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Full Length Research Paper

Toxicological and antioxidant effects of ethanolic extract of *Baphia nitida* on diazepam induced oxidative stress in rats

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The toxicological and antioxidant effects of the ethanolic extracts of *Baphia nitida* on diazepam induced oxidative stress in rats were investigated. Forty Sprague Dawley albino rats divided into eight groups of five animals each were employed in the study. Group one or control was not administered diazepam or ethanolic extract of *B. nitida* but received saline while group two received diazepam only. Groups three, four and five were administered the extract only and groups six, seven and eight were administered both diazepam and the extract, respectively. Diazepam induced oxidative stress in the rats resulted from administration of a single dose of orally administered diazepam (1 mg/100 g body weight). Ethanol was used to extract and prepare *B. nitida* leaves at the concentrations of 100, 200 and 400 mg/kg and orally administered daily to rats for 10 days. The rats were sacrificed by decapitation and blood taken and used for blood chemistry analysis. Liver anti-oxidant enzymes namely super oxide dismutase (SOD), catalase and peroxidase activities were assayed. Phytochemical screening of the ethanolic extract of *B. nitida* leaves and histopathological studies of the liver and kidney tissues were also carried out. Blood chemistry parameters, such as SOD, aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, total bilirubin, triacylglycerols, uric acid and urea levels were significantly ($p<0.05$) decreased, while albumin level was elevated in all the experimental animals administered the ethanolic extract of *B. nitida* leaves compared with the control. There were no marked significant changes ($p>0.05$) in the activities of the liver anti-oxidant enzymes of the rats administered the ethanolic extract of *B. nitida* compared with the control. The histopathological examinations revealed no abnormality in the hepatocytes and kidney tissues of the experimental animals compared with the control. Data of the study show that the ethanolic extract of *B. nitida* contained several secondary metabolites which positively affected some blood chemistry parameters in the experimental rats compared with the control rats and they did not cause liver or kidney damage. We conclude therefore that the ethanolic extract of *B. nitida* possess ability to bring about reversal of the negative effects of diazepam induced oxidative stress in rat model.

Key words: *Baphia nitida*, blood chemistry, anti-oxidant, liver, diazepam.

INTRODUCTION

Baphia nitida has a wide geographical distribution and appears mainly as a shrub or short tree, with immense benefits. It is widely endowed with a wide range of ethnopharmacological benefits hence, has been used by indigenes of many West African countries for medicinal purposes (Irvine, 1961). *B. nitida* is popularly called cam wood but among the Yoruba people of West Africa, it is called “Irosun”. They, among other uses, use parts of the plant for constipation (Irvine, 1961), ringworm, sprains and swollen joints, parasitic skin diseases (Dalziel, 1937). The leaf extract has been reported to possess a dose dependent antinociceptive (analgesic) activity (Onwukaeme and Lot, 1991). The use of the extract for treating palpitation locally has been reported (Adeyemi, 2006). *B. nitida, Cassia occidentalis* and *Boerhavia diffusal* leaves are used in food and drinks, as well as in traditional medicine, to treat rheumatic ailments which are

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an indication of oxidative stress. These plants were evaluated for their antioxidant properties through a scavenger effect on reactive oxygen species (ROS), such as hydrogen peroxide and hypochlorous acid and they all showed dose-dependent antioxidant activity. The values obtained were comparable to the antioxidant properties of pharmacological substances such as N-acetylcysteine and *Mesa*. Leaves of *B. nitida* had been used to arrest bleeding in fresh cut. Leaves of *B. nitida* are used in folk medicine for the treatment of inflamed and infected umbilical cords in Nigeria.

The methanol-acetone extract of *B. nitida* leaves is also found to inhibit gastric emptying time and intestinal motility in mice. The liver plays a central role in transforming and clearing chemicals and this makes it suceptible to the toxicity from some of these agents. Certain medicines when taken in overdoses and in some cases within therapeutic ranges may injure the organ; these chemicals that induce hepatotoxicity are called hepatotoxins. The liver plays a central role in metabolism of drug and xenobiotics, protein synthesis and in maintaining biologic equilibrium of organisms. Due to these important roles, liver enzymes are used as markers in assessment of drug or plant extract safety or toxicity (Satyapal et al., 2008). Benzodiazepines are the most frequently prescribed class of psychotropic drugs, possibly worldwide (Stokes et al., 2002). Benzodiazepines, such as diazepam (trade name Valium) are commonly used for their anxiolytic and sedative effects (Costa et al., 1995). Many studies have been conducted to ascertain any role and or involvement of free radical mediated pro-oxidative processes in the brain following diazepam administration (Musavi and Kakkar, 2003). The objective of the present study was to evaluate the antioxidant properties of ethanolic leaf extract of *B. nitida* against diazepam induced oxidative stress and tissue damage in rats different organs such as kidney, heart and liver.

