

Hepatoprotective Activities of Methanol Extract of *Hibiscus sabdariffa* Lin. in Paracetamol-induced Hepatotoxicity of Albino Rats

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Abstract: Hepatoprotective activities of methanol extract of *Hibiscus sabdariffa* L. on paracetamol induced hepatotoxicity of albino rat were investigated. A total of 20 albino rats were divided into four groups (I - IV), five rats per group. Group I and II served as the test groups, groups III as positive control, and IV as negative control. Groups I and II were treated with 250 and 500mg/kg body weight respectively of the extract for eight days after induction of hepatotoxicity while group III was treated with a standard drug (Livolin, 25mg/kg body weight) for eight days; the negative control was given distilled water. Blood samples were obtained from the rat to determine the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and total bilirubin. Liver was excised for histological studies. Hepatotoxicity induction significantly increased serum levels of ALT, AST, ALP and total bilirubin, and decreased total protein ($p < 0.05$). After eight days of orally administering the extract, ALT, AST, ALP and total bilirubin level decreased significantly ($p < 0.05$); and total protein level increased significantly ($p < 0.05$). The 500mg/kg of extract performed significantly better than 250mg/kg. Histological condition of the liver was improved by 500mg/kg of the extract. Therefore, the methanol extract of *H. sabdariffa* improved liver condition of albino rat damaged by paracetamol. The performance of the extract was dependent on its concentration.

Keywords: Acetaminophen, paracetamol toxicity, liver, dose dependent, liver health

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I. Introduction

Liver is a large organ that serves multiple vital functions in human body. By the aid of liver parenchymal (hepatocytes and cholangiocytes) and non-parenchymal cells (sinusoidal and perisinusoidal cells), the liver participates in activities such as detoxification of endogenous and exogenous substances (including drugs), carbohydrates transformation, protein synthesis, lipid synthesis and inflammatory responses (Kmièc, 2001; Gu&Manautou, 2013; Savitaet *al.*, 2014). Detoxification activities that liver undertakes release deleterious substances associated with injury or toxicity to the liver (Liska, 1998; Savitaet *al.*, 2014). Hepatotoxicity, drug or xenobiotics induced liver damage is a major setback to disease management by synthetic drug products. Drug induced liver damage is of major public health importance as the damage to liver due to drugs may sometimes necessitate liver transplant or could lead to death of patients (Sturgill & Lambert, 1997).

The ability of some botanicals to provide protection for the liver against toxicity (i.e. hepatoprotective) has elicited attention in recent times. Several plants and plant parts have been reported to be hepatoprotective. The plants included *Aphanamixis polystachya*, *Asteracanthalongifolia*, *Acacia catechu*, *Adhatodavasicca*, *Arachnidodesexilis*, *Bupleurumkaii*, *Balanitesaegyptiaca*, *Chamomile capitula*, *Cajanusindica*, *Cajanuscajan*, *Cistanchetubulosa*, *Ficusglomerata*, *Euphorbia fusiformis* and *Fumariaindica* among others (Savitaet *al.*, 2014). Currently research efforts chiefly rooted in traditional Indian medicine (TIM) and traditional Chinese medicine

(TCM) also known as Ayurveda are directed towards formulation of more humane drug products that focus more on the patient rather than the disease (Patwardhan *et al.*, 2005).

Assessment and re-assessment of these plant materials are extremely important to human health. Differences in the hepatoprotective activities of plant materials may be associated with the disparity in extraction method, extraction solvent, plant parts, mode/duration of extract administration, metabolic biomarker examined, experimental animal employed, laboratory condition in which experiments were setup, and hepatotoxicity inducing substance used. These are among factors that may affect the outcome of experiments investigating hepatoprotective activities of plant materials. For example, the hepatoprotective activities of flower *Hibiscus sabdariffa* have been reported but with some limitations. Some studies clearly indicated that calyx (Dahiruet *al.*, 2003; Adaramoyeet *al.*, 2008; Hashemi, 2014) or petal (Obuoayebaet *al.*, 2003) was flower parts investigated, others ignored the clarification (Essaet *al.*, 2005; Usohet *al.*, 2012; Alzubade, 2014; Al-Kubaisyet *al.*, 2016). Only one study by Ali *et al.* (2003) investigated *H. sabdariffa* methanol extract hepatoprotective properties in paracetamol induced hepatotoxicity in rats. This study and other studies on liver damage induced by carbon tetrachloride (Dahiruet *al.*, 2003; Usohet *al.*, 2012; Hashemi, 2014), gamma radiation (Adaramoyeet *al.*, 2008) and 2,4-dinitrophenyl-hydrazine (DNPH) (Obuoayebaet *al.*, 2003) all reported liver protection by *H. sabdariffa*. The present study intended to add to the accumulating knowledge on the hepatoprotective significance of *H. sabdariffa*. The interest in *H. sabdariffa* is due mainly to its widespread and regular consumption in Nigeria.

