

Protective Effects of Vitamin A on Paracetamol-Induced Hepatotoxicity in Rats

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Abstract: Paracetamol which is widely used as anti-inflammatory drug and pain killer is known to cause liver injury. Vitamins A and E are among the antioxidants vitamins that have attracted the attention of biochemical and toxicological researchers in the recent times as vitamin A is reported to enhance a marked reduction in CCl₄-induced hepatic damage, this study was aiming to investigate the hepatoprotective effect of vitamin A (retinol) against paracetamol-induced toxicity in Wister rats. Five groups of rats were used in a seven days experiment; The control group received no treatment, the negative control group has received single dose of PCM (1000mg/kg, orally) on day 7 and groups 3, 4, and 5 received oral doses of vitamin A of 100IU/kg, 500IU/kg, and 1000 IU/kg, respectively for 7 days and in the last day (7th day) they received a single dose of PCM of 1000mg/kg/orally. Animals were sacrificed in day 8 and blood samples were collected and used for analysis of ALT, AST, albumin, total protein, cholesterol, triacylglycerides and total bilirubin. The group of rats used as negative control showed significant elevation in serum ALT, AST, cholesterol and bilirubin levels when compared with the control group. A dose of 1000 IU/kg vitamin A has showed protective effects against PCM induced hepatotoxicity.

Keywords: vitamin A, Paracetamol, hepatoprotective,

1. INTRODUCTION

Liver is the principal organ for maintaining the body's internal environment. It plays major functions in the metabolism of carbohydrate, protein, fats and processing and excretion of xenobiotics (1). Overdoses of many medicinal drugs can cause liver injury (2) which lead to elevated levels of aminotransferases and yellowing of the skin, eyes and mucous membranes due to high level of bilirubin(3). Drug-induced liver injury is due to a drug over dose or its toxic metabolite which affect the immune response or alter the biochemistry of the hepatic tissue(4). Paracetamol (PCM) is most popular anti-inflammatory drug used as a pain killer(5). When PCM is taken in overdose it leads to liver toxicity (6) and renal tubular necrosis, which can be lethal in human and animal. Alteration in homeostatic status leads to a shift in the dynamic equilibrium of metabolism toward the ROS generation thereby oxidative stress leading to organ dysfunction(7). Several studies about protection against hepatotoxicity have been performed (8) and up to date there are no completely effective drugs that stimulate hepatic function, that offer complete protection of the organ(9). Some vitamins are known to play an important role in ameliorating the toxicity effects of reactive species generated by chemical

agents in the biological systems. It has been documented that Vitamin C, and Vitamin E minimize the hepatotoxicity (10). Vitamins A and E are among the antioxidants vitamins that have attracted the attention of biochemical and toxicological researchers in the recent times. Vitamin A is reported to enhance a marked reduction in CCl₄-induced hepatic damage (11)

This study was aiming to investigate the hepatoprotective effect of vitamin A (retinol) against paracetamol-induced toxicity in Wister rats by studying the liver function through the AST, ALT, bilirubin, Total protein, Albumin, Cholesterol and Triglyceride level and through liver histology examination.

2. MATERIALS AND METHODS

Drugs:

Paracetamol (PCM): Was obtained from local market, Khartoum, Sudan, Amipharma Laboratories Ltd. It was dissolved in distilled water and administered as a single oral dose of 1000 mg/kg body weight. According to literature, this dose is 10 times the therapeutic dose.

Vitamin "A": Was obtained from local market, Kahira Pharm & Chem.ind.co. It was dissolved in olive oil and given daily as an oral dose of 1000mg/kg.

Animals:

Twenty six apparently healthy, albino Wister male rats with an average weight of 100 gm were selected for the study. The animals were housed in the animal house of Medicinal and Aromatic Plant and Traditional Medicine research Institute under standard laboratory conditions. The rats were given standard rat pellets and water. They were optimized to laboratory conditions for one week before starting the experiment. The study was carried out in the period from November 2016 through February 2017.