MATERIALS AND METHODS

Fresh leaves of *B. nitida* were purchased from Oyingbo market in Lagos State, Nigeria. The leaves were identified and authenticated at the Department of Botany, Herbarium unit, University of Lagos, Lagos, Nigeria. The leaves of the plants were cleaned, air dried at room temperature for two weeks, ground to a powder and extracted in ethanol for 3 h using soxhlet extractor. The percentage yield for the extract was 7.0%.

Phytochemical screening

In the present study, the extract was subjected to phytochemical screening using standard tests as described by Sofowora (1993) and Edeoga et al. (2005) and adapted from Trease and Evans (1983), respectively.

Tests for alkaloids

1.0 ml of each extract was stirred with 5.0 ml of 1% aqueous hydrochloric acid on a steam bath and filtered. 1.0 ml of the filtrate was then treated with five drops of Mayer's reagent and a second 1.0 ml portion treated similarly with freshly prepared Dragendorffs and Wagner's reagents. Turbidity or precipitations with either of the reagents indicate the presence of alkaloids in the extract (Harborne, 1973; Evans, 1989).

Test for saponins

1.0 ml of the plant extract was shaken with 5.0 ml of distilled water in a test tube and filtered. Frothing which persists on warming is a preliminary evidence for the presence of saponins. 10.0 ml of the filtrate was mixed with 5.0 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion which confirms the presence of saponins.

Test for tannins

5.0 ml of the plant extract was stirred with 5.0 ml of distilled water, filtered, and 2.0 ml 5% ferric chloride reagent was added to the filtrate. A blue-black precipitate was taken as evidence for the presence for the presence of tannins.

Test for phlobatannins

Deposition of a red precipitate when 5.0 ml of aqueous extract of the plant was boiled with 2.0 ml dilute 1% hydrochloric acid indicates the presence of phlobatannins.

Tests for cardiac glycosides

5.0 ml of extract was treated with 2.0 ml of glacial acetic acid containing 1 drop of 0.1% ferric chloride, and then mixed with 1.0 ml concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

Test for flavonoids

5.0 ml of dilute ammonia solution was added to a 20.0 ml portion of the aqueous filtrate of the plant extract followed by addition of few drops of concentrated H_2SO_4. A yellow colouration observed indicates the presence of flavonoids.

Test for steroids

2.0 ml of acetic anhydride was added to 0.5 ml of the extract and then mixed with 2.0 ml H_2SO_4. The colour change from violet to blue or green in some samples indicates the presence of steroids.

Test for terpenoids (Salkowski test)

5.0 ml of the extract was mixed with 2.0 ml of chloroform while 3.0 ml of 1.0 M H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was formed which shows the presence of terpenoids.

Bioassay

Forty Sprague- Dawley albino rats weighing (170 to 260 g) were

collected from the Laboratory Animal Centre, College of Medicine, University of Lagos, Idi Araba, Lagos, Nigeria. They were all clinically healthy and maintained in standard environmental conditions of temperature (29.0± 2.0°C), 12 h dark/light cycle. They were fed a standard diet and water *ad libitum*. The bioassay was conducted in line with the internationally accepted and best practices (European Community guidelines EEC Directives of 1986, 86/609/eec; US guideline, NIII publication #85 to 23, revised in 1985) for laboratory animal care. The animals were divided into 8 groups of 5 animals each after 24 h fasting as follows:

Group 1: Saline (Control).
Group 2: Diazepam only (single dose of 1 mg/100 g body weight).
Group 3: Extract only (100 mg/kg).
Group 4: Extract only (200 mg/kg).
Group 5: Extract only (400 mg/kg).
Group 6: Diazepam, + extract (100 mg/kg extract + single dose of Diazepam, 1 mg/100 g body weight).
Group 7: Diazepam + extract (200 mg/kg extract + single dose of Diazepam, 1 mg/100 g body weight)
Group 8: Diazepam + extract (400 mg/kg + single dose of Diazepam 1 mg/100 g body weight).

