

Effect of Ascorbic acid on Mercury-induced changes on the liver in Adult Wistar Rats.

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Abstract: Mercury is among the heavy metals that have been reported to cause devastating health problems worldwide. The present work was aimed at investigating the effects of mercury chloride and Vitamin C administration on the cyto-architecture of the liver and some liver parameters. Thirty adult Wistar Rats of average weight of 200g were randomly divided into six groups of five rats per group. The animals were administered with different concentrations of mercury chloride orally for three weeks. Group one was the Control and was administered with normal saline, Groups two and three were administered with 52mg/kg body weight and 26.25mg/kg body weight of mercury chloride respectively while Groups four and five were administered with 52mg/kg of mercury chloride +5mg/kg of ascorbic acid and 26.25mg/kg of mercury chloride+5mg/kg of ascorbic acid respectively and Group 6 was administered 5mg/kg of ascorbic acid only. After three weeks of administration, the animals were sacrificed humanely, blood and tissue samples were collected for analysis. The results showed there was significant increase in liver biochemical parameters such as AST, ALT and ALP levels in the serum of treated animals ($p < 0.05$). The result showed that the administration of ascorbic acid resulted in ameliorative effects on biochemical parameters and the effect was most pronounced on the liver of treated rats at higher concentrations of mercuric chloride which was dose dependent.

Keywords: Mercuric Chloride, ascorbic acid, liver, Vitamin C, hepatocytes, Wistar rats.

I. Introduction

Mercury intoxication has been a public health problem for many decades. Consideration of the role of environmental factors in determining the susceptibility to mercury has recently been renewed by evidence from epidemiological studies (Wang, et al., 2007). Many populations Worldwide, have been exposed to doses of mercury through the consumption of fishes and sea foods (Valey et al., 1980; WHO, 2003). Some populations have experienced subsequent neuro-toxic effects and since the epidemic of mercury poisoning from contaminated fish consumption in Minamata, Japan in the late 50s, mercury has been one of the most documented examples of bio-accumulation of toxins in the environment, particularly in the aquatic food chain (ATSDR, 2011). The body accumulates ingested amounts of mercury in the kidney, brain, liver and other tissues including the hair (Burger et al., 2011).

Elemental mercury and its metabolites have the toxic effect of denaturing biological protein, inhibiting enzyme and interrupting membrane transport and the uptake and the release of neurotransmitters (Hesse, 2007; El-Shenawy and Hassan, 2008). Significant exposure to mercury can cause acrodynia involving a pink rash on the extremities, paresthesias and pain (Leong et al., 2003). Mercury is absorbed throughout the intestine and absorption is possible through biologic membranes and other sulfur-containing proteins (Sebulingum, 2006; Hesse, 2007). Mercury recycles through the entero-hepatic system in adults, and is excreted primarily in the faeces (El-Nahal, 2010). Various works had been carried out on the effects of nutrients on transport, distribution and retention of mercury and on the overall effects of mercury on the metabolism of protein, carbohydrates, lipids and other metabolites (Hesse, 2007; Quirino et al, 2012).

Vitamin C or ascorbic acid is an essential nutrient for humans and some other animal species. In living organisms ascorbate acts as an antioxidant by protecting the body against oxidative stress (Groff et al., 1995). It is also a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy (Jacob, 1999). In animals these reactions are especially important in wound-healing and in preventing bleeding from capillaries. Ascorbate is required for a range of essential metabolic reactions in all animals and plants (El-Tohany and El-Nattat, 2010). Ascorbic acid is not synthesized by some species of birds and fish. All species that do not synthesize ascorbate require it in their diet. Deficiency in this vitamin causes the disease scurvy in humans (Mader, 2001; Kumar et al., 2010).

Free radicals are molecules having an unpaired valence electron which are typically highly reactive. Antioxidants are substances that nullify the effect of free radical by either inhibiting the initial production or inhibiting the preparative phase of free radicals (Sujatha et al., 2011). Examples of antioxidants are Vitamin A, Vitamin E and Vitamin C (Vasudevan and Sreekumari, 2007). The aim of the study was to evaluate the effect of ascorbic acid on mercuric chloride-induced changes in the liver of adult Wistar rat.

