Journal of Advances in Medical and Pharmaceutical Sciences
12(3): 1-11, 2017; Article no.JAMPS.31357
ISSN: 2394-1111

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Hepatoprotective Effect of Aqueous Bark Extract of *Bosweillia dalzielii* against Paracetamol Induced Hepatotoxicity in Rabbits

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MSI and IUD managed the analyses of the study. Author JAM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2017/31357
Editor(s):
(1) Franciszek Burdan, Experimental Teratology Unit, Human Anatomy Department, Medical University of Lublin, Poland.
(2) Jinyong Peng, College of Pharmacy, Dalian Medical University, Dalian, China.
Reviewers:
(1) D. A. Omoboyowa, Akanu Ibiam Federal Polytechnic, Nigeria.
(2) Anthony E. Ojieh, Delta State University, Abraka, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/18505

Original Research Article

Received 1st January 2017
Accepted 28th March 2017
Published 4th April 2017

ABSTRACT

Aim: This study assessed the hepatoprotective potential of aqueous stem bark extract of *Boswellia dalzielii* on paracetamol-induced hepatotoxicity in adult rabbits.
Study Design: Fifteen adult rabbits weighing between 1.4-2.0 kg were divided into five groups (I, II, III, IV and V), where group I served as normal control, group II was the test control, while groups III, IV and V served as test groups. Paracetamol (300 mg/kg was administered to the test control and the test groups to induce hepatotoxicity. Groups III, IV and V were treated for 7 days with 200 mg/kg Silymarin, 150 mg/kg and 225 mg/kg/Body weight of *Boswellia dalzielii* stem bark extract respectively, while group II was left untreated and served as the test control.
Place and Duration of Study: Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University Kano between August and November, 2015.
Methodology: Using standard laboratory procedures, liver function was assessed by the assay of

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alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), total and direct bilirubin (TB and DB). Data was analyzed using one-way ANOVA using SPSS. P values ≤ 0.05 were considered significant.

Results: The two doses of the plant extract showed dose-dependent hepatoprotective effect on Paracetamol-induced hepatotoxicity, as evident by the significant reduction ($P < 0.05$) in serum levels of AST, ALT, ALP and bilirubin alongside with improved histopathological liver sections compared to paracetamol-treated animals.

Conclusion: The aqueous extract of *Boswellia dalzielii* could serve as a good alternative to relieve the toxic effect of paracetamol on rabbit liver. And also, it could provide a suitable source for new drug development.

Keywords: Hepatoprotective; Bosweillia dalzielli; paracetamol; hepatotoxicity; histopathology.

1. INTRODUCTION

The liver is a vibrant organ found in vertebrates and some animals, and is characteristically the principal visceral organ. One of the major functions of the liver is the transformation and clearance of chemicals and it is highly susceptible to toxicity from these chemicals. Some pharmaceutical agents may injure the liver even when taken in therapeutic doses not necessarily when taken in overdoses [1]. Drug-induced liver diseases are caused by both prescribed medications, over-the-counter medications, illicit drugs, herbs etc through a range of mechanisms [2]. These diseases are mainly caused by hepatotoxic chemicals (acetaminophen, carbon tetrachloride and alcohol), infections and autoimmune disorders. The hepatotoxic chemicals caused damage to liver cells mainly by inducing lipid peroxidation and other oxidative damages [3].

Medicinal plants are of great importance to individual's health of and the community at large. The main objective strategy in the management of liver diseases is to terminate the sequential consequences at the pre-fibrotic stage of the liver [4]. Naturally based compounds are now employed in the effective treatment of certain liver disease, this in turn cause contemporary medicines to have little contribution to offer for lessening hepatic diseases [4]. Also, there is an improved consideration in these natural products that may neutralize the harmful effects of environmental or toxic chemical compounds and prevent numerous hepatic disorders in humans [5].