The administration of the extract lasted for 10 days. The rats were sacrificed after last day of extract administration and blood samples were collected from each animal through ocular bleeding into two sets of plain and ethylenediaminetetraacetic acid (EDTA) treated sample bottles, respectively. The blood in the plain samples bottles were allowed to clot after 3 h. The clotted blood samples were spun in a bench top centrifuge to obtain sera. The serum samples were thereafter separated into another set of plain sample tubes and stored in the refrigerator pending enzyme assay. The whole blood collected into EDTA-treated sample bottles were used for the assay of haematological parameters. All assays were done within 24 h of the sample collection. The assay of alanine amino transferase (ALT), AST and ALP, SOD and catalase activities were done according to the procedures described by Roche Laboratories Limited, USA, total proteins, total bilirubin, urea, cholesterol, triglycerides and albumins assays were carried out according to the methods of Tietz (1995). Serum malondialdehyde (MDA) and reduced glutathione (GSH) were measured by the methods of Jiang et al. (1992) and Sedlak and Lindsay (1968), respectively.

Histology

The organs (liver and kidneys) of a rat from each group were fixed in 10% formol saline and after 72 h the organs were dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. The resulting blocks were exhaustively sectioned. The sections were randomized and selected sections were stained in haemotoxylin and eosin. The slides were then examined at magnification of × 400 under optical microscope.

Statistical analysis

The results are presented as Mean ± SEM. Analysis of variance (ANOVA) was used in the determination of the differences in the levels of the different parameters measured using graph pad prism 5.0 and differences were considered significant at ($p<0.05$).

RESULTS

The phytochemical screening of ethanolic leaf extract of *B. nitida* indicated the presence of tannin, phlobatanin, saponin, flavonoid, steroids, terpenoids, glycosides and alkaloid. The results obtained in this present study for some serum enzymes, total proteins, albumin and other serum markers of oxidative stress in normal rats treated with ethanolic extract of *B. nitida* show that treatment of rats with ethanolic extract of *B. nitida* resulted in significant ($p<0.05$) decrease in the activities of the serum liver enzymes as well as the concentrations of serum total proteins and albumins, compared respectively, with the control. This observation indicated that the ethanolic extract of *B. nitida* did not show marked hepatotoxic effect in the rat model. In the tissue analysis however, there were significant decreases ($p<0.05$) in SOD and catalase levels (18.0 units/mg protein, 85.20 units/mg protein vs 75.30 units/mg protein and 249.22 units/mg protein) for the group administered diazepam only confirming diazepam as an oxidant, while there were significant increases ($p>0.05$) for groups administered with ethanolic extract of *B. nitida* (170.45 units/mg protein, 277.15 units/mg protein). In all serum analysis reveal that markers of oxidative stress decreased in all the groups treated with ethanolic extract of *B. nitida*. Creatinine did not show any significant differences in all the groups (Figure 1).

Liver histology

Plates 1 and 2 show photomicrographs of the liver tissues of the test and the control rats, respectively. The liver section of the animal in control groups showed a central vein with prominent small-sized nuclei, with the hepatocytes well separated by sinusoids. While the tissue section of the test rats showed a prominent central vein with a relatively large-sized nuclei. Also, the sinusoids separating the hepatocytes in the test rats are observed to be relatively more prominent than that of the rats in the control groups. Generally, the liver sections of rats in the control and test groups showed that the cords of hepatocytes were well preserved, cytoplasm not vacuolated and the sinusoids well demarcated. Also, no area of infiltration by inflammatory cells and fatty degenerative changes were observed in the tissue sections. These features gave an indication of normal hepatic integrity for rats in both control and test groups while inflammatory cells were observed in all the rats administered diazepam only. Histological studies of the liver in this study then, showed that the liver of the treated experimental groups appeared to be normal compared to the control with only slight inflammatory cells seen in almost all of them.

DISCUSSION

Plants extracts are among the most attractive sources for developing new drugs and have been shown to produce promising results in antioxidant potentials (Hiruma-Lima

