Journal of Advances in Medical and Pharmaceutical Sciences
7(3): 1-11, 2016, Article no.JAMPS.25194
ISSN: 2394-1111

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anti-oxidative and Hepato-protective Effects of *Pseudocedrela kotschyi* against Paracetamol Induced Liver Damage: A Biochemical and Histological Evaluation in Rats

Suleiman Ibrahim Eleha[1], Muhammed Abdurrasheed Ola[2*], Oyedeji Samuel Oyewole[3], Adeosun Oyebola Ganiyu[4], Afolabi Olugbenga Onaolapo[5] and Adam Moyosore Afodun[6]

[1]Department of Chemical Pathology, University of Ilorin Teaching Hospital, Ilorin, Nigeria.
[2]Department of Histopathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.
[3]School of Medical Laboratory Science, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.
[4]Department of Chemical Pathology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.
[5]Department of Pathology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.
[6]Department of Anatomy, University of Ilorin, Ilorin, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author SIE designed the study, did literature search and performed some of the biochemical analysis. Author MAO wrote the manuscript and performed the histological slides photomicrography / interpretation. Authors OSO and AOG performed the biochemical and statistical analyses. Author AOO did the histological tissue processing and slides preparation. Author AMA managed the animals and performed the extracts administration. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/25194
Editor(s):
(1) Hamdy A. Sliem, Internal Medicine, Suez Canal University, Egypt and College of Dentistry, Qassim University and EL-Jouf University, Saudi Arabia.
Reviewers:
(1) Atef Mahmoud Mahmoud Attia, National Research Centre, Egypt.
(2) Julia Pérez Ramos, Universidad Autónoma Metropolitana, Mexico.
(3) Walid Hamdy El-Tantawy, National Organization for Drug Control & Research, Cairo, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/14110

Original Research Article

Received 22nd February 2016
Accepted 31st March 2016
Published 9th April 2016

**Corresponding author: E-mail: muhammed.ola@udusok.edu.ng;*

ABSTRACT

Aim: Different parts of *Pseudocedrela kotschyi* have been widely used by traditional healers for treatment of various illnesses, many of which have been scientifically scrutinized. However, this study aimed at evaluating anti-oxidative and hepato-protective potentials of its aqueous leaf (LEPK) and methanol bark (BEPK) extracts in paracetamol intoxicated rats.

Methodology: Anti-oxidative and hepato-protective study was carried out by randomly dividing Thirty six (36) rats into nine groups with four (4) rats in each group. The route of administration was intraperitoneal, except Vitamin-C that was given orally. Group-1 received 5 ml/kg bwt single dose of Normal saline on the 6th day, Group-2 received a single dose of paracetamol (750 mg/kg bwt.) on 6th day. Group 3, 4 and 5 received 500, 250 and 500 mg BEPK /kg bwt. for 6 consecutive days. Group 6, 7 and 8 received 500, 250 and 500 mg LEPK/kg bwt. for 6 days. Group-9 received 500 mg vitamin-c/kg bwt. for 6 days. Group 4, 5, 7, 8 and 9 simultaneously received single dose of 750 mg paracetamol/kg bwt on the 6th day.

Results: The degree of protection and antioxidative potentials was measured using histomorphological analysis and assessment of biochemical parameters such as, transaminases (ALT and AST), malonialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). The doses studied produced various degree of hepato-protection by favourably altering biochemical parameters and showing histomorphological improvement. The effects of both extracts were comparable to that of standard vitamin-C.

Conclusion: It has been observed that the extracts could protect the liver cells from paracetamol damage, perhaps, by its antioxidative effect. The extract produced a significant ($p<0.05$) dose-dependent protection.

Keywords: Pseudocedrela kotschyi; hepato-protective; histomorphological; biochemical.

1. INTRODUCTION

Plants form an integral part of life in many indigenous African communities. They are readily and cheaply available alternative to allopathic medicines [1]. *P. kotschyi* has numerous uses in traditional medicine, particularly its bark, roots and leaves, thus it is an important source of ingredient for local medicine [2]. *Pseudocedrela kotschyi (emigbeegi)* is one of such medicinal plant that has been in use for a long time for treatment of liver cirrhosis by traditional healers [3]. Roots of *P. kotschyi* are commonly used as chewing sticks in West Africa and its extracts have been shown to inhibit the in-vitro growth and development of the schizont stage of *Plasmodium falciparium*, thus the root may provide affordable means of treating malaria [2]. The n-butanol soluble portion of the ethanolic extract of the leaves of *P. kotschyi* has been shown to possess anti-nociceptive and anti-inflammatory activities in mice and rats, respectively [4]. The n-butanol soluble aqueous leaf extract of the plant reduced the onset and the duration of the sleeping time, induced by phenobarbitone in rats. It also increased the depression and sedation time followed by sleep [5]. In Nigeria the stem bark is used in mixtures to treat trypanosomiasis in livestock, and leaves are administered in veterinary medicine against intestinal worms [3]. In North Côte d'Ivoire, it is of value in the treatment of toothache and internal wound, the root of the plant is also used to treat intestinal helminthosis, and has been found to be a potential source of antibacterial agents [6].

In Nigeria, hundreds of plants are used traditionally for the management of liver and renal diseases. To date, only a few of these medicinal plants have received scientific scrutiny, despite the WHO recommendation that medical and scientific examinations of such plants should be undertaken [7].