Study Design

Rats were randomly divided into five groups: group 1; (control) that comprise 6 rats that sacrificed in day 8 without receiving any kind of treatment, group 2; PCM-treated group, where each rat of the 6 rats in this group, was given tap water for 7 days orally and on the 7th day rats received a single oral dose of 1000 mg/kg body weight of PCM, then sacrificed at day 8. In group 3, 4 and 5, each animal of the group was treated with a single daily 100, 500 and 1000 IU/kg body weight vitamin A orally, respectively, for one week and on day 7 received a single oral dose of PCM 1000mg/kg body weight. At the end of the treatment (day 8), animals were sacrificed and the blood was drawn out from each animal and collected in sterilized plastic plain tubes, and left to stand for 30 minutes at 25°C, then centrifuged at 3000 rpm for 15 minutes and then, the sera were collected and stored at 4°C for measurement of serum ALT, AST, total protein, albumin, triglyceride, cholesterol and total bilirubin using a ready-made kit for this purpose from Biosystems, S.A. Costa. Brava 30, 08030 Barcelona, Spain. After collecting blood samples, the abdominal cavity was immediately opened and the liver was dissected out of each animal, washed with distilled water and fixed in 10% formaldehyde at room temperature, then passed through dehydration, cleaning, embedding with paraffin wax to form paraffin blocks which sectioned to 5 µm thickness using microtome. These sections were stained with Harris hematoxylin and Eosin (H&E) stains for microscopic histological examination.

3. STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences, version 22, and Microsoft Excel software. Results were expressed as mean ± standard deviation or standard error. Independent -Samples T-test was used to compare between groups. P value of ≤ 0.05 is considered significant.

4. RESULTS

1. Biochemicals

Serum levels of AST and ALT in negative control group was found to be significantly higher than in that of the control group (P=0.038 and 0.003, respectively). The levels of serum AST and ALT in 100IU/kg vitamin A-treated group were not significantly different when compared to the enzyme levels in PCM-treated group where p values were 0.355 and 0.399 respectively. The levels of the transaminases in 500IU/kg vitamin A-treated group were clearly declined; particularly the ALT which shows significant low level compared to the negative control, P values for AST and ALT were 0.08 and 0.015, respectively. In the group treated with 1000 IU/Kg of vitamin, the levels of the enzymes AST and ALT were found to be significantly low when compared to the control group where P values were 0.034, 0.006, respectively figure (1). Serum total protein and serum albumin in PCM-treated group were less than that of the control group but the decrease was not significant (P=0.419, 0.733 respectively). Although statistically insignificant, but, in all treated groups there were observable vitamin dose-dependent increase in albumin (P=0.584, 0.654, 0.281) for a dose of 100, 500, and 1000 IU/Kg vitamin, respectively). The comparison between the values of the total protein in the vitamin-treated groups and the negative control group revealed that, the 100, 500 and 1000 IU/Kg of vitamin has observable positive effect but again statistically insignificant; P values were 0.232, 0.144, 0.952 for vitamin doses of 100, 500 and 1000 IU/Kg vitamin respectively (figure 2). Although insignificant, cholesterol concentration is higher in PCM-treated group than in both control group (P = 0.595) and vitamin A-treated groups, (P = 0.301, 0.563, 0.347) for 100, 500 and 1000 IU of vitamin, respectively. Serum triglycerides (TGs) concentration was found to be group significantly higher in control group when compared to that of the PCM-treated group (P=0.03). At low doses of vitamin (100 IU and 500 IU) there was no significant difference in the concentration of TGs compared to the PCM-treated group (P=0.484, 0.859 respectively), whereas at dose 1000 IU of vitamin, there was significant increase in TGs compared to the PCM-treated group (P=0.023) figure (3). The PCM treatment increases the serum bilirubin concentration significantly when compared to the control group (P = 0.044) whereas, the low doses of vitamin A (100 IU/kg and 500 IU/kg) did not show significant effect in decreasing bilirubin concentration induced by PCM (P = 0.261, 0.08 respectively). However, 1000 IU/kg of vitamin A has

decreased the concentration of serum bilirubin induced by PCM significantly ($P=0.008$) figure (4).

