

Hepatoprotective effect of *parkia biglobosa* stem bark methanolic extract on paracetamol induced liver damage in Wistar Rats

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Abstract: This study was designed to investigate the effect of the methanolic extract of *parkia biglobosa* stem bark on a single daily dose of oral administration of 500 mg/kg BW of paracetamol (acetaminophen, PCM) induced hepatotoxicity in wistar rats. The rats were divided into (5 groups. The rats in group I served as control and received distilled water, group II were given orally a single daily dose of 500 mg/kg BW of paracetamol for 7 days. Group III, IV, and V received a single daily dose of 500 mg/kg BW of paracetamol and then treated orally with 140 mg/kg BW acetylcysteine, 100 mg/kg BW low dose and 200 mg/kg BW high dose of *parkia biglobosa* respectively for 21 days. The activities of liver function marker enzymes were determined in the serum of the rat liver homogenate. Paracetamol caused liver damage as evident by significant increased ($p \leq 0.05$) (49.63 ± 1.99 ; 39.41 ± 1.99 ; 78.58 ± 1.72) in the serum levels of Alkaline phosphatase (AP), Aspartate transaminase (AST) and Alanine transaminase (ALT) respectively. Low dose 100mg/kg BW of *Parkia biglobosa* significantly increased ($p \leq 0.05$) serum AP levels (65.42 ± 1.6) but significantly reduced serum levels of ALT and AST (43.80 ± 2.4 ; 36.77 ± 1.58) respectively. High dose 200 mg/kg BW of *Parkia biglobosa* significantly reduced ($p \leq 0.05$) serum levels of AP, ALT and AST (26.58 ± 0.34 ; 33.68 ± 2.02 ; 31.08 ± 0.34) respectively. Acetylcysteine (standard reference drug) significantly reduced ($p \leq 0.05$) ALT and AST levels (43.46 ± 1.67 ; 30.10 ± 1.01) respectively, but the reduction in AP level (46.64 ± 1.01) was not significant. The activity of *parkia biglobosa* is comparable with acetylcysteine, a known hepatoprotective drug. Thus, *Parkia biglobosa* exhibits hepatoprotective activity against paracetamol toxicity.

Keywords: *Parkia Biglobosa*, Paracetamol, Liver Function Enzyme Markers, Acetylcysteine, Wistar Rats

1. Introduction

During the last decades considerable attention was focused on the involvement of oxygen free radical (OFR) in various diseases. Despite the presence of strong antioxidant defense mechanism to counteract the OFR and to minimize the plausible oxidative damage, OFR dependent damage to DNA and other biomolecules accumulate during the life time of organism (8). Active oxygen molecules such as superoxide and hydroxyl radicals have been demonstrated to play important role in the inflammation process produced by paracetamol (8). The liver is the most important organ concerned with the biochemical activities in the human body. It performs the normal metabolic homeostasis of the body as well as

biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals (13). Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences. A large number of xenobiotics such as paracetamol have been reported to be potential hepatotoxic agent (6). Paracetamol is a commonly used analgesic and antipyretic, and one of the most common causes of acute liver poisoning worldwide (11). It react with the basic cellular constituents of the liver like proteins, lipids, RNA and DNA to induce almost all types of lesions by causing the formation of a toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P4502E1 that can induce a dose dependent depletion of intracellular glutathione and alteration of calcium homeostasis (6).

Parkia biglobosa belongs to the plant family Fabaceae and sub family Mimosaceae of the order Leguminisae popularly known as the African locust bean tree which in Yoruba is called Igba, or Irugba, in Hausa as Dorowa and in Ibo as Origili (17; 18). The phytochemical screening of the methanolic extracts of *Parkia biglobosa* revealed the presence of saponins, tannins, terpenes, and phenols, reducing sugars, sterols, flavonoids (3). In Nigeria and other parts of West Africa where this plant *Parkia biglobosa* is grown, there have been reports from herbal medical practitioners (1) indicating that *Parkia biglobosa* stem bark was used in the treatment of diabetes mellitus, inflammatory diseases (2), pains (14), infections (15, 12) due to the presence of its active ingredients that has been reported to exhibit antioxidant properties (3), hence this study was aimed at investigating the effects of the methanolic extract of *Parkia biglobosa* on paracetamol induced liver damage in wistar rats.

2. Materials and Method

2.1. Preparation of *Parkia Biglobosa* Extracts

One kilogram of stem barks of *Parkia biglobosa* was collected and identified in the Chemistry Department of Bingham University, Karu, Nasarawa. It was rinsed and dried in the shade at room temperature and, thereafter, crushed with a mortar and pestle. The extract was obtained from the crushed bark using soxhlet apparatus and methanol as a solvent. The extract was evaporated till dryness under reduced pressure until a constant weight is obtained.

