

Original Research Article

HEPATOPROTECTIVE EFFECT OF AQUEOUS BARK EXTRACT OF *BOSWELLIA DALZIELLI* AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RABBITS

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 1.4-2.0kg were randomly divided into five groups (I, II, III, IV and V), where group I served as control, while groups II, III, IV and V served as test groups. Hepatotoxicity was induced in the test groups via oral administration of paracetamol (500mg/kg Body weight). Groups III, IV and V were treated for 7 days with 25mg/kg Silymarin, 150mg/kg and 225mg/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 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 followed by Bonferroni selected comparisons. The level of significance was set at $p < 0.05$.

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, Histology.

1. INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Okwu,1999). The World Health Organization (WHO) supports the use of traditional medicine provided they are proven to be efficacious and safe (WHO, 1985). In developing countries, a huge number of people live in extreme poverty and some are suffering and dying due to lack of medicine, as they have no alternative for primary health care (Grieve,1931). There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the

ethno-medicinal use and subsequently the isolation and characterization of compounds which will be added to the potential list of drugs.

Liver diseases are one of the major threats to public health and remains as worldwide problem. They 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 (Adewusi and Afolayan, 2010). In recent years, interest has refocused on traditional medicine due to modern drugs high cost, time and expenditure that is necessary to bring a drug to market after appropriate clinical trials, serious side-effects of some modern drugs, and drug-resistance developed by many microorganisms (Mohammed et al., 2009).

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. The small white flowers, which may appear while the tree is leafless, are fragrant. The tree is planted in the northern parts of Nigeria like Kebbi (Etuk *et al.*, 2006), Kano (Nwinyi *et al.*, 2004), Kaduna (Hassan *et al.*, 2009) and Adamawa States (Burkill, 1985) 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. The plant is regarded as 4 a veritable source of natural medicine. The local inhabitants of the northwestern part of the country have been using the decoction from various part of this plant for the treatment of a plethora of both animal and human diseases. The extract from its leaves is used in treatment of diarrhea in poultry (Nwude and Ibrahim, 1998).

The present study was conducted to evaluate the hepatoprotective effect of the aqueous extracts of stem bark of *bosweillia dalzielli* on liver function using rabbits induced with paracetamol hepatotoxicity.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

Collection 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.

Preparation of Plant Extract

50g of the dried pulverized bark of *boswellia dalzielii* was steeped in 4 liters of distilled water and kept at room temperature for 24 hours. Thereafter, the mixture was filtered using a white silky cloth and the filtrate transferred into a plastic bucket and subjected to evaporation in a water bath at 45°C, which last for 13days before completely drying off and the residue was scrapped using a spatula. Soon after, 10g of the residue was used to prepare the extract, diluted in 500ml of distilled water (with concentration of 50g/ml).

Preparation of Paracetamol Extract

5g of powdered paracetamol was diluted in 100ml of distilled water and the mixture was transferred into a dark brown bottle before use.

Experimental Animals

15 male and female adult rabbits weighing between 1.4 - 2.0kg were obtained from Sheka and Sabongari markets in Kano State. They were allowed to acclimatize with the environment for 10 days where the work was carried out, they were maintained in the experimental facility provided standard layer feeds, lettuce, spinach and regular tap water *ad libitum*. All animals were housed together using color markers to differentiate them into their individual groups of 5 containing 3 rabbits each throughout the experimental period which last for 7days.

Treatment of Animals

Five groups (1-5) of 3 rabbits each were used for the study. 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 induced control received 500mg/kg paracetamol. Then group 3,4,and 5 which served as the test

groups receives 500mg/kg of paracetamol each and 25mg/kg body weight Silymarin, then 150mg/kg and 225mg/kg of the extract, respectively for 7 days.

Biochemical Analysis:

After the period of the treatment, blood collected from the animals by decapitation were put into sterile bottles and centrifuged at a rate of 12,000 revolutions per minute (rpm) for 10 min. The clear serum obtained was analyzed for Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Albumin, total protein and Bilirubin (total and direct) using Randox and Teco Laboratory kits.

Histopathological analysis

The biopsy of the 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 haematoxyline and cosin staining technique (Auwoioro, 2010).

Statistical analysis

The results obtained were statistically analyzed by one-way ANOVA followed by Bonferroni selected comparisons, using graph pad instat software. P value < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

Generally, damage or injury to the liver impairs protein synthesis and increases serum protein concentration due to leakages from the hepatocytes (Rajesh *et al.*, 2009). The administration of acetaminophen at (500 mg/kg) in this study produced a hepatotoxic effect that resulted in the leakages of these proteins from the hepatocytes into the systemic circulation (Table. 1). This was why these proteins were detected in the serum of the treated animals. It should also be noted 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 (Patrick-Iwuanyanwu *et al.*, 2010). The tendency of these enzymes to return to near normal in the extract-administered groups is a clear manifestation of hepatoprotective effect of the extract. The results of this investigation indicated that, treatment of rabbits with the aqueous extract of *Boswellia dalzielii* 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 the table below.

