifampicin, cisplatin, isoniazid and pefloxacin)-induced hepatotoxicity [64,65,75-77]. On hypothesis to explain the beneficial effects of vitamin C in ameliorating histological changes is that vitamin E is the antioxidant and protects cellular membranes and lipoproteins against peroxidation [64,65], and would effectively scavenge free radicals within cells where reactive metabolites are being produced.

In conclusion, we suggest that Vitamin E supplementation may give beneficial results in the prevention of hepatic damage induced by the use of antibiotics (penicillin and streptomycin).

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