Chemicals

The AST, ALT and AP Kits used in this study were of analytical grade manufactured by RANDOX Laboratories Ltd and QUIMCA CLINICA APLICADA S.A respectively. The Paracetamol (acetaminophen, PCM) and Acetylcysteine were manufactured by Emzor pharmaceuticals and by Alexandria pharmaceuticals respectively.

2.2. Experimental Animals

Thirty (30) Adult wistar rats of both sexes weighing between 130-160 g were used for the study. The animals were randomly divided into 5 groups containing 6 rats per group. They were purchased from National Veterinary Research Institute (NVRI) VOM, Jos and acclimatized to animal house of the Faculty of Basic Medical Sciences, College of Medicine, Bingham University for about two (2) weeks. The animals were provided with standard diet and water *ad libitum*. The rats were kept under constant environmental condition and observed daily throughout the experimental work. The study was conducted in accordance with the ethical rules on animal experimentation and approved by the Ethical Committee of the College of Medicine, Bingham University.

2.3. Experimental Design

The extract was prepared by dissolving 2g of the extract in 50ml of distilled water and administered orally for period of 21 days. Group I received distilled water daily and serve as the normal control. Group II served as positive control and received orally a single daily dose of 500 mg/kg BW of paracetamol for 7 days. Group III served as the reference and received orally a single daily dose of 500 mg/kg BW of paracetamol and 140 mg/kg BW acetylcysteine for 21 days. Animals in Group IV and V received orally a single daily dose of 500 mg/kg BW of paracetamol and 100 mg/kg (Low dose), 200 mg/kg BW (High dose) of the methanolic extract *Parkia biglobosa* stem bark for 21 days respectively.

2.4. Animal Sacrifice and Organ Collection

Twenty four (24) hours after the last administration, the animals were sacrificed by cervical dislocation; the liver was collected and weighed. The liver samples were homogenized and centrifuged at 3000 rpm for 10 min. The serum was harvested to evaluate some liver enzymes activity such as alkaline phosphatase (AP), aspartate transaminase (AST) and alanine transaminase (ALT) (13).

2.5. Analytical Procedure

The Serum alkaline phosphatase (AP) was determined photometrically according to (9). The AST and ALT activity was estimated according to (14).

2.6. Statistical Analysis

The values are recorded as Mean \pm SEM at $p \leq 0.05$ significant different using student "t" test and one-way analysis of variance (ANOVA) and groups were compared by Duncan's multiple range test (DMRT) using SPSS Software Package 15.

3. Results

The current results showed significant increase ($p \leq 0.05$) in serum levels of AP, AST and ALT from 30.08 \pm 1.42, 24.95 \pm 1.42 and 31.64 \pm 1.06 to a 49.63 \pm 1.99, 39.41 \pm 1.989 and 78.58 \pm 1.72 respectively when treated with paracetamol as compared with the control groups (Table I). The effect of acetylcysteine on paracetamol induced liver damage observed that acetylcysteine significantly reduced ($p \leq 0.05$) serum levels of AP, AST and ALT from 49.63 \pm 1.99, 39.41 \pm 1.99 and 78.58 \pm 1.72 to 46.64 \pm 1.01, 30.10 \pm 1.01 and 43.46 \pm 1.67 respectively as compared with the positive control group (Table I). Groups treated with (low dose) 100 of the methanolic extract *Parkia biglobosa* stem bark exhibit significant increase ($p \leq 0.05$) in serum levels of AP from 49.63 \pm 1.99 to 65.42 \pm 1.58. However, a significant reduction in the serum levels of AST and ALT from 39.41 \pm 1.99 and 78.58 \pm 1.72 to 36.77 \pm 1.58 and 43.84 \pm 2.35 respectively were noticed when compared with the positive

control group (Table I). At a high dose of 200 mg/kg BW of the methanolic extract of *Parkia biglobosa* stem bark, a significant reduction ($p < 0.05$) in serum levels of AP, AST and ALT from 49.63 ± 1.99 , 39.41 ± 1.99 and 78.58 ± 1.72 to 26.58 ± 0.34 , 31.08 ± 0.34 and 33.68 ± 2.02 respectively in comparison with the positive control group (Table I).