Paracetamol is a widely used analgesic and antipyretic drug; it produces acute liver damage in high doses. Paracetamol overdose induces acute liver injury via heightened oxidative stress and glutathione (GSH) reduction [6].

Boswellia dalzielii Hutch is a tree plant of the savannah forest belonging to the family of "*Burseraceae*". It is commonly known as the "Frankincense tree". The tree has a characteristic smooth, pale brown bark that peels off in ragged papery patches, which on rapping exudes a whitish fragrant resin. It grows up to 13metre high [7]. The tree is planted in the northern parts of Nigeria like Kebbi [8] Kano, [9] Kaduna [10] where the Hausa speaking people refer to it as 'ararrabi'. It is locally abundant in Togo, Cote d' Ivoire, Cameroon, Benin, Ivory coast, Poland, Ghana, Burkina Faso, Nigeria and Czech Republic [11]. The plant is used to treat tuberculosis, [12], gingivitis, [13], skin diseases, digestive disorders, nervous disorders, musculoskeletal disorders, breathing difficulties and certain infections [14].

Ethuk et al. [8] reported that the LD_{50} of the aqueous extract of the stem bark of *Boswellia dalzielii* is above 3000mg per kilogram body weight, which was considered to be moderately non-toxic when administered orally. The effect of aqueous bark extract of *B. dalzielii* on liver function using female Wistar albino rats have been investigated where the extract was found to have no substantial effect on albumin, bilirubin content, total protein and alkaline phosphate activity at the completion of oral treatment, which lasts for five days [15]. These results suggest that the exposure didn't result in any damage to the hepatocytes and that the extract may help to strengthen the liver. In view of the potentials of Bosweillia dalzielli in medicine, the present study is aimed at evaluating the hepatoprotective effect of the aqueous extracts of stem bark of *Bosweillia dalzielii* on liver function using rabbits induced with paracetamol hepatotoxicity.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Sample

The stem bark of *Boswellia dalzielii* was collected from Fagwalawa village of Dambatta Local Government Area, Kano in July 2015. The plant was identified by Baha'uddeen Sa'id Adam, a plant taxonomist in the Department of Biological Sciences, Bayero University, Kano. A herbarium accession number 0362 was deposited at the Department's herbarium. The stem bark was dried under the shade and pulverized into powder.

Picture 1. *Bosweillia dalzielii* tree and leaf [16]

2.2 Preparation of Plant Extract

Five grams (5 g) of the pulverized stem bark of *Boswellia dalzielii* was steeped in 1000ml of distilled water and kept at room temperature for 24 hours. Thereafter, the mixture was filtered using a white silky cloth, the residue was dried and its weight taken.

2.3 Phytochemical Analysis of the Plant Extract

The aqueous extract of *Bosweillia dalzielii* was subjected to preliminary phytochemical screening to determine the presence of some phytochemical constituents. The phytochemical screening was conducted according to the standard procedures described by Trease and Evans [17] and Sofowora [18].

Picture 2. Stem bark of *Bosweillia dalzielii* [16]

2.4 Preparation of Paracetamol

Paracetamol tablets BP (500 mg) GlaxoSmithkline Consumer Nigeria Plc was purchased from a pharmacy shop. Five (5 g) of paracetamol was reconstituted in 100 ml distilled water to make a 5% solution. The preparation was made fresh whenever treatment was to be introduced.

2.5 Animals

Male and female adult rabbits weighing between 1.4 - 2.0 kg were obtained from Sabongari market in Kano State. The animals were allowed to acclimatize with the new environment for two weeks. The males were separated from the females and kept in cages. They were maintained on feed and in the experimental facility provided standard layer feeds, lettuce, spinach and water *ad libitium* throughout the experimental period. The ethical guidelines regarding animal care as approved by the Institutional Animal Ethics Committee of the Department of Biochemistry, Bayero University, Kano were observed during the study.