II. Materials And Methods

Experimental Animals

Thirty adult Wistar rats of average weight of 200g were used for this study and were acclimatized for three weeks and kept in the Animal house of the Department of Human Anatomy Ahmadu Bello University Zaria. After acclimatization, the rats were divided into six groups of five rats per Group for the experiment.

Experimental Chemicals

Twenty grams of Mercury chloride manufactured by May and Bakers Chemical Laboratory Limited Dagenham England while Vitamin C tablets manufactured by Jopan Pharmaceuticals Ltd. were purchased and used for the experiment.

Animal Experimentation

Thirty Wistar rats were divided into 6 Groups of 5 animals each. Group 1 (Control) was administered with distilled water, Group 2 was given 52.5mg/kg body weight of mercury chloride (Hg), Group 3 animals were administered with 26.25mg/kg body weight of Hg, corresponding to 25% and 12.5% of LD₅₀ of Hg respectively (Lucky, 1987). Animals in Group 4 were given 52.5mg/kg body weight of Hg and 5mg/kg body weight of ascorbic acid, while Group 5 animals were administered with 26.25mg/kg body weight of Hg and 5mg/kg body weight of ascorbic acid and Group 6 rats were administered with 5mg/kg body weight of ascorbic acid only corresponding to 10% of LD₅₀ of ascorbic acid (FSAUK, 2007). The administration was by oral route daily and lasted for 3 weeks while the animals feed and drinking water was allowed *ad libitum*.

Animals Sacrifice

After the administration, the animals were weighed and anaesthetized by inhalation of chloroform in the sacrificing chamber. Incision was made through the skin of the abdominal wall. The abdomen was opened through a mid sagittal incision and the spleen was removed and fixed in Bouin's fluid. The tissues were routinely processed and stained using haematoxylin and eosin and crystal violet methods.

Estimation of Haematological parameters

Blood was collected by means of Cardiac puncture and blood cell count was done using an auto-analyzer. Red blood cell count (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white Blood cell count (WBC), packed cell volume were analyzed and differential white blood cell count was done.

Estimation of oxidative parameters

Determination of Catalase activity

Catalase activity was determined using the method described by Sinha, (1972) and the absorbance was read at 570 nm. Standard curve was made by plotting the absorbance obtained at various levels of the assay. The Catalase activity was obtained from the graph of the standard curve.

Determination of superoxide dismutase (SOD) activity

Superoxide Dismutase (SOD) activity was determined by a method described by Fridovich, (1989). Absorbance was measured every 30 seconds up for a total of 150 seconds at 480 nm from where the SOD activity was calculated.

Assessment of lipid peroxidation

Lipid peroxidation as evidenced by the formation of TBARS was measured by the method of Niehaus and Samuelson (1968). The absorbance of the pink supernatant was measured against a reference blank using a spectrophotometer at 535nm.

Assay of reduced Glutathione concentration

Reduced glutathione (GSH) concentration measurements were done according to the method of Ellman (1959) as described by Rajagopalan et al. (2004), and the absorbance was read at 412 nm.

Statistical Analysis

All data were presented as mean \pm SD. For establishing significant differences, data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test. Values were considered statistically significant if P value is less than or equal to 0.05 ($p \leq 0.05$).