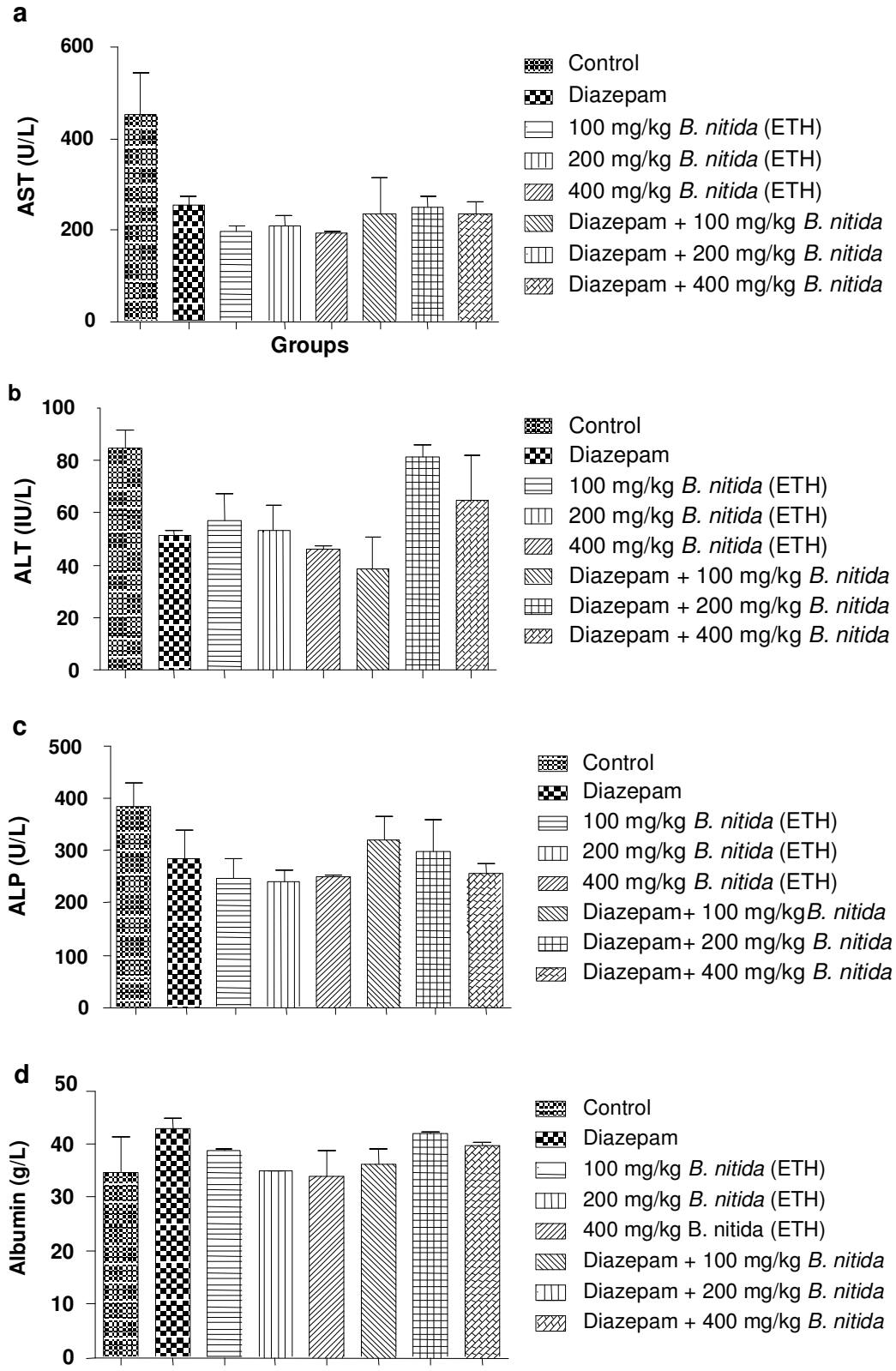