2.6 Study Design

Fifteen male and female rabbits were divided into five groups of 3 rabbits each. Group 1 did not receive any dose of the extract neither did they receive any induction thus, serves as the normal control, group 2 which served as the test control received 300 mg/kg paracetamol. Then groups 3,

4, and 5 which served as the test groups receives 300 mg/kg of paracetamol each and 200 mg/kg body weight Silymarin, then 150 mg/kg and 225 mg/kg of the extract, respectively for 7 days.

Twenty four hours after the last administration, samples of blood were collected from the animals in which serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were determined, and the concentration of bilirubin (direct and total) were also determined.

2.7 Biochemical Analysis

Serum AST, ALT and ALP activities, Albumin, total protein and Bilirubin (total and direct) concentrations were determined using reagents and kits obtained from Randox Laboratories, UK.

2.8 Determination of Serum AST and ALT

For serum AST and ALT estimation, reagent blank was prepared by mixing 0.5 ml of reagent 1 with 0.1 ml of distilled water. While for serum samples, 0.1 ml of each sample was mixed with 0.5 ml of reagent. These mixtures were incubated for exactly 30 mins at 37ºC, then 0.5 ml of reagent 2 was added to both the reagent blank and sample test tubes and allowed to stand for 20 mins at 23ºC. Sodium hydroxide (5.0 ml) was added and mixed, then absorbance of the samples against the reagent blank was read after 5minutes at 546 nm [19].

2.9 Determination of Serum ALP

For each sample, 0.5 ml of alkaline phosphatase substrate was dispensed into labeled test tubes and equilibriated to 37ºC for 3 minutes. At timed intervals, 0.05ml of each standard, control, and sample were added to the labeled test tubes and mixed gently. Deionized water was used as sample for reagent blank. The mixtures were incubated for 10minutes at 37ºC and 2.5 ml alkaline phosphatase color developer was added to all test tubes and mixed carefully. Absorbance of the coloured solutions was measured at 590 nm spectrophotometrically [20].

2.10 Determination of Total Protein

In total protein determination, 0.02 ml each of distilled water, standard and serum was pipetted into labeled test tubes. Then 1.0 ml of reagent 1 was added to each of the labeled test tubes. The mixtures were incubated at for 30 minutes at 22ºC. Absorbance of the sample was read against reagent blank at 550 nm [21].

2.11 Determination of Albumin

For albumin estimation, 0.01 ml each of reagent, standard and sample was pipetted into labeled test tubes. Subsequently, 3.0 ml of of BCG (bromocresol green) reagent 1 was added. The mixture was incubated for 5 minutes at 22ºC. Absorbance of the sample was read against reagent blank at 630 nm [22].

2.12 Determination of Serum Bilirubin

For estimation of total bilirubin, 200 µl of reagent 1, 50 µl, 1000 µl and 200 µl of sample were mixed for each sample and allowed to stand for 20 minutes at 22ºC. Reagent 4 (1000 µl) was added to the mixture and allowed to stand at 23ºC for 20 minutes. Absorbance of the sample was read at 578 nm against sample blank (A_{TB}).

Total bilirubin (µmol/l) = 185 × A_{TB} (578 nm).

For direct bilirubin, 200 µl of reagent 1, 50 µl of reagent 2, 2000 µl of 0.9% NaCl and 200 µl were mixed in a cuvette and incubated at 22ºC for 10 minutes. The absorbance of the sample was read at 546 nm against sample blank (A_{DB}).

Direct bilirubin (µmol/l) = 246 × A_{DB} (546 nm).

Unconjugated bilirubin = Total bilirubin – Direct bilirubin [23].

2.13 Histopathological Analysis

The rabbits were sacrificed and the liver was harvested and fixed in 10% formal saline. The biopses of liver of the rabbits were fixed with 10% formal saline, dehydrated with ascending grade of alcohol cleared with toluene, infiltrated with molten paraffin wax. The microtome sections were stained with haematoxylene and eosin staining technique [24].