Acetaminophen (Paracetamol) is one of the more potentially dangerous analgesic drugs; it is mostly converted to inactive compounds via Phase II metabolism by conjugation with sulfate and glucuronide, with a small portion being oxidized via the cytochromes P450 enzyme system [8]. Cytochromes P450 2E1, (CYP2E1) and 3A4, (CYP3A4) convert approximately 5% of paracetamol to a highly-reactive intermediary metabolite, N-acetyl-p-benzo-quinoneimine (NAPQI) [9]. Under normal conditions, NAPQI is detoxified by conjugation with glutathione (GSH) to form cysteine and mercapturic acid conjugates which are excreted [10]. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue

macromolecules such as lipid or -SH group of protein and alters the homeostasis of calcium, thus resulting in widespread hepatocytes damage and death, leading to acute hepatic necrosis [11].

In spite of numerous established uses of *P. kotschyi* in treatment of various diseases; its anti-oxidative and hepato-protective effects has not been investigated, hence the essence of this study. There are scanty literatures on hepatoprotective activities of the BEPK and LEPK for this purpose, *Pseudocedrela kotschyi* was given prophylactically to rats which were then treated with acetaminophen.

2. MATERIALS AND METHODS

2.1 Plant Materials

Different plant parts; leaves and bark of *P. kotschyi* were collected from Kisi, Oyo State, South West Nigeria (equitorial vegetation). The plant was identified by a plant Taxonomist at University of Ilorin, Ilorin, Kwara State, Nigeria. The leaves and stem bark were separated from the plants and washed in tap water, rinsed with distilled water. The plant parts were then shade dried at room temperature till there was no loss of weight. They were grinded into coarse powder and stored at room temperature for further study.

2.2 Extraction Procedure

The methanol and aqueous extracts were prepared by soaking 100 g each of the dry powdered plant materials in 1 L of methanol and water at room temperature for 72 hours with occasional shaking for effective extraction. The extracts were filtered after 72 hours, first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40℃. The percentage yield of extracts ranged from 7–19%w/w.

2.3 Animals Treatment

Thirty six (36) healthy Wistar strain albino rats weighing between 150-200 g were obtained from the Laboratory Animal Centre of Osun State University, Osogbo, Osun State Nigeria. The rats were housed in clean metallic cages and kept in a well-ventilated room and allowed to acclimatize to the laboratory condition for two weeks before commencement of the study. They were fed with standard animal pellet and water *ad libitum*.

2.4 Drugs and Chemicals

Acetaminophen injection was purchased from DRUGFIELD Pharmaceutical Ltd, Sango-Otta, Nigeria. SGOT, SGPT kits were procured from DIALAB Diagnostics, Austria. Thiobarbituric acid (TBA), Pyrogallol, Hydrochloric acid and the rest of the chemicals utilized were of analytical grade and were purchased from ROVERT Scientific Limited, Edo, Nigeria.

2.5 Anti-oxidative and Hepato-protective Studies

The animals were weighed before the experiment and the difference in weight was not statistically significant. The animals were randomly divided into nine groups with 4 rats in each (n=4). Group 1 served as normal and positive control, while group 2, 3 and 6 served as negative, BEPK and LEPK controls respectively. Group 1 was given single dose of normal saline, 5 ml/kg bwt on the 6th day of the experiment; group 2 was given single dose of 750 mg/kg bwt of paracetamol on 6th day of the experiment; group 3 and 6 rats were given daily 500 mg/kg bwt of BEPK and LEPK respectively for 6 consecutive days. Group 4 and 5 were given BEPK 250 and 500 mg/kg bwt daily for 6 days. Group 7 and 8 were pretreated with 250 and 500 mg BEPK/kg bwt respectively for 6 days. Group 9 was pretreated with 500 mg/kg bwt Vitamin-C for 6 days.

On the 6^{th} day, Single dose of 750 mg/kg of acetaminophen was concomitantly administered to group 4, 5, 7, 8 and 9 animals. The entire animals were sacrificed by cervical dislocation 30 hrs post-treatment. Blood was drawn by cardiac puncture and its extremities were fixed to the dissection board with drawing pins. Vertical midline incision was done and skin and abdominal muscles were retracted laterally. Liver tissue were gently removed, weighed and examined macroscopically, they were then fixed immediately in 10% formol saline.

To ascertain hepatic damage induction, group 2 rats were firstly administered with 750 mg paracetamol/kg body weight intraperitoneally (ip). After 24 h, blood was withdrawn for ALT and AST estimations.

2.6 Biochemical Studies

The blood was obtained from all animals by cardiac puncture. The blood samples were

allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30℃ for 15 min and utilized for the estimation of various biochemical parameters namely ALT, AST using DIALAB commercial kits [12], serum SOD by the method of Marklund and Marklund, [13], MDA was measured by the method of Pasha and Sadasivadu [14]. After the separation of serum the residue thus obtained was lysed and heamolysate used for the estimation of catalase activity by the method of Sinha [15].

2.7 Histopathological Study

5 mm^2 thick tissue pieces were excised from the liver and fixed in 10% formol saline solution, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4 µm thickness were cut and stained with hematoxylin and eosin (H&E). These sections were examined microscopically for histopathological changes.