III. Results

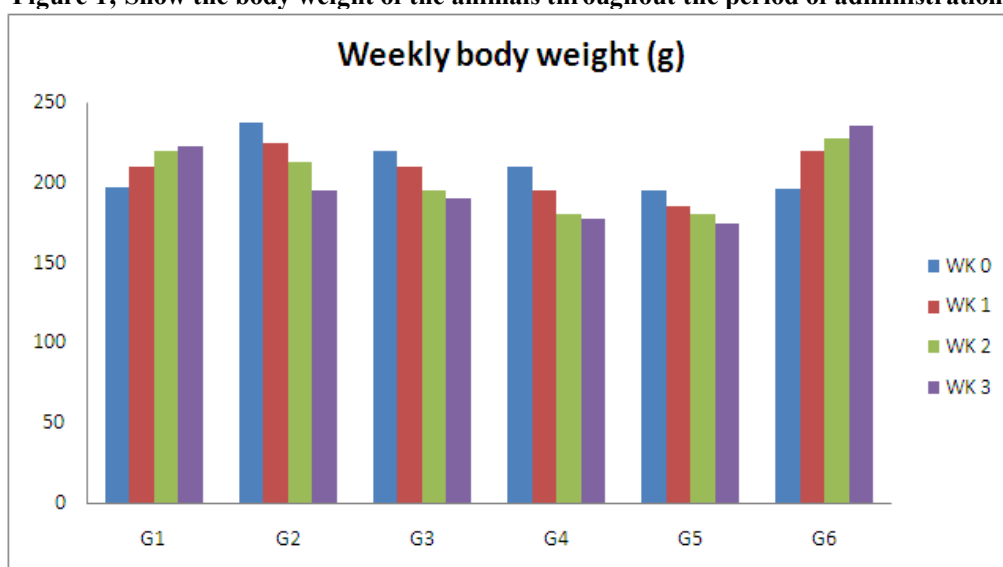
Physical Observation

During the period of administration, the animals were observed to be using their forelimbs to scratch their mouth. On mercuric chloride administration, the animals got weakened which was observed due to the reduction of their physical activities. There was decrease in body weight in the animals administered with mercuric chloride only, especially after the first and second weeks of treatment whereas there was increase in body weight in the Control group.

Body Weight Difference

There was decrease in body weight in Groups 2, 3, 4 and 5 during the twenty one days of administration, however the degree of decrease was less in Groups 4 and 5 when compared to Groups 2 and 3 suggesting the ameliorative effect of Vitamin C. The results showed that the body weight of animals in Groups 2 and 3 were decreased significantly during the 21 days of treatment when compared to the Control group. Groups 4 and 5 showed decrease in weight but less decrease when compared to Groups 2 and 3 indicating the ameliorative effect of vitamin C while Groups 1 and 6 showed increase in body weight as shown in Fig.1.

Figure 1; Show the body weight of the animals throughout the period of administration.



Hematological Parameters

The mean value of packed cell volume (PCV) of Group 1 showed a decrease when compared to Groups 2, 3, 4 and 5 indicating the toxic effect of mercuric chloride while the mean value of hemoglobin concentration in Group 1 showed a decrease when compared to Groups 2, 3, 4 and 5, indicating the toxic effect of mercuric chloride. The mean values of neutrophil, lymphocyte and eosinophil in Group 1 showed a significant decrease when compared to Groups 2, 3 and 5, and a statistically significant increase when compared to Groups 4 and 6 as shown in Table 1.

Table 1; Showing the results of Hematological Indices due to mercuric Chloride

GP	PCV(1/L) Mean±Sem	Hb(g/1) Mean±Sem	NEUTROPHIL Mean±Sem	LYMPHOCYT Mean±Sem	EOSINOPHIL Mean±Sem
1	0.43±0.01	14.42±0.30	21.80±1.53	76.20±2.01	1.40±0.60
2	0.41±0.02	13.53±0.63	22.00±2.00	73.70±1.85*	3.00±0.60*
3	0.42±0.12	14.00±0.40	23.00±2.10*	74.00±2.58*	2.50±0.30
4	0.40±0.15	13.32±0.50	20.50±3.80	79.25±1.88*	2.00±0.41
5	0.42±0.02	13.83±0.70	22.50±1.93*	73.50±2.25*	2.75±0.48*
6	0.40±0.01	12.90±0.24	18.24±2.32*	78.50±1.32*	2.50±0.65

*P<0.05; Packed cell volume (PCV), Hemoglobin concentration (Hb).

Biochemical Parameters

The results of the Biochemical analysis showed a marked decrease in the liver enzymes, AST and ALT in the Groups treated with mercuric chloride only (Groups 2 and 3) when compared to the Control Group while Groups 4 and 5 showed mean values of AST and ALT close to that of the Control group. Significant difference was seen in ALT and AST levels (P≤0.05) between Groups 3 and 2, 3 and 1, 3 and 4, 4 and 2 and 4 and 1 as

shown in Table 2. There was a significant increase ($P \leq 0.05$) in the mean values of ALP in Groups 3, 4, and 5 when compared to Group 1.