Table I. Effect of methanolic extract of *Parkia biglobosa* stem bark on hepatic marker enzymes in the serum of paracetamol (acetaminophen, PCM) induced hepatotoxic and control rats

Groups	Alkaline Phosphatase (IU/L)	Aspartate transaminase (IU/L)	Alanine transaminase (IU/L)
I	30.08 ± 1.42	24.95 ± 1.42	31.64 ± 1.06
II	$49.63 \pm 1.99^*$	$39.41 \pm 1.99^*$	$78.58 \pm 1.72^*$
III	46.64 ± 1.01	$30.10 \pm 1.01^+$	$43.46 \pm 1.67^+$
IV	65.42 ± 1.58	$36.77 \pm 1.58^+$	$43.80 \pm 2.4^+$
V	$26.58 \pm 0.34^{**}$	$31.08 \pm 0.34^+$	$33.68 \pm 2.02^+$

Each value is mean \pm S.E.M for six rats in each group. * indicate values that are significant different when compared with the control group at ($p < 0.05$), + indicate values that are significant different when compared with the induced group at ($p < 0.05$).

4. Discussion

Paracetamol is a commonly used analgesic and antipyretic drug. Over dosage of it leads to the saturation of conjugation pathway leading to glutathione depletion and increase in the formation of toxic reactive metabolites (6). High level of reactive metabolites increase the level of hepatotoxicity with increased level of protein adducts formation, mitochondrial dysfunction and oxidative stress (6). Hepatotoxins may react with the basic cellular constituents like proteins, lipids, RNA and DNA and induce almost all types of lesions of the liver. Liver is among the organs most susceptible to the toxic effects of paracetamol overdose which occur as a result of the formation of a toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P4502E1 that can induce a dose dependent depletion of intracellular glutathione and alteration of calcium homeostasis (6). Increased using of synthetic drug therapy leads to many side effects and undesirable hazards. So there is a world wide trend to return to natural resources, which are culturally acceptable and economically viable (10). Acetylcysteine also called N-acetylcysteine or NAC, works to reduce paracetamol toxicity by replenishing body stores of the antioxidant glutathione. Glutathione react with the toxic NAPQI metabolite so that it does not damage cells and can be safely excreted (5). Many medicinal plants contain substantial amounts of antioxidants other than vitamin C, vitamin E and flavonoid, carotenoids which have been reported to prevent oxidative damage caused by free radical and may prevent the occurrence of disease, cancer and aging (21). The efficacy of the various preparations of *Parkia biglobosa* is widely acclaimed by the Hausa communities of northern Nigeria for the treatment of

some diseases like malaria, diabetes mellitus and pains due to its various components (7). The phytochemical screening revealed the presence of terpenes, saponosides, tannins, reducing sugar compounds and flavonoids (3). Flavonoids belong to the polyphenol family and are found in most plant materials. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral (4). Liver enzymes (ALT and ALP) are normally found in circulation in small amounts because of hepatic growth and repair. They are good indicators for liver state that increased when hepatocytes are damaged and they suffer from exacerbation and remissions irrespective to liver condition (19). On the other hand, ALP belongs to a group of enzymes catalyze the hydrolysis of phosphomonoesters at alkaline pH. ALP present in cell surface in most human tissues. The highest concentration is found in the intestine, liver, bone, spleen and kidney (21). The specific location of the enzyme within both sinusoidal and bile canalicular membranes accounts for the more predominant elevations in certain disorders (19). Flavonoids are powerful antioxidants against free radicals and are described as free-radical scavengers (4). This activity is attributed to their hydrogen-donating ability (21). Flavonoids are capable of modulating the activity of enzymes and affect the behavior of many cell systems and exerting beneficial effects on body (19). They terminate chain radical reaction by donating hydrogen atom to a peroxy radical as thus, forming flavonoids radical, which, further reacts with free radicals thus terminating propagating chain (20) leading to suppression of oxidative stress maintaining liver function and its enzyme activity. Hence due to the antioxidant effect that flavonoids exhibit, the presence of flavonoids might account for the therapeutic effect that was observed in *Parkia biglobosa*. The efficacy of the therapeutic effect of *Parkia biglobosa* extract was observed to have increased with increased concentration (dose-dependent manner). This may be due to the increased concentration of the active component of the extract thereby preserving the structural integrity of the hepato cellular membrane which was evident from the reduction in the enzyme activities against the paracetamol induced rise in the enzyme levels in the serum. It could be suggested that the leakage of enzymes because of liver injury was prevented by the liver cell membrane stabilizing action of flavonoids from the extract.

5. Conclusions

Therapies developed in line with modern medicine are often limited in their efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. Therefore, treating liver diseases with natural compounds, which are easily available and do not require laborious pharmaceutical synthesis seems highly attractive. From the results obtained in this study, it can be concluded that the methanolic extract of *Parkia biglobosa* stem bark exhibited therapeutic properties against

paracetamol induced liver damage. Therefore, the therapeutic effect of this extract will encourage its use in the treatment and management of various insult induced on the liver.

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