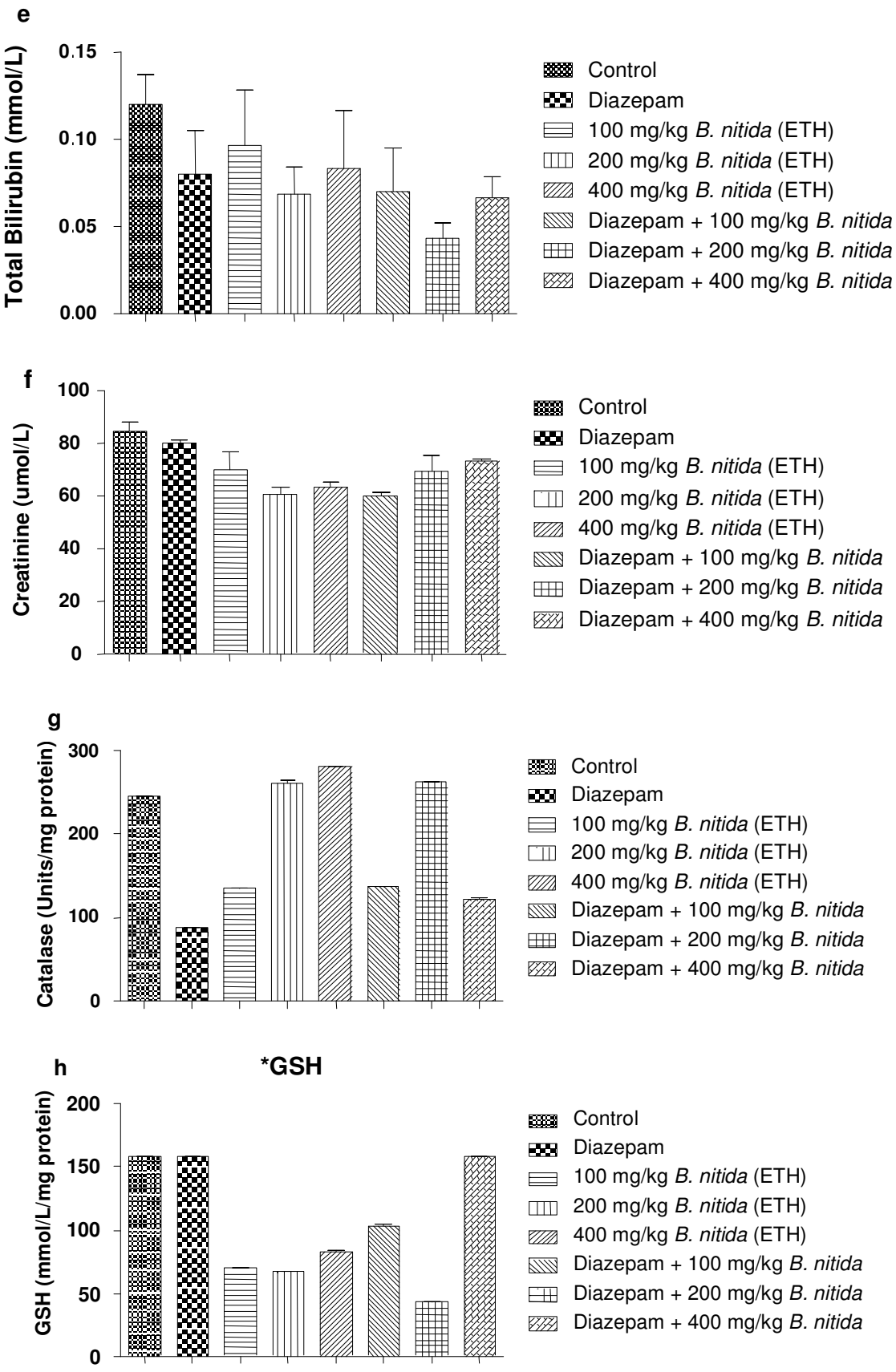