Table 2: Showing the result of biochemical parameters

GROUP	AST(IU/L) MEAN±SEM	ALT(IU/L) MEAN± SEM	ALP(IU/L) MEAN± SEM	AST/ALT RATIO
1	41.00±2.04	76.25±2.50	59.25±10.35	0.54
2	56.00±3.03*	113.25±5.83*	55.75±8.70*	0.50
3	55.75±1.11*	117.25±4.60*	78.75±10.26*	0.48
4	57.50±2.63*	107.00±2.88*	79.75±6.35*	0.54
5	45.00±2.68*	73.75±2.32*	87.27±4.67*	0.61
6	43.00±1.96*	75.00±2.34	57.25±10.07	0.57

Mean; standard error and statistical significant different * $P < 0.05$.

Histological Changes

The results of histological observations showed normal architecture of the liver with central vein, hepatic cords and sinusoidal spaces in Control Group animals as shown in Figure 1 while there were congestion of Central Vein and enlarged sinusoids, widespread mononuclear infiltration, and necrosis of hepatocytes in Group 2 animals as shown in Figure 2. The result from Group 3 showed mononuclear cell infiltration, congestion of Central Vein and diffused necrosis of hepatocytes as shown in Figure 3 and Group 4 animals showed mononuclear infiltration, focal areas of necrosis and congestion of central vein and dilated sinusoids as in Figure 4. Group 5 showed dilated sinusoids, necrosis of hepatocytes and a less congested central vein when compared to Figures 2 and 3 as shown in Figure 5 while Group 6 showed a normal liver architecture with normal Central vein, sinusoids, hepatocytes and kupffer cells as shown in Figure 6.

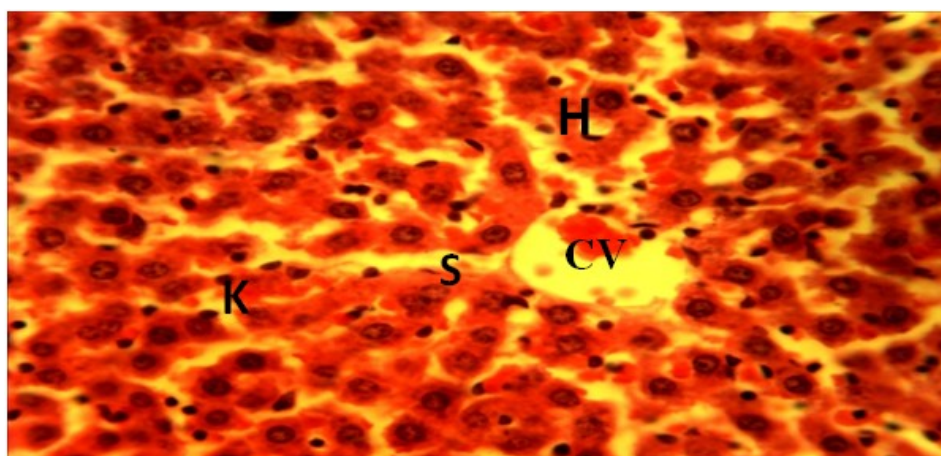


Fig. 1: A section of the Liver from Control group showing normal liver architecture with Central vein (CV), hepatocytes (H), Sinusoid (S) and kupffer cells (K). H&E x400.

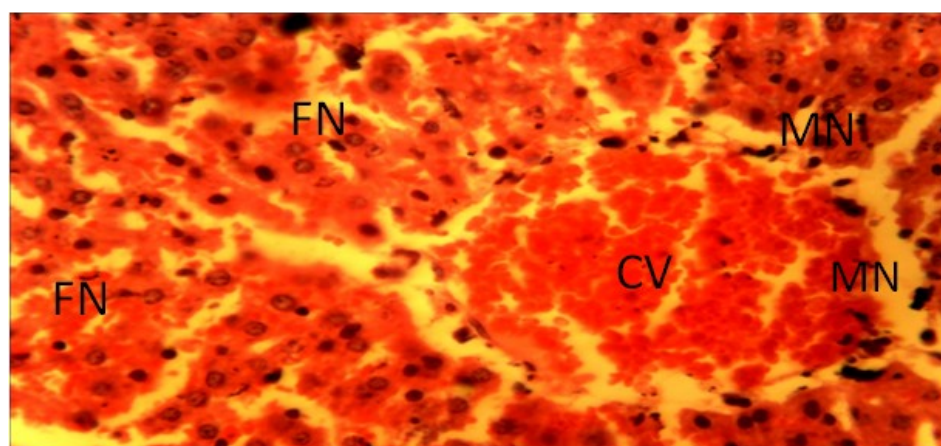


Fig. 2: A section of Liver from Group 2 with Congested Central Vein (CV), Focal area of necrosis (FN), and mononuclear infiltrations (MN). H&E Staining x400.