II. Materials And Methods

2.1 Plant Sample Collection and Identification

Fresh plants of *Hibiscus sabdariffa* L. used in the study were purchased from the metropolitan market in Nsukka, Enugu State. The leaves were authenticated by plant taxonomists in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

2.2 Preparation of Extract

The calyces of *Hibiscus sabdariffa* L. were dried under shade at room temperature in the laboratory and ground using a mechanical grinder. The resultant powder was weighed and put in a labeled receptacle. In a beaker, 500g of the powder was macerated in 2500ml of methanol (Analytical grade from BDGP) for 72 hours at room temperature. The mixture was filtered using porcelain cloth and the filtrate obtained was filtered again using Whatman filter paper No. 1 and then allowed to evaporate to dryness in open air. The dried extract obtained was stored in a tightly corked container in a refrigerator at -4°C until required. Stock solution of the extract was prepared by dissolving 1g weight of the powdered extract in 10ml of normal saline.

2.3 Experimental Animals

Male albino rats weighing 200 - 280g were obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were kept in metal wire cages in a room with 12-hour light-dark cycles, at room temperature and allowed free access to food and water. The animals were acclimatized for 14 days under these conditions before the start of the experiments. Care was taken to ensure rats were handled in compliance with standard animal welfare regulations.

2.4 Drugs and Chemicals Procurement

Paracetamol (Emzor Pharmaceuticals, Nigeria) purchased from Model Pharmacy, University of Nigeria, Nsukka; and methanol purchased from Jeo Chemicals located in University of Nigeria, Nsukka were used for the experiment.

2.5 Induction of Hepatotoxicity in Rats using Paracetamol (Acute model)

Hepatotoxicity in the rats was induced by the oral administration of 2000mg per kilogram body weight of Paracetamol (Emzor Pharmaceuticals, Nigeria). Hepatotoxicity was established before the commencement of subsequent stages of the study.

2.6 Experimental Design

Animals were randomized into four groups (I – IV) comprising five animals in each group. Rats in all the groups were given Paracetamol to induce hepatotoxicity. Group I and II were treated with 250 and 500mg/kg body weight of methanol extract of *H. sabdariffa*. Group III served as positive control, they were treated with standard drug Livolin (25mg/kg body weight). Group IV served as the negative control, they were left untreated till the end of the experiment. Drug and extract was administered orally once daily. All the rats were allowed access to food and water during the course of treatment.

2.7 Assays for Biochemical Parameters

Blood samples collected from the retro median canthus of the retrobulbary plexus of albino rats eyes were put into sterile plain centrifuge bottles and centrifuged at a rate of 12 000RPM for 10mins. The clear serum obtained was analysed for aspartate transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and total bilirubin. Enzymatic and spectrophotometric assay methods by means of Randox Commercial Assay Kits were used. Liver enzyme assays were based on the method of Reitman & Frankel (1957); total bilirubin on method described by Jendrassik&Grof (1938); and total protein based on method by Tietz (1995).

2.8 Statistical Analysis

Two way analysis of variance (ANOVA) was used to compare extract concentration and duration of treatment dependent changes in biochemical characteristics of the rats. Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, New York). Charts were plotted in Microsoft Excel (Microsoft Inc., Redmond). Level of significance was set at $p < 0.05$.

III. Results

3.1 Changes in the Serum Biomarkers of Paracetamol-induced Hepatotoxicity in Albino rats treated with *Hibiscus sabdariffa* Methanol Extract.