2.8 Statistical Analysis

The experimental results were expressed as the Mean ± SEM for animals in each group. The biochemical parameters were analysed statistically using one-way analysis of variance ANOVA, followed by Dunnett's multiple comparison test (DMCT). P value of < 0.05 was considered as statistically significant.

3. RESULTS

The results of biochemical and histological analyses are presented in figures and tables below:

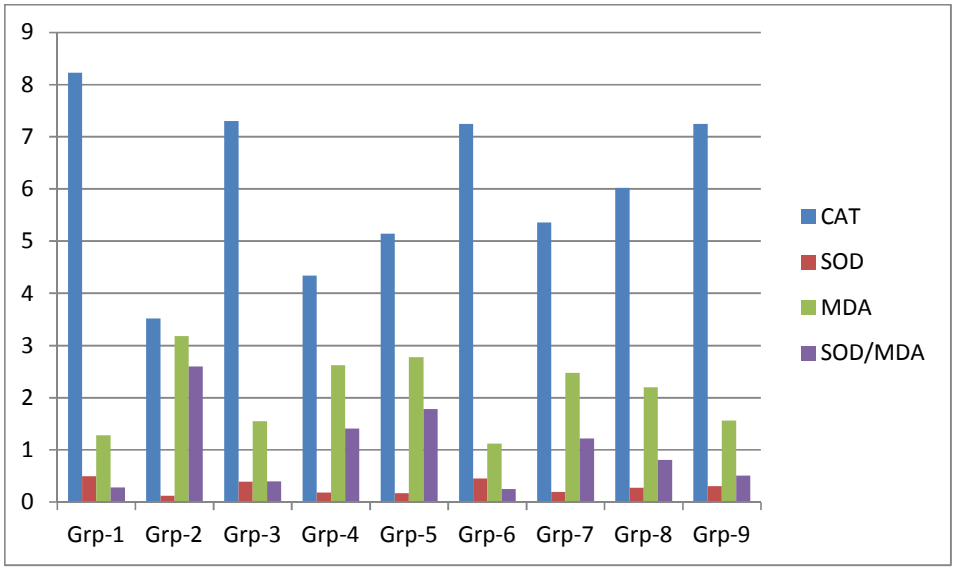

Fig. 1. Pictorial representation of redox parameters in various groups

For CAT and SOD/MDA, the value from Fig.-1 has to be multiply by ×10 to arrive at the actual value

Table 1. The result of redox biochemical parameters in various groups

Group	CAT (unit/ml of H_2O_2 decomposed)	SOD (unit/mg protein)	MDA (nmol/dl)	SOD/MDA
1	82.76±7.22	0.50±0.17	1.28±0.34	2.80±0.39
2	35.20±5.03*	0.12±0.13*	3.18±0.85*	25.98±3.19*
3	72.94±3.90*	0.39±0.14*	1.55±0.28	3.95±0.71
4	43.45±2.85**	0.18±0.05**	2.62±0.27	14.09±2.79**
5	51.37±2.41**	0.17±0.45	2.78±0.15	17.80±2.75**
6	72.47±3.00*	0.45±0.45	1.12±0.13	2.49±0.47
7	53.55±5.04**	0.20±0.15**	2.47±0.80**	12.20±0.81**
8	60.23±3.58**	0.27±0.17**	2.20±0.33**	8.17±1.45**
9	72.54±4.15**	0.31±0.11**	1.56±0.23**	5.06±0.29**

*Values are Mean ± SEM; n = 4 animals in each group; *P<0.05 is considered significant when compared with group I; **P<0.05 is considered significant when compared with group 2 by Dunnett's multiple comparison test*