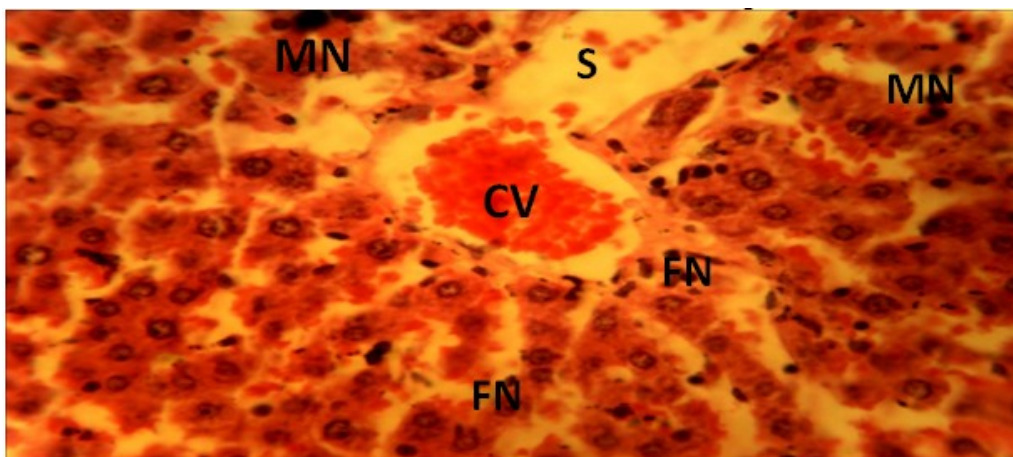


Fig.3: A section of Liver from Group 3 with Congested Central Vein (CV), enlarged Sinusoid, Focal area of necrosis (FN) and mononuclear cellular infiltrations (MN).H&E x400

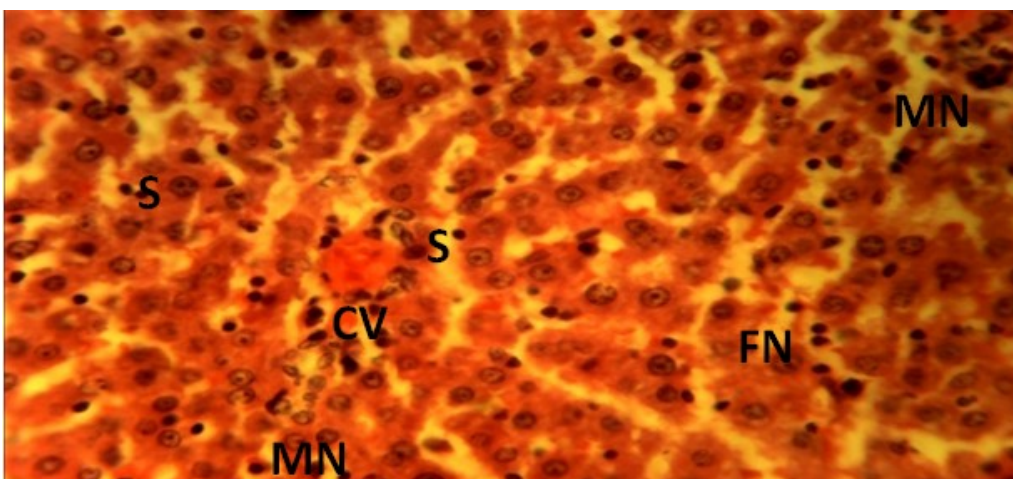


Fig.4: A section of the Liver from Group 4 with Congested Central Vein (CV), enlarged (S) Sinusoids, focal area of necrosis (FN) and mononuclear cellular infiltration (MN).H&E x400

