# HEPATOPROTECTIVE EFFECT OF *MANGIFERA-INDICA* STEM BARK EXTRACTS ON PARACETAMOL-INDUCED OXIDATIVE STRESS IN ALBINO RATS

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**Abstract**

This study was conducted to evaluate the hepatoprotective activity of aqueous and ethanol extract of *Mangifera-indica* stem bark in paracetamol-induced liver injury in winstar albino rats. Aqueous and ethanolic extracts of *M. indica* stem bark at a dose of 200mg/kg were administered for 7 days to rats in groups II and III respectively while vitamin C (200mg/kg) was given as a standard antioxidant to group IV along with water and standard feed. Groups I and V received water and standard feed for 7day. On the 8$^{th}$ day all groups except group I received paracetamol at a dose of 2g/kg body weight and all rats were sacrificed 12hrs after paracetamol administration. Serum total protein (TP), albumin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were determined to assess the effect of the extract on the paracetamol induced hepatic damage in addition to hepatic antioxidant status. The ethanolic extract was found to be more effective than the aqueous extract (200 mg/kg) in paracetamol induced liver damage by decreasing the activity of serum enzymes AST, ALT and ALP while increasing significantly ($P<0.05$) the levels of total proteins and albumin. Glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) activity were significantly increased ($P<0.05$) in the treated groups while malondialdehyde (MDA) levels were reduced. From this studies it is concluded that both aqueous and ethanol extract of *M. indica* stem bark possesses hepatoprotective activity.

**Keywords:** Hepatoprotective, *Mangifera indica*, paracetamol, oxidative stress

## Introduction

Liver, the largest organ of human body is saddled with the responsibility of detoxifying chemicals and other xenobiotics by inactivating and metabolizing those substances (Izunya et al., 2010). Thus, it is a direct victim for toxicity leading to drug induced liver disorders of which 39% are due to paracetamol toxic dose (Metha et al., 2012). Paracetamol is an analgesic taken with or without prescription by a large population including infants and adults in Nigeria as first aid treatment for the relief of pain, fever and cold. Though it is safe at therapeutic dosage (Ryder and Beckingham, 2001), its misuse results to hepatotocixity (Ahmed and Khatar, 2001). In modern day medicine, no drug is available for exciting liver function, proffer protection to the liver and revitalize hepatic cells (Metha et al., 2012). Hence, the basis on which various researchers across the world focus on deriving new natural products from medicinal plants with hepatoprotective activity.

*M. indica* commonly called mango is a fruit crop grown widely in Nigeria for its edible fruits (Nwinuka et al., 2008) and medicinal values associated with different parts of the plant (Malami et al., 2014). Various works have reported the antidiabetic activity (Ojewole, 2005; Muruganandan et al., 2005) and antianaemic activity (Ogbe et al., 2010) while only a few have reported its hepatoprotective activity. All these important uses of *M. indica* stem bark is credited to their phytochemical constituents. This work is aimed at investigating the ability of hot water extract and ethanolic extract of *M. indica* stem bark to protect the liver cells from damage.

## Materials and methods

### Chemicals

All chemicals used were of analytical grade.

### Collection of plant material

*M. indica* stem bark were harvested on the field within the premises of Lagos State Polytechnic, Ikorodu, Lagos State, Nigeria.

### Preparation of plant extract

The stems were cut into small pieces, air-dried under the shade and pulverized in to coarse powder using wooden pestle and mortar and stored until required for use. 100g of powdered *M. indica* stem bark was taken into two different beakers. The samples were soaked with 500 ml of ethanol and boiled distilled water respectively. All the above extracts were concentrated

to a small volume by the use of rotary evaporator and dried at $50^0$C in a water bath. The extract containing the bioactive compounds were stored at -$20^o$C until the period of analysis.

**Phytochemical Screening**

Standard test procedures were used to determine the presence of phytochemicals in the ethanolic and aqueous stem extract of *M. indica*. The alkaloids, flavonoids, saponin, tannins and phenolics were determined according to the methods of Harborne (1973) and Sofowora (1992)

**Animals**

Thirty (30) male albino winstar rats weighing between 120 to 150g were procured from the animal house of Lagos University teaching Hospital, Nigeria. The animals were kept in plastic cages with free access to standard laboratory pellet diet and water throughout the experimental period while maintaining a 12 hours dark and 12 hours light cycle at room temperature. The animals were handled according to ethical guidelines of Zimmerman (1983).