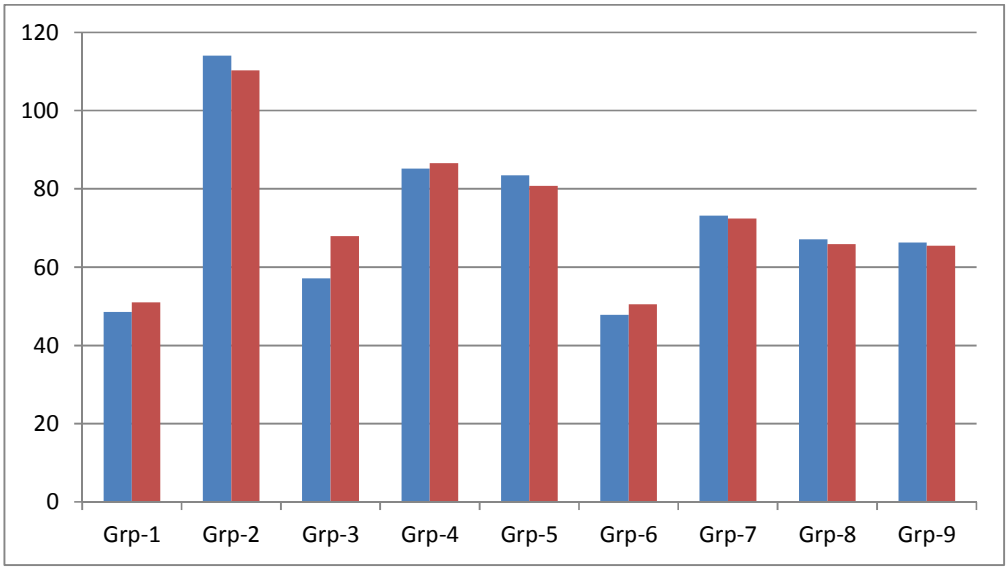

Fig. 2. Pictorial representation of liver function test in various groups

3.1 Result of Liver Histopathology

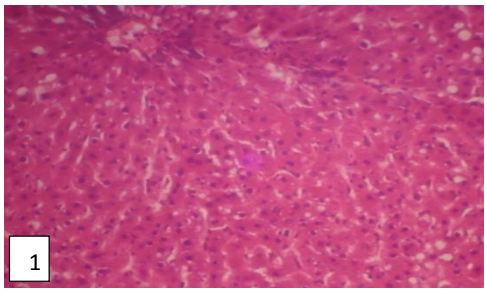

Fig. 3a. Photomicrograph of liver section of normal control rats (group-1) showing normal hepatic cells with central vein and sinusoidal dilation (H & E ×100)

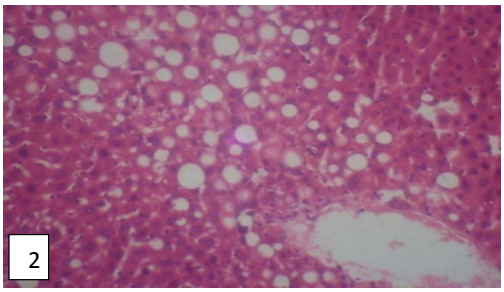

Fig. 3b. Photomicrograph of liver section of paracetamol control rats (group-2) showing severely degenerated hepatic cells, portal inflammation with considerable architectural distortion

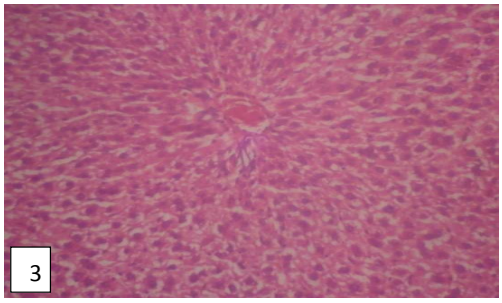

Fig. 3c. Photomicrograph of liver section of rats treated with 500 mg BEPK/kg bwt. only (group 3); note mild portal inflammation, with narrowing of sinusoid, normochromic with preserved hepatic architecture

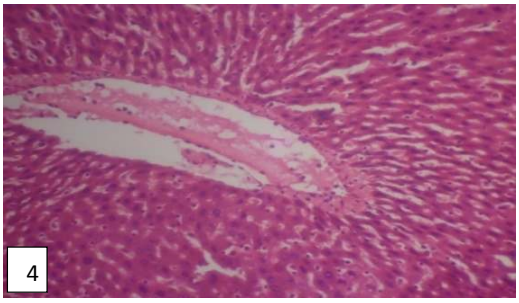

Fig. 3d. Photomicrograph of liver section of rats pretreated with 250 mg BEPK /kg bwt. (group 4); note moderately degenerated hepatic cells, moderate portal inflammation with slight architectural disorganization

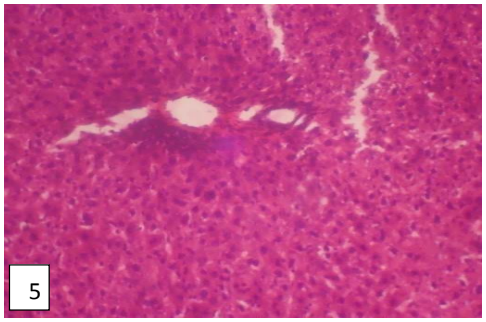

Fig. 3e. Photomicrograph of liver section of rats pretreated with 500 mg BEPK/ kg bwt. (group 5); note mild portal inflammation and very mild degenerative changes in comparison to the section in Fig. 3d.

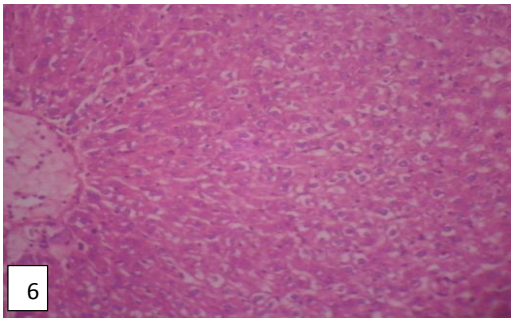

Fig. 3f. photomicrograph of liver section of rats treated with 500mg LEPK/kg bwt. only (group 6); histopathological appearances are similar to that of normal rats except with very mild perilobular necrosis.