Induction of hepatotoxicity caused noticeable changes in the biochemical profile investigated. Administration of methanol extracts of *H. sabdariffa* calyces significantly improved the liver condition of the rats as indicated by reversion of the changes induced by the toxicant. There were no significant variations in the levels of the serum biochemical investigated among the treatment groups (controls and other groups) prior to the induction of hepatotoxicity ($p > 0.05$). The alanine aminotransferase (ALT) level after induction of hepatotoxicity increased significantly in all the treatment groups ($p < 0.05$) (Figure 1). There were no significant differences between the ALT levels of the treatment groups after hepatotoxicity induction ($p > 0.05$). At the end of 8 days treatment of the hepatotoxic rats, the ALT levels in all the treatment groups except the negative control dropped significantly than its levels immediately after hepatotoxicity ($p < 0.05$). The levels of ALT in the rats treated with 250 and 500 mg/kgb.wt of methanol extract of *H. sabdariffa* dropped significantly compared to the negative control, but were significantly higher than that of the standard control ($p < 0.05$).

After induction of hepatotoxicity, the aspartate aminotransferase (AST) concentration increased significantly ($p < 0.05$) (Figure 2). There were no significant differences between the AST levels of the four treatment groups after hepatotoxicity induction. At the end of 8 days curative treatment, the AST level of the 250 and 500 mg/kgb.wt *H. sabdariffa* methanol extract treated albino rats dropped significantly ($p < 0.05$). The AST level of the rat treated with 250mg/kgb.wt of the extract was, however, not significantly lower than that of the negative control ($p < 0.05$). Those treated with 500 mg/kgb.wt of the extract had a significant decrease in their AST level than in the negative control group ($p < 0.05$); but the standard control group had a significantly lower AST level ($p < 0.05$).

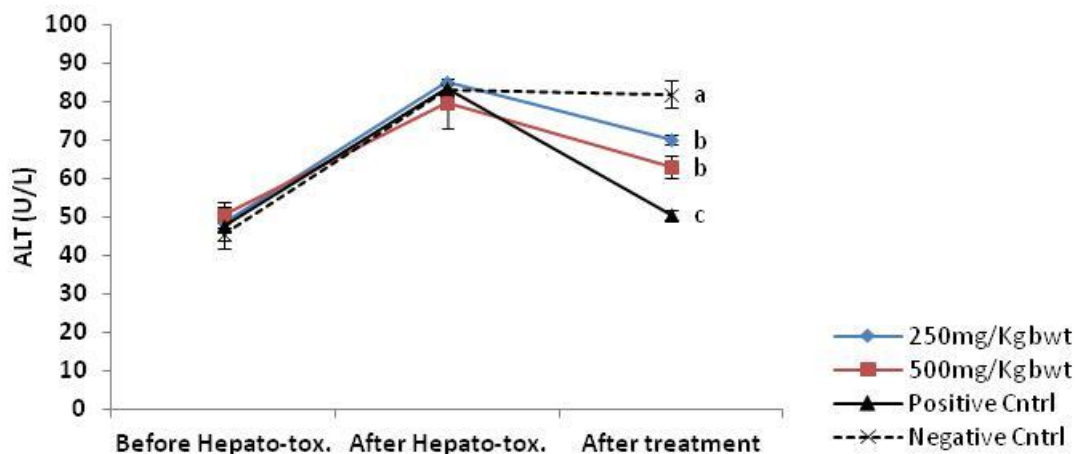


Figure 1: *Hibiscus sabdariffa* extract reduced alanine aminotransferase (ALT) level in paracetamol induced hepatotoxicity of albino rats. Hepato-tox. = hepatotoxicity. Values with different alphabet were significantly different after treatment at $p < 0.05$.