**Animal grouping and scheduling**

The animals were randomly divided into five groups of six (6) rats. Aqueous and ethanolic extracts of *M. indica* stem bark at a dose of 200mg/kg were administered for 7 days to rats in groups II and III respectively while vitamin C (200mg/kg) was given as a standard antioxidant to group IV along with water and standard feed. Groups I and V received water and standard feed for 7day. On the $8^{th}$ day all groups except group I received paracetamol at a dose of 2g/kg body weight and all rats were sacrificed 12hrs after paracetamol administration. The blood was collected by cardiac puncture into heparin bottles and samples were centrifuged at 2500 rpm for 15 min to obtain the plasma and stored at $20^o$C until ready for analysis.

**Assessment of hepatoprotective activity**

The serum levels of total protein, albumin and activities of ALT, AST and ALP were determined using Randox assay kits

**Preparation of liver homogenates**

The liver was perfused with 0.86 % cold saline to completely remove all the red blood cells. 0.1g of the liver was homogenized in ice-cold 0.1M phosphate buffer (pH 7.4), centrifuged and the supernatants were collected for estimation of superoxide dismutase (SOD), catalase, reduced glutathione and malondialdehyde (MDA) levels by spectrophotometric methods.

**Assessment of antioxidant activity**

The activities of SOD (Kakkar et al., 1984) and CAT (Sinha, 1972) were assayed in the liver. SOD was assayed by the inhibition of the formation of NADH-phenazine methosulphate nitroblue tetrazolium formazan which was measured at 520 nm. One unit of SOD activity is defined as the enzyme concentration required inhibiting the chromogen production by 50 % in 1 min under the assay conditions. CAT activity was determined by measuring the amount of hydrogen peroxide ($H_2O_2$) consumed in the reaction. The remaining $H_2O_2$ was reacted with dichromate-acetic acid reagent and monitored spectrophotometrically at 590 nm. Activity was expressed in μmol $H_2O_2$ consumed/ min/mg protein. Lipid peroxidation was estimated by adopting the method of Vashney and Kale (1990)**.** This was estimated by measuring the absorbance of the stable pink MDA-TBA complex at 532nm. The reduced glutathione (GSH) content of the liver was measured following the method of Ellman (1959). This method measures the absorbance of chromophoric product, 2 – nitro – 5 – thiobenzoic acid, resulting from the reaction of Ellman's reagent with GSH at 412nm.

**Statistical Analysis**

The experimental results were analyzed using One Way Analysis of Variance (ANOVA) on Microsoft excel 2010 and expressed as Mean ± SEM. Differences between means were regarded significant at $P < 0.05$ using t- test.

**Result and Discussion**

From table 1, alkaloids, tannins, flavonoids, saponins, terpenoids and steroids were detected in both aqeous and ethanolic extracts of *M. indica* stem bark. Phenolic compounds including flavonoids, tannins, have been reported to possess antioxidant property (Aljadi and Kamaruddin, 2004). Therefore, hepatoprotective activity of *M. indica* stem bark extracts could be as a result of the presence of phenolic compounds.

Paracetamol toxicity results from formation of its intermediate, NAPQI an oxidant which depletes the liver's antioxidant system leading to attacks on components of healthy cells such as lipids, proteins, nucleic acids and membranes leading to loss of structural integrity and functions (Mark, 1998; Poli et al., 2004).

The ability of plant extracts to protect liver against paracetamol-induced toxicity is utilized in screening plants for hepatoprotection potential (Zakariah et al., 2011). ALT and AST are amino acids metabolizing enzymes localized within the liver and their levels in the serum together with ALP levels are indicators of the liver status. From figure 2, rats treated with paracetamol only showed significant increase ($P<0.05$) in the activities of

ALP, AST and ALT. Rats in groups pretreated with aqueous, ethanol and vitamin C produced a significant decrease in the activities of ALP, AST and ALT. Increased serum levels of these enzymes indicate hepatic injury which is evident in paracetamol treated groups because these enzymes leak into the serum when hepatic membrane damage occurs (Pari and Murugan, 2004; Izunya et al., 2010). However, a significant reduction in values of ALT, AST and ALP in rats treated with aqueous and ethanol extracts of *M. indica* showed that the plant has the ability to protect rats' hepatocytes from damage.

Total protein reflects the functional status of the liver (Pachathundikandi and Varghese, 2006) because the liver is furnished with machineries for synthesizing serum proteins excluding γ-globulins, thus, liver damage is characterized by hypoproteinemia and decreased albumin which can affect the whole physiological status of animals (Kanchana and Mohammed, 2011; Iweala and Osundiya, 2010). A significant reduction ($P<0.05$) in TP and albumin observed in paracetamol only treated groups in relation to control group indicates hypoproteinemia while the significant increase ($P<0.05$) in the treated groups suggests increased protein synthesis. The reports of Malami et al., (2014) is in accordance with our findings showing a decreased activity of AST, ALT, ALP and increased content of TP and albumin levels in $CCl_4$ induced hepatotoxicity when treated with aqueous extract of *M. indica* stem bark.