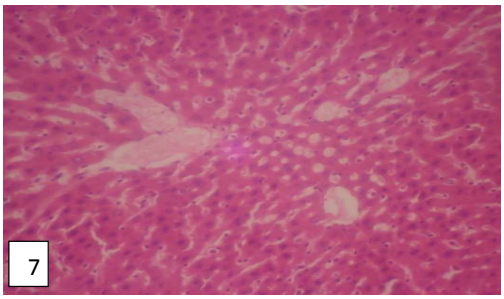

Fig. 3g. Photomicrograph of liver section of rats pretreated with 250 mg LEPK/ kg bwt. (group 7); showing mild necrosis, normochromic with preserved hepatic architecture

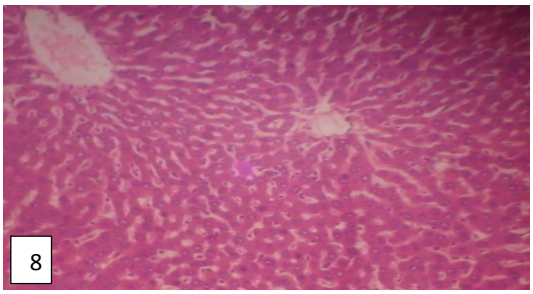

Fig. 3h. Photomicrograph of liver section of rats pretreated with 500 mg LEPK/kg bwt. (group 8); note moderate antagonism of paracetamol induced degenerative changes, except with very mild centrilobular necrosis

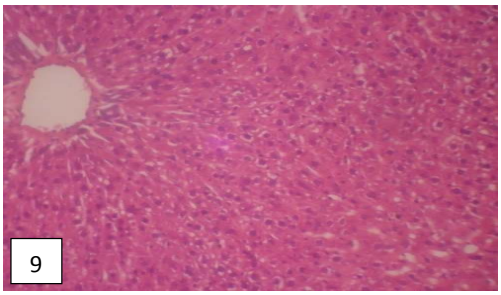

Fig. 3i. Photomicrograph of liver section of rats pretreated with 500 mg Vit-C/ kg bwt. (group 9); hepatocellular appearances are almost similar to that of normal rat (group-1)

4. DISCUSSION

Paracetamol is the most commonly prescribed analgesic and antipyretic drug [16]. The drug is safe at therapeutic levels, but its overdose can lead to hepatic and renal damage in both humans and experimental animals [17]. The blood draining the stomach and small intestine is delivered directly to the liver via the hepatic portal vein, thus exposing the liver to relatively large concentrations of ingested drugs or toxicants [18]. The central role of the liver in the drug metabolism further predisposes it to toxic injury, because drug metabolism may sometimes go awry, leading to the formation of electrophilic toxic metabolites [19]. The electrophiles interact with cellular proteins to cause direct cellular injury, it may also form protein-drug adducts that become targets for immune-mediated injury [19]. Liver injury associated with overdose is

frequently due to ingestion of paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs), along with antimicrobial agents and antiepileptic's, these are the most frequent causes of liver injury with conventional doses [20].

4.1 Hepatic Biochemical Assesment

Paracetamol induced hepatotoxic effect was assessed by the level of released cytoplasmic enzymes in circulation. The study of different enzymes activities such as ALT, AST and other hepatic parameters have been found to be of great value in the assessment of clinical and experimental liver damage [21].

The outcome of this study showed that the plasma levels of ALT and AST were significantly elevated ($P < 0.05$) above two fold in group-2 rats in comparison with the positive control group (Table 2). The increased levels of plasma enzymes indicate an enhancement of permeability, damage or necrosis of hepatocytes by toxic intermediate of acetaminophen (NAPQI) [22]. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane [23]. The result is in agreement with those reported by earlier researchers [19,22,24].

Table 2. The result of liver function parameters in various groups

Group	ALT (U/L)	AST (U/L)
1	48.5±3.20	51.00±2.60
2	114.07±8.77*	110.23±8.80*
3	57.17±1.90*	67.94±4.45*
4	85.18±4.58**	86.58±4.19**
5	83.44±2.10**	80.77±2.51**
6	47.87±3.10	50.54±7.42
7	73.19±3.10**	72.36±2.19**
8	67.07±5.13**	65.83±4.90**
9	66.28±2.28**	65.47±2.13**

*Values are Mean ± SEM; n = 4 animals in each group; * P<0.05 is considered significant when compared with group 1; ** P<0.05 is considered significant when compared with group 2 by Dunnett's multiple comparison test*

Results indicated that treatment with BEPK only at 500mg/kg body weight for 6 consecutive days exerted a significant ($P < 0.05$) change in the plasma level of ALT and AST when compared to group 1 (Table 2). This showed that BEPK may possess mild hepatic toxicity at this dose. Contrarily, equal treatment with LEPK resulted in changes in the enzymes level in group 6 rat, the difference of which was statistically insignificant ($P > 0.05$) with respect to group 1.

The results of the enzymes assay in group 4, 5, 7, and 8 were reduced and their differences with respect to the paracetamol treated group were statistically significant ($P < 0.05$). The changes were more pronounced in group 8 than others. This showed that the formulations were slightly and variedly effective in attenuating acute increase in enzymes level. The normalization of the hepatic markers at the concentrations of the extracts is an indication that the extracts proffer mild protection to hepatocytes against acetaminophen induced leakage of enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes.

Oral administration of Vitamin-C at dose of 500mg/kg was able to prevent the toxic effect of Acetaminophen on the liver as demonstrated by normalization of ALT and AST activity in group 9 in relation to the negative control. There is scanty available literature on the comparative hepato-protective effect of leaf and bark extracts.