Table 1: Effect of treatment with the aqueous extract of *boswellia dalzielii* stem bark against paracetamol Induced hepatotoxicity on biochemical parameters

GROUP S	ALT	AST	ALP	T/P	ALB	TBIL	DBIL
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 (induced control)	16.00±6.55 ^a	82.50±9.19 ^b	38.53±15.72 ^c	88.72±25.58 ^a	43.61±10.40 ^a	77.46±18.14 ^a	55.83±41.62 ^a
GROUP III (test group)	15.33±4.04 ^a	74.00±21.21 ^c	30.20±3.60 ^b	82.81±10.48 ^b	42.39±14.38 ^a	71.23±17.25 ^b	53.55±15.09 ^a
GROUP IV (test group)	15.00±11.31 ^a	50.33±39.52 ^d	23.43±2.21 ^a	80.05±7.38 ^b	40.73±1.10 ^a	64.73±23.12 ^c	42.74±8.17 ^b
GROUP V (test group)	10.50±0.07 ^b	24.66±15.50 ^a	6.25±0.00 ^d	72.67±11.53 ^c	29.80±6.07 ^b	62.51±15.28 ^c	35.27±3.93 ^c

Values are expressed as mean ± SD; n = 3. Values with different superscripts are significantly different at 95% confidence limit (P<0.05).

Histopathological analysis

Paracetamol is a common antipyretic agent which is safe in therapeutic doses but can produce fatal hepatic necrosis in toxic doses. Paracetamol (acetaminophen) induced hepatic injury has been reported by several studies (Schnellman, 2001; Bessems and Vermeule, 2001; Plevris *et al.*, 1998) and this was justified in view of the hepatocytotoxic indications observed in the liver sections of group II (figure 2) in this study. The hepatotoxic mechanism of paracetamol is said to be mediated through direct liver toxicity of the parent drug or its metabolites (Pham *et al.*, 1997), increase peroxidation (Schnellman, 2001; Bessems and Vermeule, 2001; Plevris *et al.*, 1998), and formation of toxic metabolite; N-acetyl-p-benzoquinone imine (Lee and Marks, 2007; Jafri *et al.*, 1999).

The histological findings (H and E) showed normal histological features in the liver hepatocytes of the control (group I) (figure 1), while that of the hepatotoxic control (group II) presented marked degeneration of hepatic cord (green arrow), (figure 2). On the other hand, liver features of the treated groups (III IV and V) showed moderate degeneration of hepatocytes (green arrows), and fibrosis (red arrows) for the group III treated rabbits (figure 3), while the 150mg/kg *boswellia dalzielii* extract treated rabbits showed milder degeneration of hepatocytes (green arrows) and fibrosis (red arrow) (figure 4). And the 225mg/kg *boswellia dalzielii* extract treated rabbits showed no degeneration of hepatocytes, hence shows area of fibrosis. 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). Also, the observed efficacy of *Boswellia dalzielii* bark extract in the treatment groups was dose dependent (figures 4 and 5). These lessened hepatic damage by the administration of *Boswellia dalzielii* bark extracts in group III IV and V compared to that of group II, indicates recovery from paracetamol hepatic damage and suggests its healing potentials as well. This finding is in line with other studies in which different types of *Boswellia dalzielii* extracts have showed hepatoprotective activity against CCl₄, ethyl alcohol and diethylnitrosamine (Bhakta *et al.*, 2001; Jafri *et al.*, 1999; Parthasarathy and Prasanth, 2009; Rani *et al.*, 2010; Sastry *et al.*, 2011).

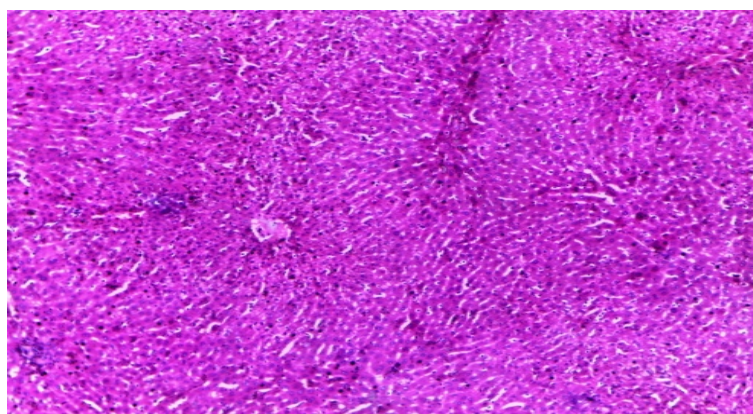


Figure 1 (Group I): Section shows unremarkable liver biopsy with hepatocytes arranged in cord radiating from central vesicles (plate 1, H and E, mag ×100)