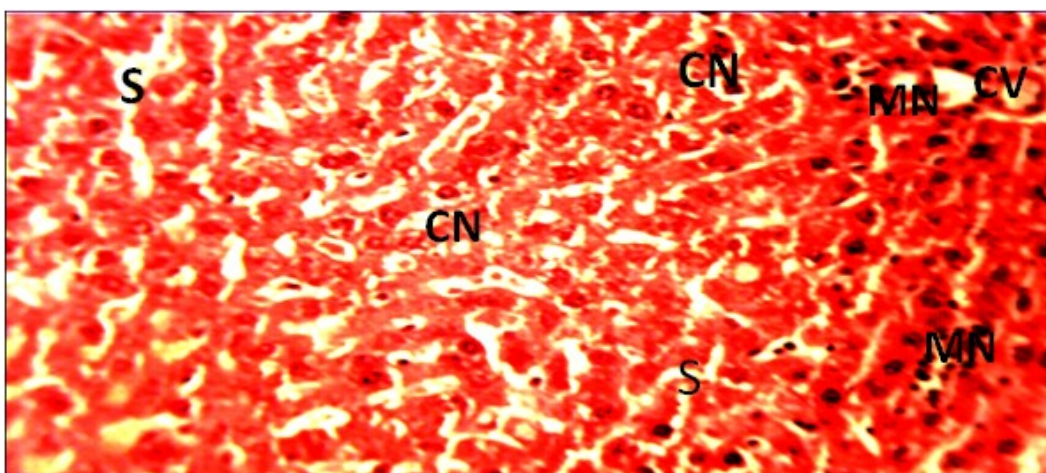


Fig.5: A section of the liver from Group 5 with Cellular necrosis (CN), Central Vein (CV), enlarged sinusoids (S), Mononuclear cellular Infiltration (MN). H&E x400

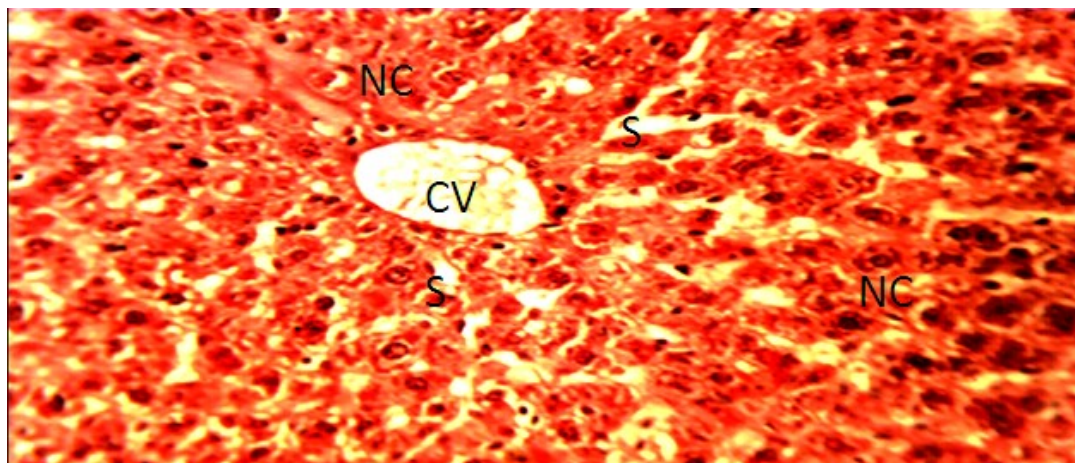


Fig.6: A section of the liver from Group 6 with Normal Cellular arrangement (NC), Central Vein (CV) and sinusoids (S). H&E x400