4.2 Redox Biochemical Assessment

4.2.1 Serum SOD activity

The Free radicals and disorders of antioxidant defense system have a pathogenic impact on human tissues and hence are seen as important factors in the development of various diseases [25]. Molecular oxygen produces toxic compounds such as superoxide, hydrogen peroxide, hydroxyl and hydroperoxyl radicals, which are very active oxidants and have the potential to destroy cell components such as unsaturated lipids, proteins, and DNA [26].

Administration of a single dose of 750 mg paracetamol /kg body weight was found to lower the serum level of SOD (Table 1); the difference was statistically significant ($P < 0.05$) when compared to control group 1. This finding is in agreement with the observation of Francessco and colleagues [19].

The effect of various treatments on plasma SOD level in rats is shown in Table 1. In this study, plasma level of SOD in group 3 was slightly lowered with respect to group 1 (Table 1); the difference was statistically significant ($P < 0.05$). Contrarily, the difference observed in group-6 was statistically insignificant ($P > 0.05$) in relation to the normal control group. It could be inferred

then that BEPK induced mild oxidative stress and not LEPK.

The SOD contents in various groups were variedly consumed, thus there were decreases in SOD level in group 4, 5, 7 and 8, (Table 1). However, the differences were statistically significant ($P < 0.001$) in group 4, 7 and 8, while in group 5 the difference was insignificant ($P > 0.05$) with respect to paracetamol intoxicated group.

Pretreatment with Vitamin-C showed significant increase ($P < 0.05$) in SOD activity despite administration of toxic dose of paracetamol (Table 1), when compared with group 2.

The result of this study showed that BEPK and LEPK at the doses study possess a moderate potential to reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of antioxidant enzymes.

4.2.2 Catalase activity in lysate

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [27].

The levels of CAT activities in various groups are shown in Table 1. Like other parameters, CAT activity in paracetamol intoxicated rats group was found to be significantly lower ($P < 0.05$) than in normal group. The reduction in the activity of these enzymes as evident in this study may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide [26].

In the present study, the percentage decrease in catalase level in group 3 and 6 was slightly high, (11.9% and 12.4% respectively); however, the differences were statistically insignificant ($p > 0.001$) in relation to group 1 (Table 1).

Total CAT activities in group 4, 5, 7 and 8 were discovered to be significantly higher with respect to paracetamol toxic group; all the differences were statistically significant ($P < 0.001$).

In addition, pretreatment with Vitamin-C at 500 mg/kg almost cushion changes in the catalase activity, the difference was statistically significant ($P < 0.01$) in relation to group 2 (Table 1).

Conclusively, pretreatment with BEPK and LEPK was observed to antagonize the effect of paracetamol action on catalase activity, thus prevent the accumulation of excessive free radicals and to some extent protected the liver from paracetamol intoxication.

4.2.3 Serum lipid peroxidation

Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. Malondialdehyde (MDA) is one of the end products in the lipid peroxidation process [27].

The outcome of this study showed that MDA contents were significantly increased ($P < 0.05$) in paracetamol control group when compared to normal group (Table 1). The increase in MDA levels, as evident in this study suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [26]. Comparable and similar observations have been earlier reported [28,29].

Treatment with each of the extract, BEPK and LEPK in group 3 and 6 respectively resulted in an insignificant ($P > 0.05$) increase in MDA level compared to normal control. The percentage increase observed were 21.0% and 12.5% respectively.

The treatment administered to rats in group 4 and 5 failed to counterbalance the increase in lipid peroxidation process, as a result of that, a significant ($P < 0.05$) rise in MDA level was observed in the two groups (Table 1).

However, pretreatment with LEPK both at 250 and 500 mg/kg with that of Vit-C at 500 mg/kg resulted in significant ($P < 0.05$) decrease in MDA with respect to group 2. Thus those formulations mitigated acute increase in MDA level; so, it may be possible that the mechanism of hepato-protection by LEPK is due to its antioxidative effect.

4.2.4 MDA/SOD ratio

Tightly controlled ROS generation appears to be one of the central elements in the mechanisms involved in cell function, growth, differentiation and death [30]. Maintaining a physiological equilibrium between intracellular levels of antioxidants and the production of reactive oxygen species (ROS) is crucial for the survival of organisms. An increase in ROS generation

beyond the ability of antioxidative protection, i.e. oxidative stress, potentially leads to cellular damage [30]. The ratio of MDA/SOD may be considered as an index of OS. [30,31].

The MDA/SOD ratio in different rat groups is shown in table 1. The ratio was significantly ($P < 0.05$) high in paracetamol treated group when compared to the positive control; the percentage increase observed was 828% indicating high oxidative stress. This is due to high peroxidation rate.

The treatment outcome in group 3 and 6 revealed slight decrease in MDA/SOD values in the two groups, the differences were however not significant ($P > 0.05$) statistically in relation to group 1. This is an indication that administration of the extract did not induce oxidative stress at the doses study.

The percentage changes in MDA/SOD ratio level were found to be high in group 4, 5, 7 and 8 (Table 1), the differences were significant ($P < 0.05$) in relation with paracetamol toxic group.

The MDA/SOD values in vitamin-c pretreated group as shown in Table 1 was raised, the differences were statistically significant ($P < 0.05$) in relation to group-2.