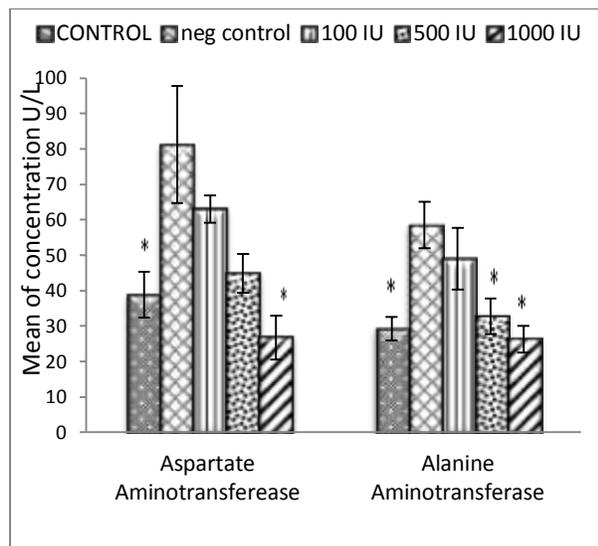


Figure1:Effect of different doses of vitamin A on serum ALT, AST levels of PCM-treated group compared to PCM-treated group (neg. control). *; indicates the significant difference compared to the negative control.

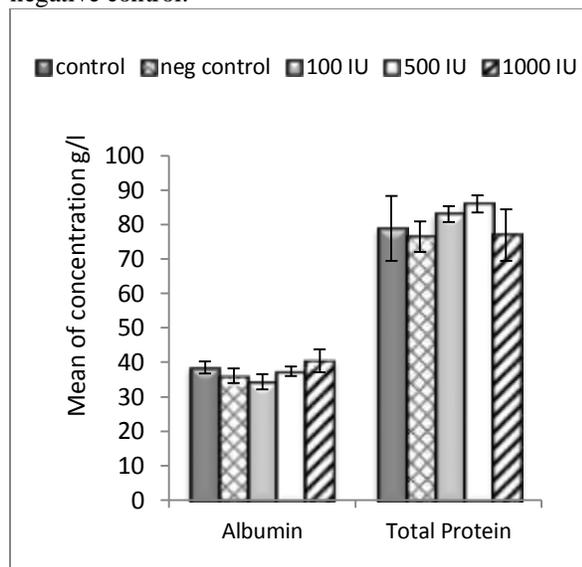


Figure2: Effect of different doses of vitamin A on serum total protein and albumin levels of PCM-treated compared to PCM-treated (neg. control).

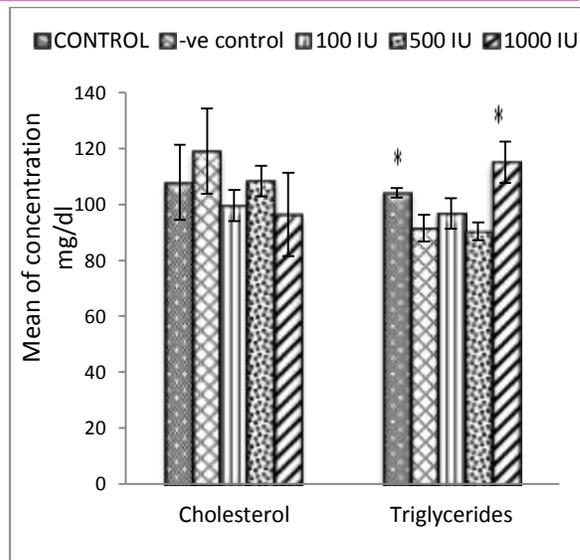


Figure3:Effect of (different doses of vitamin A on serum cholesterol and Triglyceride levels compared to PCM-treated group.*;indicates the significant difference compared to the negative control.