2.14 Statistical Analysis

The results obtained were expressed as mean ± SD for each group. Data was statistically analyzed with one-way ANOVA followed by Benforoni multiple comparisons, using Graphpad instat software. *P* value < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

The phytochemical analysis carried out on the extract revealed the presence of tannins, saponins, flavonoids, alkaloids, terpenes, saponin glycosides and glycosides.

Table 1. Phytochemical constituents of *B. dalzielii* stem bark

Phytochemical constituents	Result
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Glycosides	++
Saponin glycosides	+
Anthraquinone	-
Terpenes	+

+: Present; -: Absent

Serum levels of ALT, AST, ALP, Total protein, Albumin, Total and Direct Bilirubin obtained were significantly elevated in the test (paracetamol) control group. The Silymarin treated group (group III) showed significant reduction in AST, ALP, TP and TBil. All treatment groups (group III, IV and V) showed significant reduction in all the parameters when compared to group II with highest reduction recorded in group V (225 mg/kg of *B. dalzielii* stem bark aqueous extract). Results were presented in Table 2.

All biochemical parameters recorded at the end of the treatment, showed that, the standard drug (Silymarin) and the plant extracts (150 mg/kg and 225 mg/kg of *B. dalzielii* stem bark) lowered the elevated levels of liver enzymes following paracetamol administration in comparison to paracetamol-only treated group (group II). The different concentrations (150 and 225 mg/kg) of the plant extracts used in the study indicated a dose dependent effect.

The histological findings (H and E) showed normal histological features in the liver hepatocytes of the control group (A) (Fig. 1), while that of the hepatotoxic control (group II) presented marked degeneration of hepatic cord (B), (Fig. 2). On the other hand, liver features of the treated groups (III IV and V) showed moderate degeneration of hepatocytes and fibrosis (C) for the group III treated rabbits (Fig. 3), while the 150 mg/kg *Boswellia dalzielii* extract treated rabbits showed milder degeneration of hepatocytes and fibrosis (D) (Fig. 4). And the 225 mg/kg *Boswellia dalzielii* extract treated rabbits showed no degeneration of hepatocytes, hence shows area of fibrosis (E). The hepatoprotective effects of *Boswellia dalzielii* bark extract was confirmed by the comparative histopathological changes presented by the treated groups (III IV and V) in relation to the features presented by group I (control) and group II (untreated).

3.2 Discussion

Paracetamol is a common analgesic that is safe in therapeutic doses. But with overdose it can leads to hepatic necrosis and fatal hepatic failure. Paracetamol hepatotoxicity is caused by its reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion, a prerequisite for paracetamol-induced hepatotoxicity [25,26]. In overdoses, it is a potent hepatotoxin, producing fulminant hepatic and renal tubular necrosis, which can be lethal in human and animal.

Drugs such as paracetamol are mostly hepatotoxic, and are known to cause noticeable elevation in serum level of enzymes, such as ALT, AST, ALP, and bilirubin, indicating substantial hepatocellular injury [27]. Depending on the extent of liver damage, these enzymes leak into the blood stream when there is a liver disease [28,29]. Also, damage or injury to the liver impairs protein synthesis and increases serum protein concentration due to leakages from the hepatocytes [30]. The administration of acetaminophen in this study produced a hepatotoxic effect that resulted in the leakages of these proteins from the hepatocytes into the systemic circulation (Table 2). This was why these proteins were detected in the serum of the treated animals.

It is eminent that all the groups that were given acetaminophen (Groups II, III, IV and V) produced high values of all the parameters when compared to Group I. The result of this study shows that paracetamol elevated the plasma levels of AST, ALT, ALP, total protein, albumin and bilirubin (total and direct) in rabbits. Such elevation is indicative of liver injury [31]. The findings in this study were also in agreement with previous reports made by several authors [32-34], who demonstrated that the elevations in liver markers in rats, mice and rabbits are due to liver injuries.