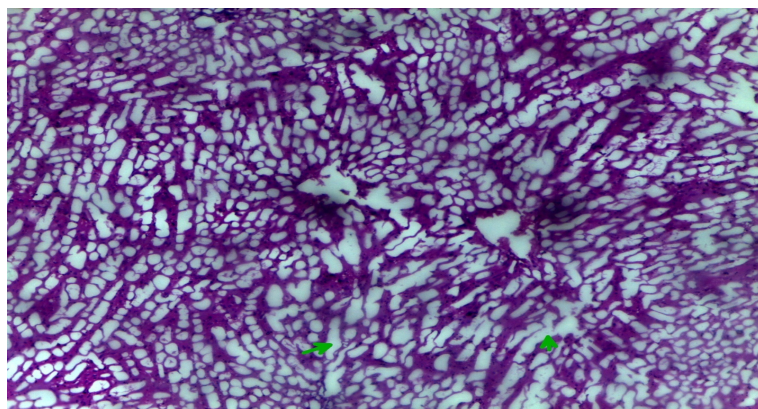


Figure 2 (Group II): Section shows marked degeneration of hepatic cord. (plate 2, H and E, mag ×100).

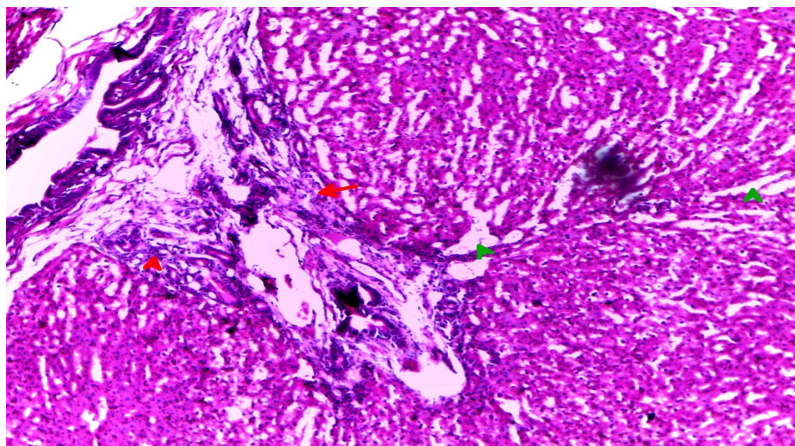


Figure 3 (Group III): Section shows area of moderate degeneration of hepatocytes, and fibrosis (plate 3, H and E, mag $\times 100$)

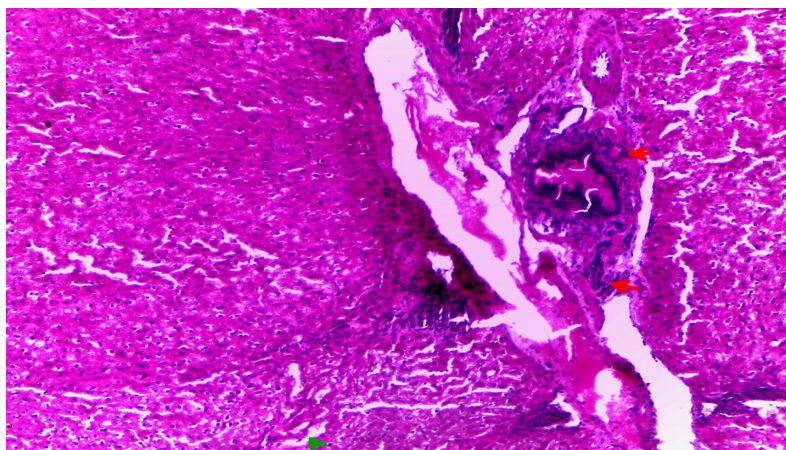


Figure 4 (Group IV): Section shows area of mild degeneration of hepatocytes, and fibrosis (plate 4, H and E, mag $\times 100$).

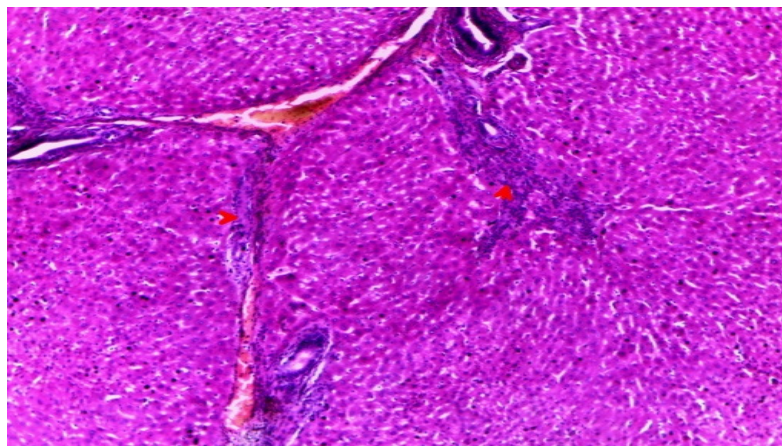


Figure 5 (Group V): Section shows areas of fibrosis (plate 5, H and E, mag $\times 100$)

4. CONCLUSION

From the findings of this study, it could be concluded that, the oral administration of aqueous extract of *B. dalzielii* exhibited its hepatoprotective potentials on Paracetamol-induced hepatotoxicity in rabbits.

Therefore, efficacy, safety, and the possible role of *B. dalzielii* in treatment of liver disorders should be further evaluated by ingenious controlled studies.

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