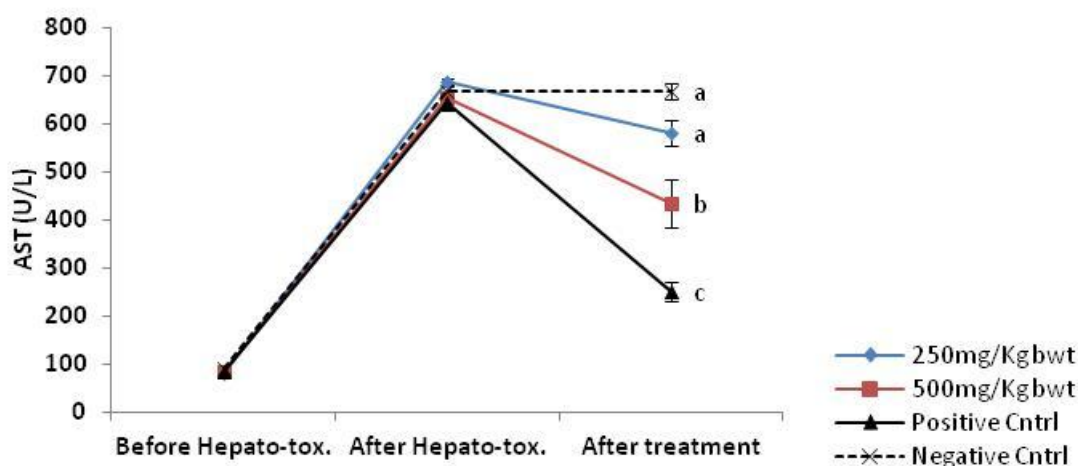


Figure 2: *Hibiscus sabdariffa* extract reduced aspartate aminotransferase (AST) level in paracetamol induced hepatotoxicity of albino rats. Hepato-tox. = hepatotoxicity. Values with different alphabet were significantly different after treatment at $p < 0.05$.

The values of alkaline phosphatase (ALP) were not significantly different among the treatment groups after hepatotoxicity ($p > 0.05$), but increased significantly than it were before it ($p < 0.05$) (Figure 3). When the rats were treated, the ALP level in all the groups (including negative control) dropped significantly than they were after hepatotoxicity ($p < 0.05$), but remained significantly higher than they were before it induction ($p < 0.05$). The ALP level of the rats at the end of treatment with 250 or 500 mg/kgb.wt of the extract was not significantly less than the negative control ($p > 0.05$).

The total bilirubin level followed a similar trend as the other parameters (Figure 4). The level in the treatment groups increased significantly than it was before hepatotoxicity ($p < 0.05$); and dropped significantly after treatment with either methanol extracts of *H. sabdariffa* (250 mg/kgb.wt or 500mg/kgb.wt) or the standard drug (Livolin) ($p < 0.05$). While the negative control remained at similar level 8 days post hepatotoxicity as it was just after it ($p > 0.05$).

The concentration of total protein unlike other biochemical followed a different trend (Figure 5). It concentration after hepatotoxicity dropped significantly ($p < 0.05$); and increased significantly in all the groups treated either with standard drugs or 250 and 500 mg/kgb.wtmethanol extracts of *H. sabdariffa* than the negative control ($p < 0.05$). In the group treated with 500 mg/kgb.wt of the extract, the concentration of total protein was at the same range as the standard control group ($p > 0.05$).

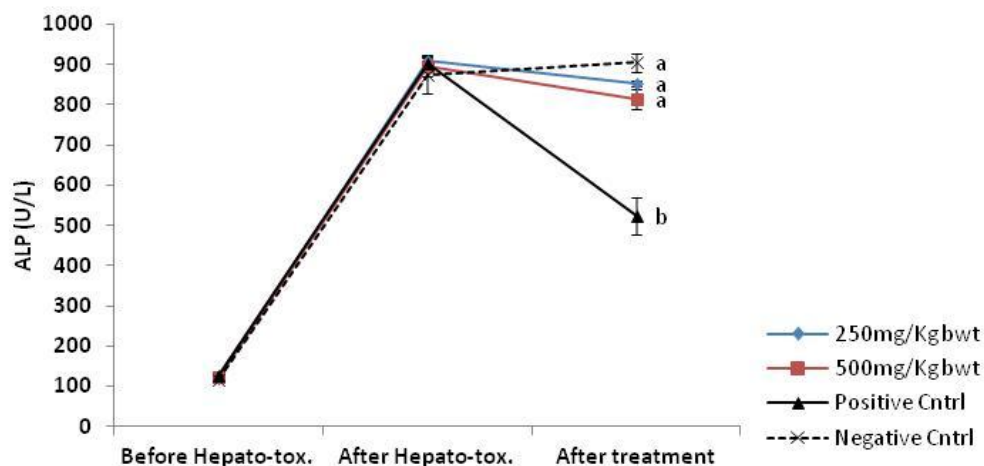


Figure 3: *Hibiscus sabdariffa* extract reduced alkaline phosphotase (ALP) level in paracetamol induced hepatotoxicity of albino rats. Hepato-tox. = hepatotoxicity. Values with different alphabet were significantly different after treatment at $p < 0.05$.