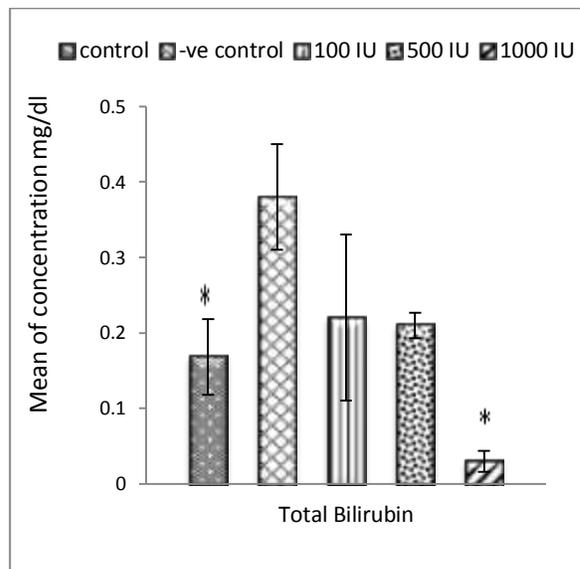


Figure4:Effect of (different doses of vitamin A+PCM) on serum bilirubin levels compared to PCM-induced group (neg. control).*;indicates the significant difference compared to the negative control.

2. Liver histology

As shown in the photomicrograph of the liver section from normal control group stained by H&E (figure 5), the hepatocytes showed normal tissue architecture and cellular outline, the nuclei are normal in shape and size seen around the central vein region (CV) (Figure 5A). While the histopathological examination

of the liver tissue of rats treated with single dose of PCM of 1000mg/kg body weight (figure 5B) showed marked congestion of central vein with hemorrhage, degenerative hepatocytes (destruction of cell wall and absence of nuclei), various degree of cellular necrosis evident by the present of different form of nucleus changes which include: karyopyknosis, karyogensis and karyorrhexis. Liver sections from rats pretreated with vitamin A 1000IU/kg for one week followed by 1000 mg PCM/kg body weight showed a normal view of hepatic tissue with mild degenerative and necrotic changes, some degree of fatty change and some nuclei are variable in size and shape (Figure 5C).

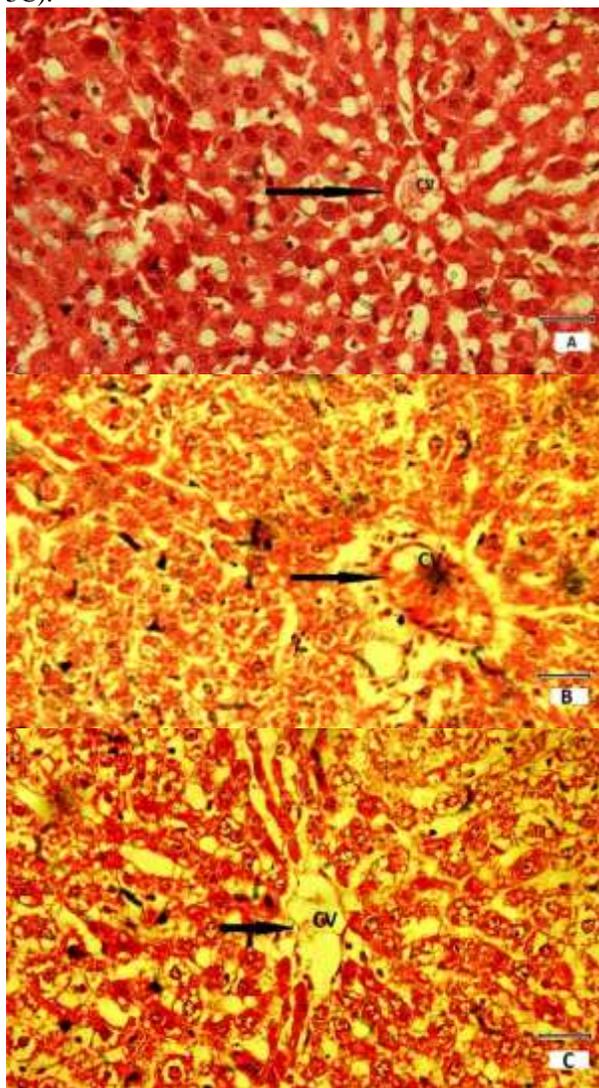


Figure5: A; Normal control group, B; PCM-treated group. C; (vitamin A, 1000 IU/kg + PCM) group.