IV. Discussion

Mercury is a common environmental and occupational toxic heavy metal, which is known to have direct and indirect effects on biological systems and cells (Hesse, 2007; Bjomberg et al., 2011). One of the ways that mercury exerts its toxic effects is through oxidative stress that may be an important contributor to the negative pathogenesis observed after mercuric chloride exposure (Valera et al., 2008; ATDRS, 2011). Result from the present study showed congested central vein and sinusoids, widespread mononuclear infiltration and more diffused necrosis of hepatocytes as a result of mercury exposure in the experimental rats. In the present study, the results demonstrated that mercuric chloride decreased biochemical parameters such as alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The reduction in PCV and Hb in this study revealed microcytic hypochromic anemia. This observation was consistent with an earlier observation of others on genotoxic potential of mercuric chloride and different anti-oxidants alone or in combined form in the liver and other tissues (Wang et al., 2007; Burger et al., 2011; Quirino et al., 2012). Co-administration of mercuric chloride with ascorbic acid in the present study resulted in ameliorative effects on haemato-biochemical parameters. However, the overall mean value of the group treated with ascorbic acid tend to move towards normal, indicating the recuperative effect of this anti-oxidant against mercury toxicity.

In the present study, the Liver of Groups 2 and 3 rats showed congestion of hepatoportal blood vessels, congestion of central vein, edema in the portal tract and fatty changes indicating the toxic effect of mercuric chloride. It has been shown that in chronically diseased liver, some cells are activated by factors released by the liver hepatocytes and Kupffer cells, proliferate, and acquire the features of myofibroblasts, with or without the lipid droplets (Kumar et al., 2010; Sujatha et al., 2011). Under the conditions of liver toxicity, some cells become infiltrated close to the damaged hepatocytes and play a major role in the development of fibrosis, including the fibrosis secondary to alcoholic liver disease (Agarwal et al., 2010; Ibegbu et al., 2013). This fibrosis may become irreversible, leading to Liver cirrhosis, (Junqueira and Carneiro, 2005; Hesse, 2007; Quirino et al., 2012).

Moreover, the liver of the of Group 3 presented with focal accumulation of inflammatory cells and necrotic hepatocytes in addition to the observed changes in Group 2. This could be due to ability of mercury to activate signals that increase tumor necrosis factor-Alpha, a substance in the liver that causes inflammation, malignancy and cell death (Hesse, 2007; Burger et al., 2011). Mercuric chloride also induces a significant increase in DNA damage and apoptosis of liver cells (Wang et al., 2007; Bjomberg et al., 2011). This corresponds to the current report that, different doses of mercuric chloride in Wistar rats presents with congestion of hepatoportal blood vessels and edema in the portal tract, focal hepatic hemorrhage and vacuolar degeneration of hepatocytes (El-Nahal, 2010). Cirrhosis is the end result of several conditions that affect the liver architecture, usually a consequence of sustained progressive injury to hepatocytes produced by several agents, such as ethanol, drugs or other chemicals, hepatitis virus mainly types B, C or D, and autoimmune liver disease, (Junqueira and Carneiro, 2005; El-Tohamy and El-Nattat, 2010).

However, liver of Groups 4 and 5 rats co-administered Vitamin C with mercuric chloride showed lesser effects in Group 4 when compared to Group 2 and a slight congestion of central vein in Group 5 as compared to Group 3. This could be associated with the ability of Vitamin C to ameliorate the inhibitory action of mercury by removing the reactive oxygen species (ROS) once formed, via very rapid electron transfer system that inhibits lipid peroxidation, thus preventing radical chain reaction (El-Tohamy and El Nattat, 2010; Agarwal et al., 2010).

While the present study indicated that co-administration of mercury with Ascorbic acid had some effects on haemato-biochemical parameters, it also showed a significant ameliorative effect on the liver given higher doses (Group 5), showing that its effects are dose dependent. Although it is biologically plausible that Vitamin C may affect Mercury absorption and excretion, the effect was more obvious in low-exposed subjects with higher Vitamin C supplementation (Vapputuri et al., 2000; Agarwal et al., 2010). This result agrees with previous report that, Vitamin C supplementation resulted in some reduction in mercury retention in 85 human volunteers who consumed a mercury containing drink (Vapputuri et al., 2000; El-Shenawy and Hassan, 2008).

V. Conclusion

Mercuric chloride has been shown to have degenerative changes on the liver and causes decreases in biochemical parameters while no significant change was observed on the hematological parameters. Ascorbic acid has been shown to have ameliorative effects and that was most pronounced on the liver of mercuric chloride treated rats at higher doses, suggesting that it was dose dependent.

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