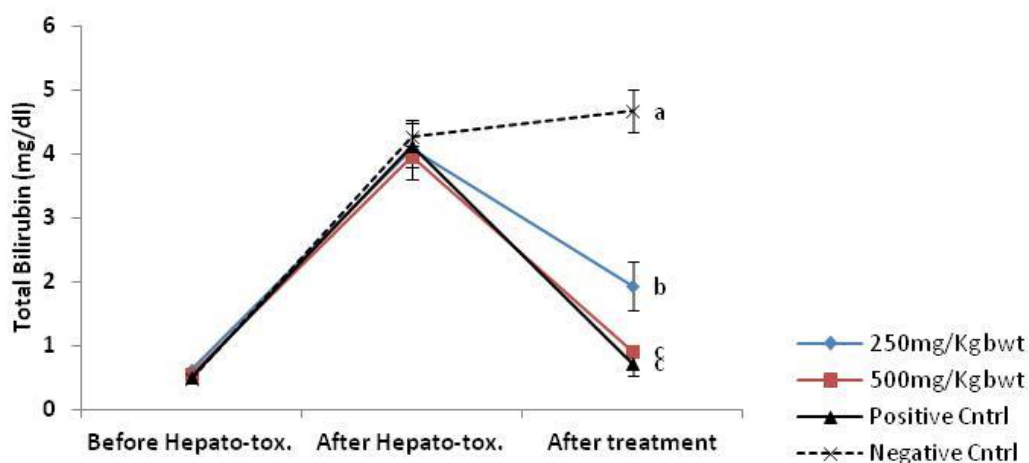


Figure 4: *Hibiscus sabdariffa* extract reduced total bilirubin level in paracetamol induced hepatotoxicity of albino rats. Hepato-tox. = hepatotoxicity. Values with different alphabet were significantly different after treatment at $p < 0.05$.

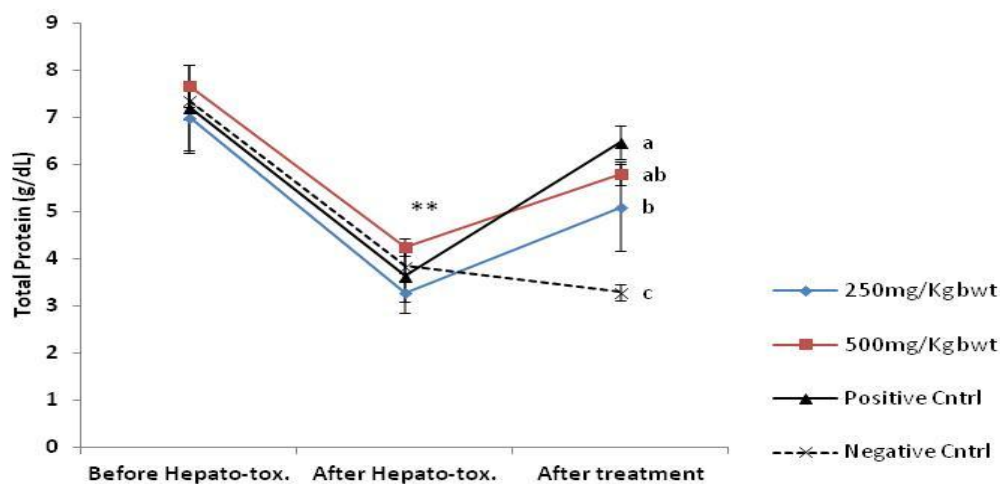


Figure 5: *Hibiscus sabdariffa* extract improve total protein level in paracetamol induced hepatotoxicity of albino rats. Hepato-tox. = hepato-toxicity. **All values after hepato-toxicity significantly less than values before hepato-toxicity at $p < 0.01$. Values with different alphabet were significantly different after treatment at $p < 0.05$.