Table 2. Effect of aqueous extract of *Boswellia dalzielii* stem bark and Silymarin against paracetamol Induced hepatotoxicity on biochemical parameters

	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/dL)	ALB (g/dL)	TBIL (µmol/L)	DBIL ((µmol/L)
Group I (Normal control)	8.50±6.36	8.50±2.12	28.12±4.41	56.78±44.66	30.67±3. 46	52.04±4.66	34.26±3.70
Group II (Test control)	16.00±6.55	82.50±9.19	38.53±15.72	88.72±25.58	43.61±10.40	77.46±18.14	55.83±41.62
Group III (Paracetamol+25 mg/kg Silymarin)	15.33±4.04[a]	74.00±21.21[a,b]	30.20±3.60	82.81±10.48[a]	42.39±14.38[a]	71.23±17.25[a]	53.55±15.09[a]
Group IV (Paracetamol + B. dalziellii 150 mg/kg)	15.00±11.31[a]	50.33±39.52[a,b]	23.43±2.21[b]	80.05±7.38[a,b]	40.73±1.10[a]	64.73±23.12[a,b]	42.74±8.17[b]
Group V (Paracetamol + B. dalziellii 225 mg/kg)	10.50±0.07[b]	24.66±15.50[a,b]	26.25±0.00[b]	72.67±11.53[a,b]	29.80±6.07[b]	62.51±15.28[a,b]	35.27±3.93[b]

n = 3

a = Significant at 95% confidence limit (P<0.05) when compared with the normal control group

b = Significant at 95% confidence limit (P<0.05) when compared with the test control group

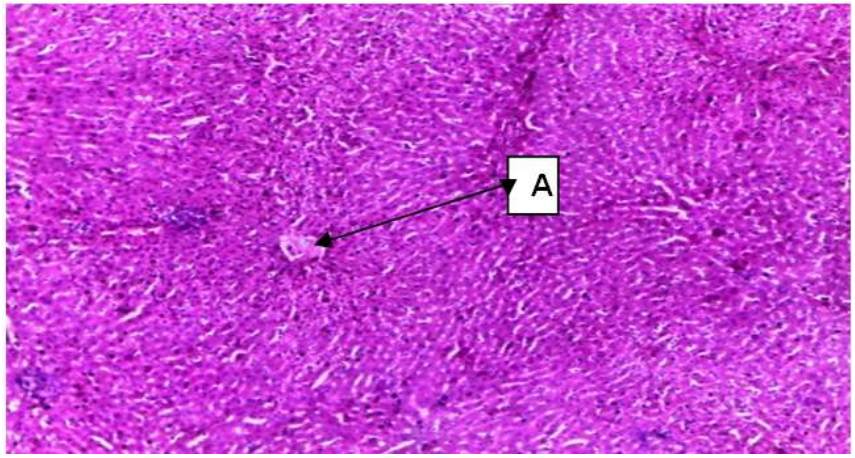

Fig. 1. (Group I): Section shows unremarkable liver biopsy with hepatocytes arranged in cord radiating from central vesicles