Figure 1. Effects on some serum enzyme activities and biomolecule concentrations in rats orally administered diazepam and *Baphia nitida*. (a) AST activities, (b) ALT activities, (c) ALP activities, (d) albumin level, (e) total bilirubin concentration, (f) creatinine concentration, (g) catalase activity, (h) GSH,(i) MDA level, (j) SOD activity, (k) uric acids concentration, (l) Urea concentration, (m) Total cholesterol level, (n) Triglyceride concentration, (o) Total protein level.



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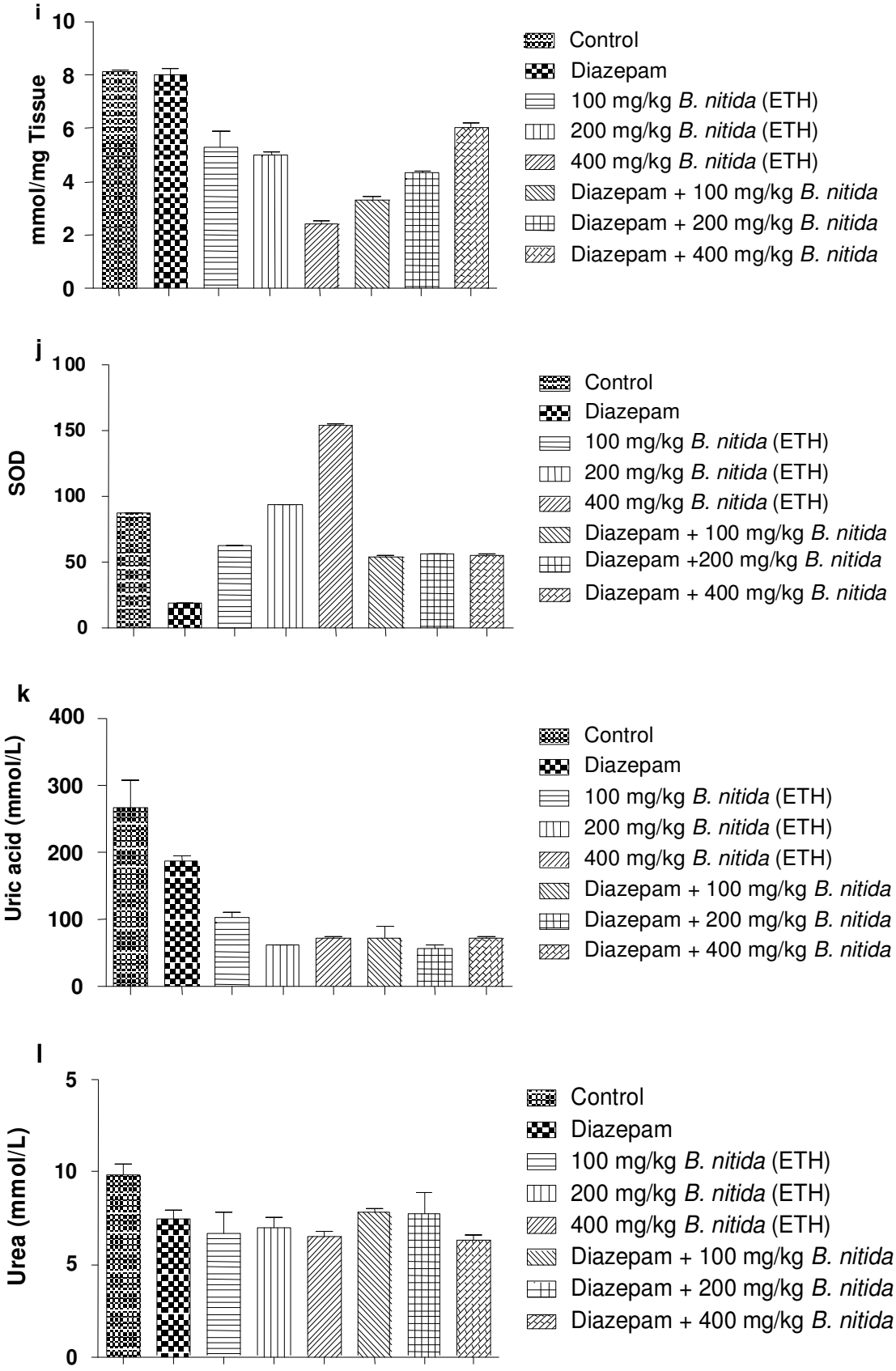