IV. Discussion

Paracetamol (N- acetyl *p*-amino phenol, acetaminophen) is a widely used analgesic and antipyretic drug well-tolerated in prescribed doses, but causes hepatotoxicity in experimental animals and humans at high doses (Kumar *et al.*, 2004; Singh *et al.*, 2011). It is mainly metabolized in the liver to excretable glucuronide and sulphate conjugate (Vermeulinet *al.*, 1992). Hepatotoxicity of paracetamol has been attributed to formation of the toxic metabolites, N-acetyl *p*-benzoquinoneimine (NABQI) when a part of paracetamol is activated by Cytochrome-p 450 enzymes in the liver (Isao *et al.*, 2004; Singh *et al.*, 2011). Generation of this toxic metabolite overwhelm the detoxification process of the liver. Introduction of precursors of antioxidants such as acetylcysteine (a precursor of glutathione) can limit the severity of toxicity (Singh *et al.*, 2011). From studies, paracetamol overdose caused acute centrilobular necrosis in rats, mice, pig, guinea pig, hamsters, rabbits, cats and dog (Savitaet *al.*, 2014), and centrilobular necrosis in humans mostly characterized by pyknosis and eosinophilic cytoplasm (Kmiiec, 2001). Overdose of paracetamol leads to mitochondrial dysfunction followed by acute hepatic necrosis (Espostiet *al.*, 2012). The elevated levels of serum liver enzymes from this study are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Ganong, 2003). Damage to liver cells caused leakage of cellular enzymes into serum.

In the assessment of liver damage certain biomarkers of hepatotoxicity are measured and one of such biomarkers are enzymes levels such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) because liver damage arising from necrosis or membrane damage lead to release of these enzymes into circulation; therefore, measurement of these enzymes in serum gives an indication of the health status of the liver. ALT is the standard clinical biomarker of hepatotoxicity as it is found majorly in the liver; leakage into the circulation distinctly indicates liver damage. Leakage of ALT and AST from the liver into circulation is indicative of hepatocellular necrosis; elevation of serum ALP and total bilirubin is an indication of hepatobiliary injuries which consequently interferes with bile movement (cholestasis) (Kumar *et al.*, 2004; Garbaet *al.*, 2009; Singh *et al.*, 2011). Total protein decline is another indicator of liver damage from the paracetamol overdose as hepatotoxicity interferes with protein synthesis activities in the liver (Thapa&Walia, 2007).

Methanol extract of *H. sabdariffa* ameliorated paracetamol-induced hepatotoxicity effect on measured biomarkers. The hepatoprotective effect was observed as decrease in the hepatotoxicity-induced elevated levels of total bilirubin and the enzymes ALT, AST, ALP, and increase in the decreased level of total protein following treatment with the extract. The possible mechanism responsible for the amelioration of the paracetamol induced liver damage by the extract may result from the extract acting as a free radical scavenger by intercepting the radicals involved in paracetamol metabolism by microsomal enzymes (Ay, 1976; Robert, 2000; Singh *et al.*, 2011). The extract may also contain important hepatoprotective phytochemicals. A number of scientific reports indicated the role of flavonoids, triterpenoids and steroids in hepatoprotection against hepatotoxins (Kmiiec, 2001; Hassan *et al.*, 2010; Kumar *et al.* 2012, Agbaforet *al.*, 2014; Da-Costa-Rocha *et al.*, 2014; Obuoayebaet *al.*, 2014; Rehmanet *al.*, 2015). The presence of these compounds in *H. sabdariffa* was reported by Kumar et al. (2012) and Obuoayebaet *al.* (2014).

V. Conclusion

In conclusion, the results of this study demonstrate that the methanol extract of *H. sabdariffa* has possible curative action on paracetamol induced hepatotoxicity after 8 days of treatment with 250 and 500mg/kg b.wt, as observed by the effects on the enzymes that are markers of liver damage in albino rats.

CONFLICT OF INTEREST DECLARATION

The authors declared no conflict of interest.

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