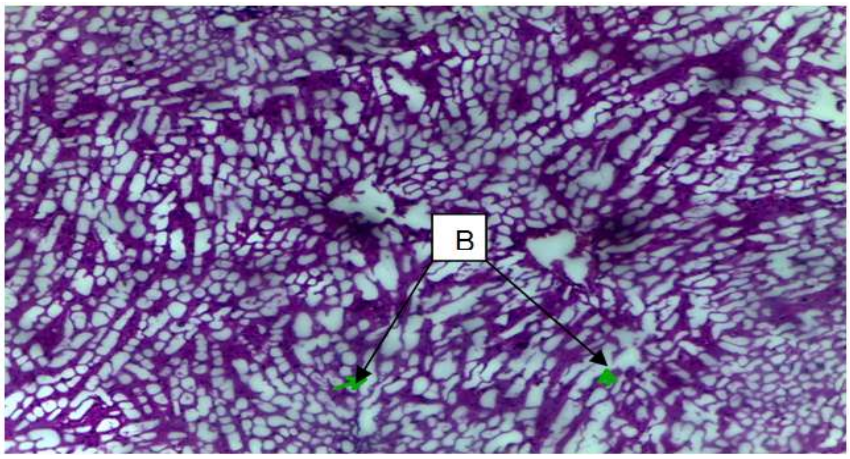

Fig. 2. (Group II): Section shows marked degeneration of hepatic cord

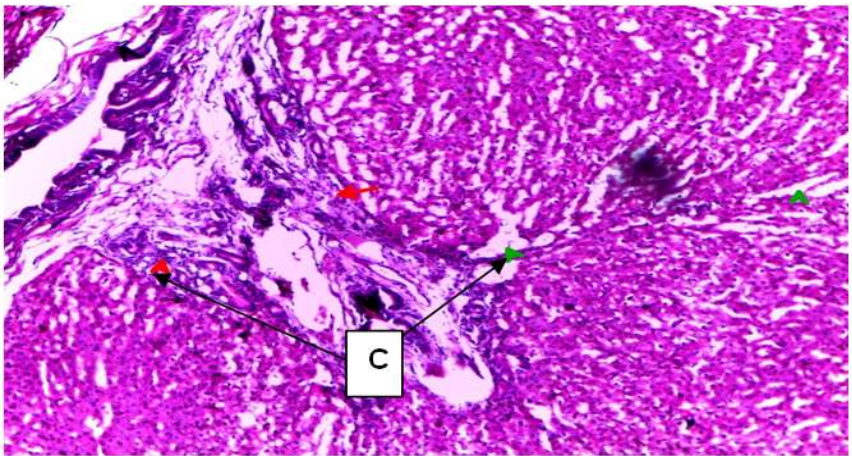

Fig. 3. (Group III): Section shows area of moderate degeneration of hepatocytes, and fibrosis

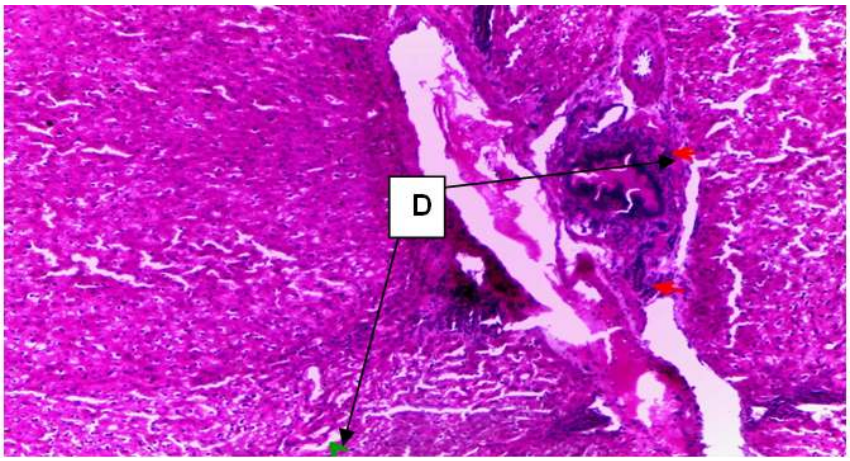

Fig. 4. (Group IV): Section shows area of mild degeneration of hepatocytes and fibrosis