4.3 Liver Histopathology

The basic structural component of the liver is the hepatocyte and it grouped in interconnected plates, constituting two-thirds of the mass of the liver. The liver structural units is called liver lobules, the liver lobule is formed of a polygonal mass of tissue about 0.7 x 2 mm in size, with portal spaces at the periphery and a vein, called the central or centrilobular vein, in the center [32]. Blood flows from the periphery to the center of the liver lobule**.** Consequently, oxygen and metabolites, as well as all other toxic or nontoxic substances absorbed in the intestines, reach the peripheral cells first and then reach the central cells of the lobule. This direction of blood flow partly explains why the behavior of the perilobular cells differs from that of the centrilobular cells [18]. This duality of behavior of the hepatocyte is particularly evident in pathological specimens, where changes are seen in either the central cells or the peripheral cells of the lobule [33]. Hepatic necrosis can be classified by the zone of the Liver tissue affected. Xenobiotics, such as acetaminophen, that undergo bioactivation to toxic intermediates cause necrosis of the cells surrounding the central veins (centrilobular) because the components of the cytochrome P450 system are found in those cells in abundance [18]. At higher doses or in the presence of agents that increase the synthesis of cytochrome P450 (inducers), the area of necrosis may incorporate the peripheral region [18].

Histopathological studies of rat liver tissue from Group1 show normal hepatic cells with central vein (V) and sinusoidal dilation (Fig. 3a).

In paracetamol treated rats (Group 2), severe hepatocellular damage was observed; histological findings revealed a remarkable diffuse necrosis, disappearance of nuclei with hepatic architectural disorganisation (Fig. 3b). Similar observations have earlier been reported by Gulnaz et al. [34]. Some Authors also said that Paracetamol intoxication can result in severe hepatic damage characterized by hemorrhagic centrilobular necrosis in both humans and animals [35].

Histomorphological evaluation of the liver sections in group 3 and 6 revealed very mild hepatocellular changes; such as mild portal inflammation. These observations further support the absence of significant hepatotoxicity in both BEPK and LEPK at 500 mg/kg body weight formulation (Fig. 3c and 3f respectively).

Pretreatment with BEPK at 250 and 500 mg/kg bwt resulted in moderate centrilobular necrosis. Necrotic area is wider in group 4 than 5 (Fig. 3d and 3e respectively). The levels of damage in both cases are minimal in comparison to that of group 2, thus the two formulations mildly mitigated toxic effect of paracetamol.

The photomicrograph of group 7 and 8 showed minimal distortion in the hepatic architecture with respect to the group 2 (Fig. 3g and 3h respectively). This observation proved that both doses were able to cushion the effects of paracetamol toxicity.

In group 9, the micrograph revealed apparently normal hepatic cells, with preserved histoarchitecture (Fig. 3i). This finding showed that vitamin-C at the dose studied completely prevent the structural toxic effect of paracetamol.

The observed activity in liver histopathology profile is supportive of the fact that there is hepatoprotective activity in these test formulations, to some extent.

5. CONCLUSION

In conclusion, the results of this study demonstrate that both BEPK and LEPK have a potent hepato-protective action against paracetamol-induced hepatic damage in rats. Our results also show that the hepato-protective effects of both extracts may be due to its antioxidant and free radical scavenging properties. Further, investigation is underway to determine the exact phytoconstituents that is responsible for its hepato-protective activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

DECLARATION

All Authors hereby declared that “Principles of laboratory animal care” (NIH publication No.85 - 23, revised 1985) were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wagate CG, Mbara JM, Gakuya DW, Nayingi MO, Kareu PG. Screening of some Kenyan medicinal plants for antibacteria activity. Phytoth Res. 2010;24:150-153.
2. Kassim OO, Loyevsky M, Amonoo H, Lashley L, Ako-Nai KA, Gordeuk VR. Inhibition of *in-vitro* growth of *Plasmodium falciparum* by *Pseudocedrela kotschyi* extract alone and in combination with *Fagar azanthoxyloides* extract. Trans R Soc Trop Med Hyg. 2009;103:698-702.
3. Udeme OG, Owunari AG. Effect of extract of *Pseudocedrela kotschyi* on blood glucose concentration of alloxan induced diabetic albino rats. Eastern Journal of Medicine. 2009;14:17-19.
4. Musa YM, Haruna AK, Ilyas M, Yaro A, Ahmadu AA, Usman H. Phytochemical, analgesic and anti-inflammatory effects of the ethyl acetate extract of the leaves of *Pseudocedrella Kotschyi.* African Journal of Traditional, Complimentary and Alternative Medicines. 2008;5(1):92-96.
5. Anuka JA, Ijezie DO, Ezebnik ON. Investigation of pharmacological actions of the extract of *Pseudocedrela kotschyi* in laboratory animals. Annual Regional Conference of WASP. 1999;9-10.
6. Koné WM, Atindehou KK, Terreaux C, Hostettmann K, Traore D, Dosso M. Traditional medicine in North Côte d’Ivoire: Screening of 50 medicinal plants for antibacterial activity. J. Ethnopharmacol. 2004;93:43-49.
7. World Health Organization. Expert committee on Diabetes Mellitus: Second Report. Technical Report Series. WHO, Geneva. 1980;646-661.
8. Heard KJ. Acetylcysteine for acetaminophen poisoning. The New England Journal of Medicine. 2008;359(3): 285–292.
9. Richardson JA. Management of acetaminophen and ibuprofen toxicoses in dogs and cats. J Vet Emerg Crit Care. 2000;10:285-291.
10. Rumbeiha WK, Lin YS, Oehme FW. Comparison of N-acetylcysteine and methylene blue, alone or in combination, for treatment of acetaminophen toxicosis in cats. American Journal of Veterinary Research. 1995;56(11):1529–1533.
11. Mary EL. The pharmacotherapy of chronic pain. Rheum Dis Clin N Am. 2008;34:369-385.
12. Bergmeyer HU, Bernt E, Grassi M, Micheal G. GOT and GPT in UH Bermmeyer. Methods of enzymatic analysis. Verlag Chemie Weinhelm. Academic Press New York. 1978;458-460.
13. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry. 1974;47:469–474.
14. Pasha KV, Sadasivadu B. Intracellular content of thiol compounds, thiobarbituric acid reactive substances and gamma-glutamyl transpeptidase in rat brain during anoxia. Neurosci Lett. 1984;46:209-214.
15. Sinha KA. Colorimetric assay of catalase. Anal Biochem. 1972;47:389-394.
16. Bertolini A, Ferrari A, Ottani A, Guerzoni S, Tacchi R, Leone S. Paracetamol: New