The antioxidant enzymes, superoxide dimutase is responsible for converting superoxide anion to hydrogen peroxide and oxygen (Zelko et al., 2002) while catalase spontaneously converts hydrogen peroxide to water and oxygen respectively (Chelikani et al., 2004) in order to prevent hydroxyl radical formation closely related with loss of hyaluronic acid in joints resulting to inflammatory disorders such as arthritis (Panda et al., 2011). Hence, reduction results in accumulation of superoxide radical and hydrogen peroxide which are harmful to the cell. Figure 4 exposed the effect of treatments on SOD and CAT activity in the liver of treated rats. SOD and CAT levels were significantly increased ($P<0.05$) in treated rats compared to untreated rats which received paracetamol only.

The reduced activity of SOD and CAT after paracetamol administration in this present study signifies that the oxidant production have overwhelmed the available antioxidant enzyme synthesized in response to presence of oxidants. This is confirmed by the result obtained from assessing the amount of MDA available in the liver homogenates (figure 5). MDA is the end product of lipid peroxidation; therefore an increased level in hepatocytes implies that lipid peroxidation has occurred resulting from failure of antioxidant system, hence liver damage (Ojo et al., 2006). GSH, a non-protein sulfhydryl compound plays an important role in maintaining

hepatocyte integrity by conjugating NAPQI (Manyinke et al., 2000) leading to formation of GSSG (oxidized formof GSH). This process depletes GSH level (Jaeschke et al., 2002) which is evident in paracetamol treated groups while a significant increase ($P<0.05$) was produced in the treated rats. This suggests that the extracts could spare or aid GSH in detoxifying the metabolites by being oxidized itself.

This study reflects higher hepatoprotective activity of ethanol extract of *M. indica* stem bark when compared to the hot water extracts. Ethanol extracts have been reported as potent antioxidants capable of removing free radicals than hot water extracts due to low solubility of secondary metabolites in water relative to ethanol extract which the effect is attributed (Lapornik et al., 2005; Omololu, 2011; Ogunmoyele et al., 2012). In conclusion, both hot aqueous and ethanolic extract of *M. indica* stem bark can be used in the prevention of paracetamol-induced oxidative stress.

Table 1: Phytochemical screening of water and ethanol extract of *Mangifera indica* stem bark

| Phytochemicals | Aqueous extract | Ethanol extract |
|---|---|---|
| Alkaloids | + | + |
| Tannins | + | + |
| Flavonoids | + | + |
| Anthraquinones | -- | -- |
| Phlobatannins | -- | -- |
| Saponin | + | + |
| Terpenoids | + | + |
| Steroids | + | + |

**Key:** + = positive -- = negative

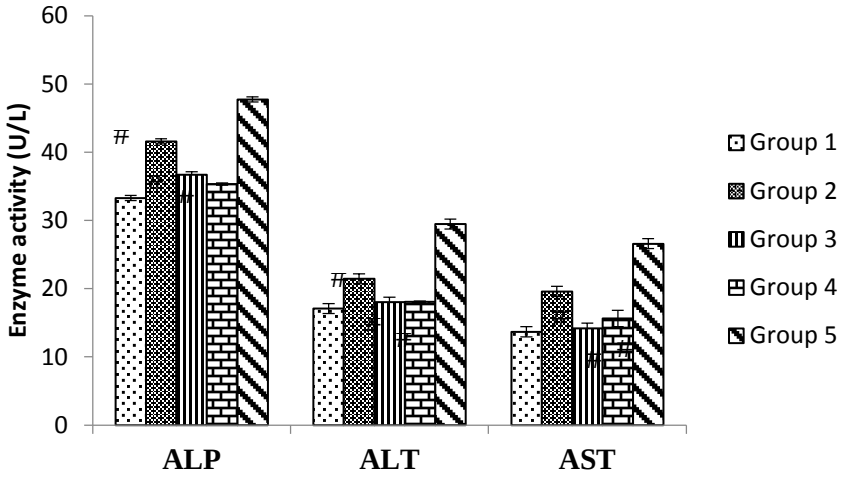


Fig 1: Effect of treatment on serum enzyme activity (ALT, ALP AND AST)