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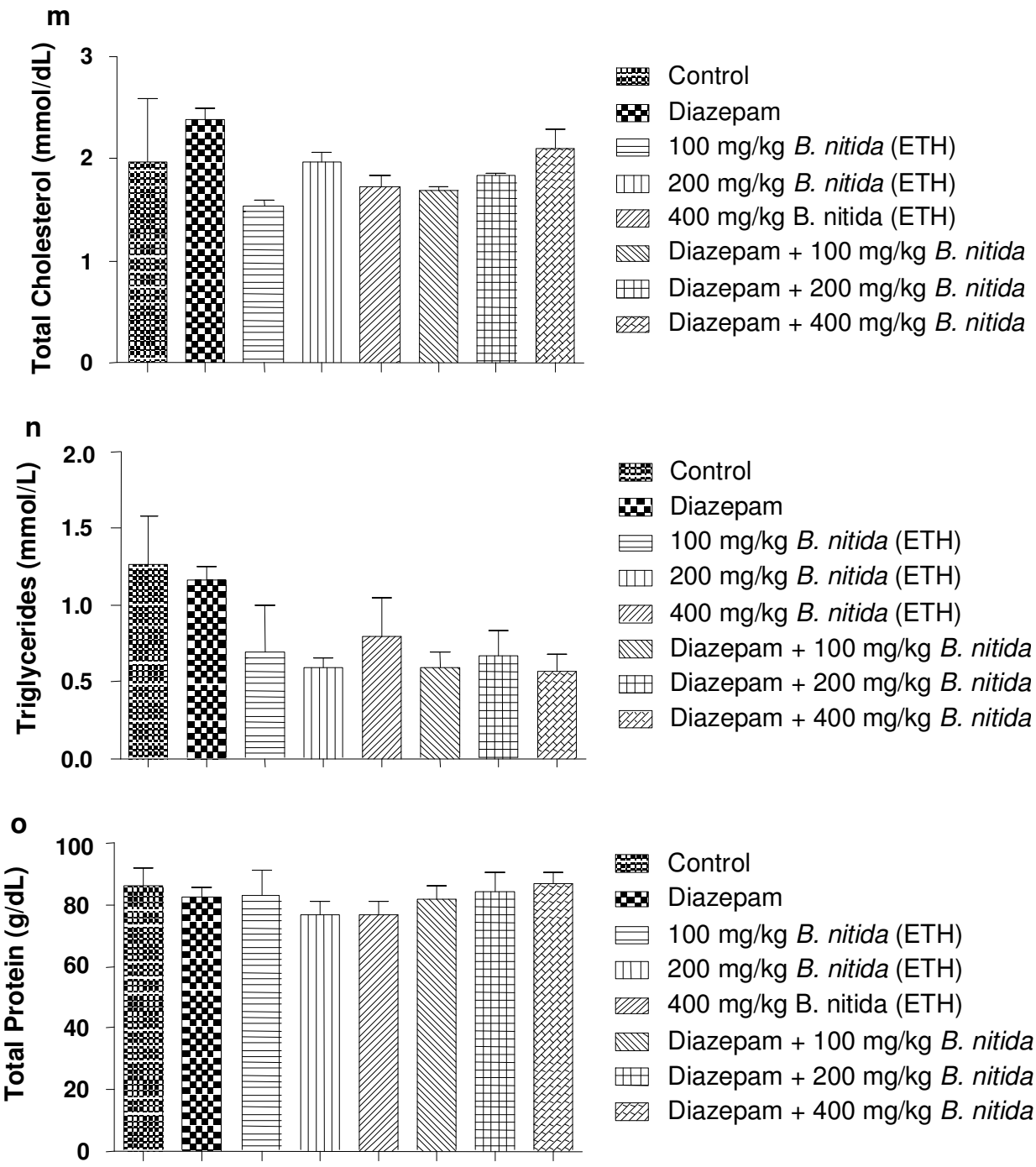

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et al., 2001; Toma et al., 2002). Flavonoids are secondary metabolites present in plants and are known to possess wide range of biological activities (Harborne, 1996). These phytochemicals are known to perform many functions in plants, and may exhibit different biochemical and pharmacological actions in animal species wheningested (Duke and Wain, 1981). Saponins are known to posses hypocholesterolemic effects (Price et al., 1987) and as such its presence in the leaf extracts of *B. nitida* may aid in lessening the oxidative stress induced on the liver as a result of the administration of diazepam shown by marked elevated activity of MDA in the rat organs such as kidney and liver in this study. It is known that exogenous antioxidant from vegetable

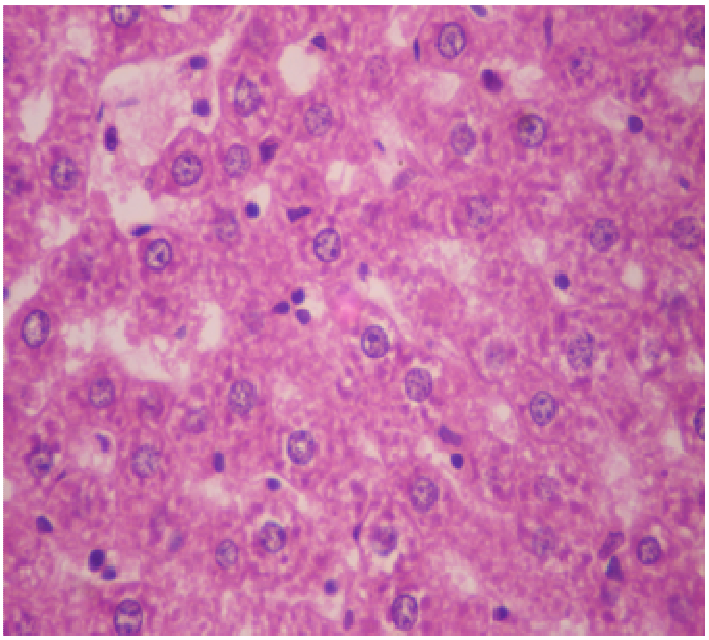

Plate 1. Photomicrograph of rat hepatocytes showing the normal liver of the rat (x 400) with intercellular organization, cells have distinct nuclei and intact cellular membrane showing no signs of necrosis.