vistas of an old drug. CNS Drug Rev. 2006;12:250-275.
17. Slitt AL, Naylor L, Hoivik J, Manautou JE, Macrides T, Cohen SD. The shark bile salt 5 beta-scymnol abates acetaminophen toxicity, but not covalent binding. Toxicol. 2004;203:109-121.
18. Charles RC, Robert ES. Modern pharmacology with clinical application. Principle of Toxicology, 5th edition 2006;56-67.
19. Francesco M, Harium Y, Upendra G, Amr H, Shalini J, Emilio M. Protective effect of phytocompound on oxidative stress and DNA fragmentation against paracetamol-induced liver damage. Annals of Hepatology. 2009;8:1.
20. Andrade RJ, Robles M, Fernández-Castañer A, López-Ortega S, López-Vega MC, Lucena MI. Assessment of drug-induced hepatotoxicity in clinical practice: A challenge for gastroenterologists. World J Gastroenterol. 2007;13(3):329–40.
21. Vaishwana I, Kowale CN. CNS effect of two ayurvedic drugs Shilajeet and Edinolon changes in liver and serum lipids produced by carbontetrachloride. Ind J Exp Biol. 1976;14:58-61.
22. Malaya G, Upal KM, Thangavel SK, Periyasamy G, Ramanathan SK. Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetra-chloride induced liver damage in rats. Iranian Journal of Pharmacology & Therapeutics. 2004;3(1): 12-20.
23. Fan G, Tang JJ, Bhadauria M, Nirala SK, Dai F, Zhou B, Li Y, Liu ZL. Resveratrol ameliorates carbon tetrachloride-induced acute liver injury in mice. Environ Toxicol Pharmacol. 2009;28(3):350-356.
24. Lee WM. Acetaminophen and the US acute liver failure study group: Lowering the risks of hepatic failure. Hepatology. 2004;40:6-9.
25. Mahadk SP, Evans D, Lal H. Oxidative stress and role of antioxidant and omega-3 essential fatty acid supplementation in schizophrenic proga neuropscycho pharmacol. Biol Pschiciatry. 2001;25:463-493.
26. Catherine G, Paul AF, Kais HA. Developmental changes in antioxidant enzymatic defences against oxidative stress in sheep placentomes. Journal of Endocrinology. 2010;205:107–116.
27. Kurata M, Suzuki M, Agar NS. Antioxidant systems and erythrocyte life span in mammals. Biochem Physiol. 1993;106: 477-487.
28. Vermeulen NPE, Bessems JGM, Vandestreat R. Molecular aspects of paracetamol induced hepatotoxicity and its mechanism based prevention. Drug Metab Rev. 1992;24:367-407.
29. Deepak KD, Veerendra CY, Siva SN, Tirtha G, Rajalingam D, Pinaki S, et al. Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R. Br. on paracetamol-induced hepatotoxicity in rats. Tropical Journal of Pharmaceutical Research. 2007;6(3):755-765.
30. Jyothi P, Riyaz N, Nandakumar G, Binitha MP. A study of oxidative stress in paucibacillary and multibacillary leprosy. Indian Journal of Dermatology, Venereology and Leprology. 2008;74(1):80-83.
31. Bhadwat VR, Borade VB. Increased lipid peroxidation in lepromatous leprosy. Indian J Dermatol Venereol Leprol. 2000;66:121-125.
32. Guyton AC. Text Book of medical physiology; Kidney Disease and Diuretics, Elsevier Sunder 16th Edition. 2006;438-452.
33. Junqueira L, Carneiro J. Basic histology; Text and Atlas. Liver. Lange, Ottawa, USA. 2005;158-161.
34. Gulnaz H, Tahir M, Munir M, Sami W. Protective effects of garlic oil on acetaminophen induced nephrotoxicity in male albino rats. Biomedica. 2010;26:9-15.
35. Valentovic PL, Kolarovic M, Gasparikovs LO. Effect of osmotic stress on stability and water relations in two maize cultivas. Plant Soil Environ. 2006;52:186-191.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14110