5. DISCUSSION:

The liver is involved in the biochemical processes such as growth, supplying energy as well as it participate in the metabolism of carbohydrates, proteins and fats, bile acids, and in the storage of vitamins(9). Drug-induced hepatotoxicity is a common cause of liver injury(4). Paracetamol (PCM) hepatotoxicity is due to the reaction of its metabolite which causes oxidative stress and glutathione (GSH) depletion (12). PCM is also shown to directly inhibit cellular proliferation resulting in lipid peroxidation, depletion of ATP, and alteration of homeostasis. All of these changes are considered potentially fatal to the cell (13). The sensitivity of liver to the toxic agents is usually seen in the levels of different enzyme activities such as AST, ALT, and in the levels of total bilirubin and total protein (14). In this study it was observed that giving vitamin A orally at doses of 100, 500, 1000 IU/kg, on daily basis resulted in a dose-dependent decreases of serum aminotransferases compared to the paracetamol-induced liver injury group. This indicated that vitamin A has positive effect in liver cells regeneration. The actual mechanism of decreased levels of the enzymes concentration were not studied but, vitamin A may cause regeneration of damaged hepatocytes through one of the several signaling pathways known to stimulate regeneration in the liver including cytokines, growth factors, hormones, and nuclear receptors, or it may act as scavenger of free radicals that cause liver injury like the action that exerted by tocopherols (15).

It was also observed that PCM has lowering effect of both the total serum protein and albumin. As the liver synthesizes many species of plasma proteins including the Albumin, α globulins, β -globulins, fibrinogens and others, the damaged cells were incapable of synthesizing normal amount of proteins. Although the difference was not significant when compared with the control group but the measured concentrations were observed to be less in the PCM-treated group. In all vitamin A-treated groups, the liver has retained its function and the levels of both albumin and total proteins were increased to the levels of controls. This is clearly indicates that vitamin A pretreatment can prevents the liver from being injured and improve the liver function. As the liver play vital role in the metabolism, conjugation of bilirubin to glucouronic acid and its regulation and secretion into the bile, the PCM treatment in this study was shown to negatively affect this function. As shown in the results section, total bilirubin concentration was significantly increased in PCM group compared to the normal healthy control group.

The damage of the rat hepatocytes as seen in the histopathology slides may be the direct cause of this dysfunction of the hepatocytes, thus, the failure of hepatocytes to conjugate the bilirubin and to excrete it in the bile. On the other hand, the significant decline of serum bilirubin in vitamin A treated groups confirms the hepatoprotective activity of vitamin A which agreed with finding reported by Effiong *et al* that rats exposed to gasoline vapors and treated with retinol has recovered from the hepatotoxicity and retain normal serum bilirubin (17). Both the triacylglycerols (TGs) and cholesterol were affected by both PCM and by vitamin A treatment, where an oral administration of overdose of PCM significantly decreased serum triglyceride ($P=0.03$). This decrease in TGs could be due to hepatotoxicity that has been associated with excessive intake of PCM (18). In all vitamin A-treated groups there were increase in triglyceride concentration and particularly in the group treated with 1000IU/kg ($P = 0.023$). This may be due to increased lipid soluble vitamin A uptake by liver. Although insignificant, there was an increase in the cholesterol levels of the PCM treated group when compared with the normal group. The liver is central to the regulation of cholesterol levels in the body, not only does it synthesize cholesterol for export to other cells but it also removes cholesterol from the body by converting it to bile salts and excreting it into the bile, so, this function may altered or the impaired catabolism of chylomicron remnant gives rise to increase in serum cholesterol. The attenuation in serum total cholesterol concentration observed in the vitamin A treated rats may be due to increase in the serum HDL-cholesterol concentration and TG, because vitamin A supplement was provided with fat, which aids the esterification and packaging of retinol into chylomicron. HDL-cholesterol mobilizes cholesterol into the cells (19).The biochemical results were also proved by histological manifestations. The changes mostly comprise hepatocellular necrosis, fatty accumulation and other histological observations added more verification to the hepatoprotective influence of vitamin A particularly at dose 1000IU/kg.

6. CONCLUSION

The present investigations indicated that vitamin A at all doses exhibits significant barrier against paracetamol-induced toxicity. A dose of 1000IU/kg of vitamin A is more effective than doses of 100 and 500IU/kg of the vitamin. Further investigation is necessary to determine the effect of vitamin A at the molecular level and the mechanism of liver protection against paracetamol toxicity.

7. ACKNOWLEDGEMENT

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