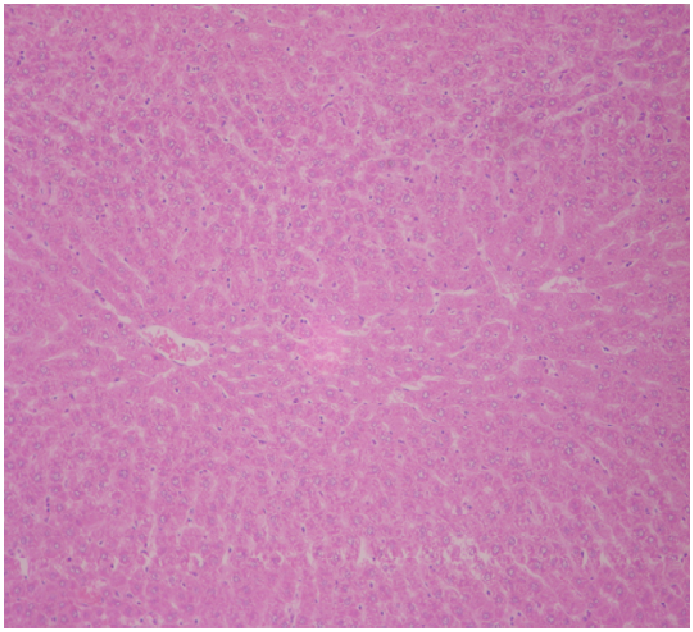

Plate 2. Photo micrographs of rat hepatocytes showing the liver of the rat administered diazepam only magnification x 400. There was no intercellular organization; cells have distinct nuclei and intact cellular membrane showing no marked necrosis.

sources may influence both the antioxidant capacity of blood (Cao et al., 1998) as well as oxidative stress biomarkers (Tesoriere et al., 2004). The results of this study showed that ethanolic extract of *B. nitida* has hepatoprotective properties in the rat. There were decreases in ALT, AST, ALP, total bilirubin, urea, total

cholesterol, triglyceride, albumin and uric acid in all groups compared to the control but the decrease was less in control group administered with diazepam, thus suggesting that *B. nitida* had an effect on these serum markers of oxidative stress thus reinforcing the finding by Onwukaeme (1995).

The creatinine level in the serum suggests that *B. nitida* prevented possible wastage in muscle mass caused by the potential drug dependent effect on the basal metabolic rate.

The histology of liver cells showed cellular organization, distinct and circular nuclei with no cellular swelling, no destruction of plasma membrane and organelle membrane and there was also no sign of cell distruption or necrosis showing that *B. nitida* extract had no negative effect on the liver at end of ten days, an observation that had been made by Nadia- Abdelmajeed (2009). Tissue analysis of the levels of SOD, GSH, and MDA show that *B. nitida* improved the liver functions by mopping up free radicals capable of inducing oxidative stress. The phytochemical analysis showed that ethanolic extract of *B.nitida* contain tannin, phlobatannin, saponin, flavonoid, steroids, terpenoids, cardiacglycosides and alkaloid. Saponins and flavonoids possess anti inflammatory ability which is dose related, and this might account for the retention of liver and kidney tissues integrity. Mehdi et al. (2010) reported isolation of two new acylated flavonol penta glycosides which possess antioxidant activity. It is probable that hepatoprotective activity of the extract of of *B. nitida* is largely due to the pronounced active plant chemicals such as flavonoids, alkaloids and terpenoids among others. It is also clear from the analysis that it is a very safe medicinal plant that could be exploited in treating variety of known diseases as earlier few studies had suggested. This study has shown that ethanolic extract of *B. nitida* contains active constituents that can be used as antinociceptive (analgesic). The hepatoprotective potentials of *B. nitida* is connected with antioxidant mechanism, it could therefore, be a good source of analgesic extract. However, further research is required in order to further ascertain the active antinociceptive and other bioactive constituents.

Abbreviations: SOD, Super oxide dismutase; **ALT,** alanine amino transferase; **AST,** aspartate amino transferase; **ALP,** alkaline phosphatase; **ROS,** reactive oxygen species; **GSH,** glutathione; **EDTA,** ethylenediaminetetraacetic acid; **MDA,** malondialdehyde; **ANOVA,** analysis of variance.

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