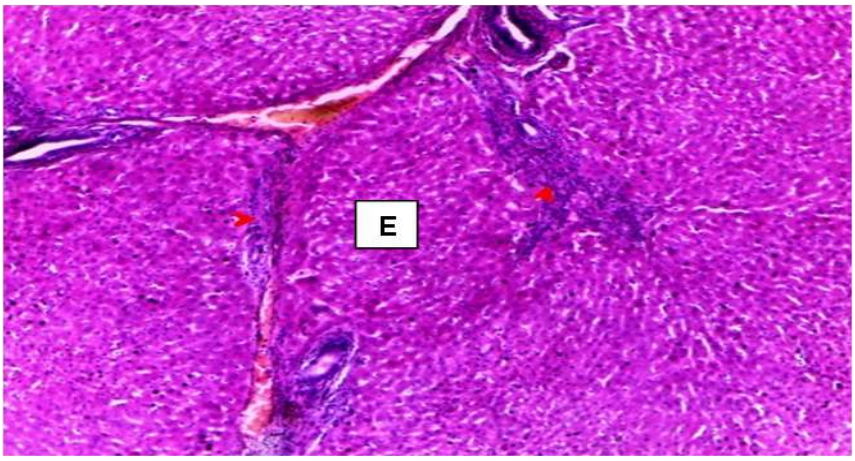

Fig. 5. (Group V): Section shows areas of fibrosis

The possibility of these enzymes to change to normal state in the extract-administered groups is a strong indication of hepatoprotective effect of the extract. The results of this investigation indicated that, treatment of rabbits with the aqueous extract of *Boswellia dalzielii* and silymarin (a standard drug) hutch after paracetamol administration caused a decline in hepatotoxicity of the rabbits. This is evidenced in the significant decrease in serum AST, ALT, ALP, TBIL, DBIL, total protein (TP) and albumin (ALB) relative to the paracetamol treated group as shown in Table 2.

It should also be noted that group IV and V were treated with the extract of *B. dalzielii* at 150 and 225 mg/Kg body weight, respectively. However, the values of AST and ALT were seen to be lower in Group V which took 225 mg/kg of the extract. This result shows that the hepatoprotective or healing effect of the administered *B. dalzielii* was best at 225 mg/kg.

Silymarin has hepatoprotective properties and is used in treatment of various liver diseases [35]. Various studies indicated that Silymarin exhibits strong antioxidant activity [36] and shows protective effects against hepatic toxicity induced by a wide variety of agents by inhibiting lipid peroxidation.

Histopathological studies supported the evidence of biochemical parameters analysed in this study. Histological analyses of rabbit liver treated with paracetamol showed significant hepatotoxicity, characterized by inflammatory hepatic tissues, including the presence of moderate infiltration of neutrophils. Extensive infiltration of inflammatory cells around the central vein and loss of cellular boundaries was observed in the treated groups, after hepatotoxicity induction by paracetamol [37]. Treatment with the extract reduced the severity of hepatic damage, when compared to that of the test control, decreasing neutrophil infiltration in the hepatic tissue which further indicated their significant hepatoprotective effect. The result of the histopathological studies showed that the observed efficacy of stem bark extract in the treatment groups was dose dependent (Figs. 4 and 5), as the hepatoprotective effect was paramount at 225 mg/kg. These result of the gross and histopathological examinations can be seen to be in line with the result of the liver function enzyme assay, showing that the best hepatoprotective effect of the aqueous extract of *B. dalzielii* was observed in the group of rabbits administered 225 mg/kg extract (Group V). This is also in accordance with the findings of several authors [38-41].

The hepatoprotective or healing effect of the crude extract of *B. dalzielii* was thought to be due to the normalization of impaired membrane function activity of the liver. And the tendency of the liver tissue to rejuvenate after it has been damaged might be related to the healing process.

4. CONCLUSION

The result of this study indicates that the biologically active antioxidative phytoconstituents of the aqueous extract of *Boswellia dalzielii* stem bark could relieve the damaging effect of paracetamol in the liver of rabbits. The result also

shows that the hepatoprotective or healing effect of the administered aqueous stem bark extract of *B. dalzielii* increased with increase in the extract concentration. Therefore, efficacy, safety, and the possible role of *B. dalzielii* treatment of liver disorders should be further evaluated by ingenious controlled studies.

CONSENT

It is not applicable.

ACKNOWLEDGEMENT

This work was partially supported by Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University Kano.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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