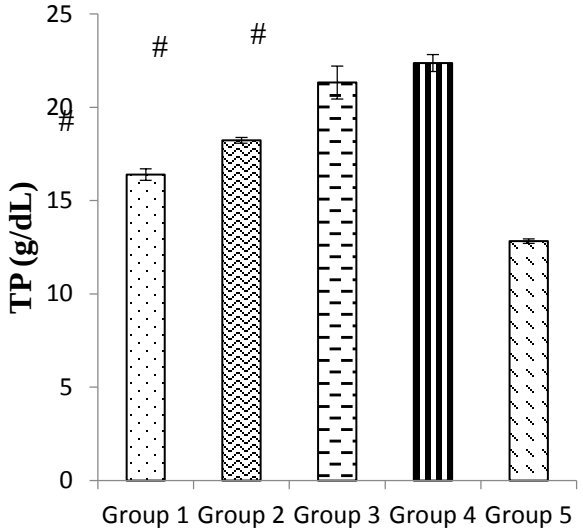


Fig 2: Effect of treatment on serum total proteins concentration

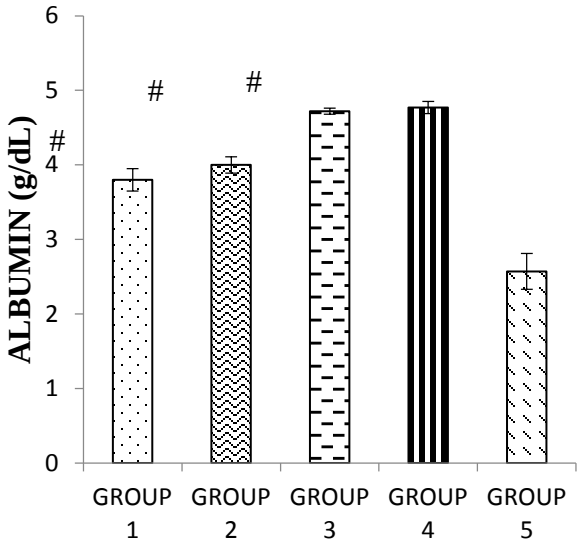


Fig 3: Effect of treatment on serum albumin concentration

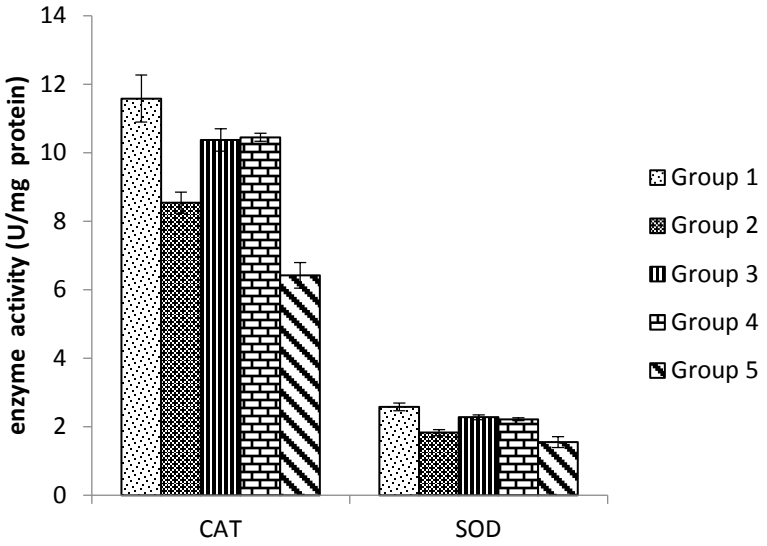


Fig 4: Effect of treatment on SOD and Catalase activity

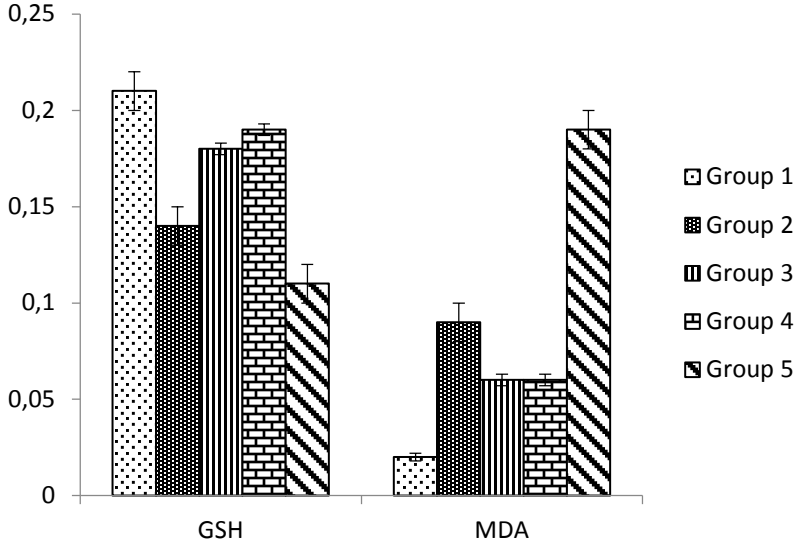


Fig 5: Effect of treatment on concentration of GSH (µg/ml) and MDA (nmol MDA/mg protein)

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