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HEPATOPROTECTIVE AND NEPHROPROTECTIVE ACTIVITY OF BARK EXTRACT OF *BRIDELIA RETUSA* SPRENG IN CCL_4 TREATED FEMALE MICE

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Abstract-The present study was undertaken to investigate hepatoprotective and nephroprotective activity of ethanolic and aqueous extracts of the stem bark of *Bridelia retusa* in carbon tertrachloride treated female mice. The protective activity of the extracts was justified by the significant decrease in the weights of adrenal glands when compared to CCL_4 treated group and the weights of ovary was increased to that of the normal control group. The enzyme activity of ALAT (Alanine transaminase) and LDH (Lactate dehydrogenase) in the liver while that of ALAT, ASAT (Aspartate transaminase), LDH and AKP (Alkaline phosphotase) in the kidney were significantly lowered ($p<0.05$) in all the groups treated with extracts when compared to CCL_4 treated group and were found to be brought almost to normal control. When compared to mice treated with CCL_4, group IV and V showed significantly ($p<0.05$) lowered ASAT activity while group III and V showed significantly ($p<0.05$) lowered AKP enzyme activity in the liver and the values were normalized to that of group I. The enzyme activity of ACP in the liver and kidney was significantly increased ($p<0.05$) in all the groups treated with extracts when compared to CCL_4 treated group and were found to be brought almost to normal control. The ATPase enzyme activity of liver and kidney was significantly ($p<0.05$) increased with respect to that of CCL_4 treated group. The concentration of protein, carbohydrate, DNA, RNA and cholesterol in the liver and kidney were estimated to be significantly ($p<0.05$) increased when compared to that of CCL_4 treated group except that of cholesterol in kidney and the values were almost equivalent to that of normal group. In general, both the extracts possessed protective activity though ethanolic extract was found to exhibit greater protection.
Keywords: *Bridelia retusa* Spreng, sequential extraction, carbon tetrachloride, hepatoprotective and nephroprotective activity.

Introduction

According to WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. The chemical constituents obtained from plants may be pharmacological screened for developing novel agents [1, 2]. Phytochemicals are compounds found in plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. Phytonutrients have various health benefits, for example, they may have antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effects to mention but a few [3].

Liver is the largest organ in the vertebrate body and also an important organ actively involved in metabolic functions such as production and secretion of bile, prothrombin and fibrinogen. The liver is also responsible for detoxifying poisonous substances in the body by transforming and removing toxins, waste, and pollutant xenobiotics. In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief [4]. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost [5]. Carbon tetrachloride also known as tetra chloromethane is known to have hepatotoxic effects. When exposed, the liver is inflamed and the hepatocytes destroyed [6]. *Bridelia retusa* S. is a moderate sized tree or a shrub belonging to Euphorbiaceae family found growing throughout India. The species in Southeast Asia are usually part of the primary and secondary forest vegetation either as big trees or as smaller trees or shrubs. Decoction of stem bark with country liquor is used for diarrhea, ear ache and prevents pregnancy. Pounded bark is mixed with gum of *Steroulia urines* Roxb. and the mixture is prescribed orally 2-3 days after menstruation for complete infertility [7]. Extract

from the stem bark has antiviral, anticancer and hypotensive properties. Paste of the stem bark is applied to wounds and bark juice taken internally in case of snake bite [8]. The liver is a major detoxifying organ in vertebrate body, which involves intense metabolic activities. Certain toxic chemicals and medicines can cause liver damage, which has been recognized as a toxicological problem. However, herbal medicines are known to play an important role in the treatment of various ailments, including hepatopathy. Many traditional practitioners have claimed that numerous medicinal plants and their formulations can be effectively used for the alleviation of different types of liver diseases [9]. The objective of this study was therefore to evaluate the hepatoprotective and nephroprotective activity on bark extracts of *Bridelia retusa* S.

Materials and methods

Plant materials and preparation of bark extracts

The bark of *Bridelia retusa* S. was obtained from the jungles of Western Ghats (Amboli) under the guidance of Forest Officer. The plant material was cut into pieces and subjected to shade drying. On complete drying the pieces were powdered and stored in air tight containers at room temperature for future use. The powder (50 gm) was subjected to extraction in soxhlet apparatus using various solvents of petroleum ether (40-60^{o}C), chloroform (60-62^{o}C distillation) and ethanol in order to obtain organic extract while distilled water was used for aqueous extract which was carried out one after the other in a sequential manner based on their polarity. All the extracts were filtered using Whatman filter paper no.1 and concentrated. The filtered extracts were evaporated and dried extracts obtained from each of the solvents were labeled, weighed and stored at 4^{0}C in air tight containers.

Animals

Laboratory bred adult virgin Swiss albino mice aged 90 days weighing between 25-35 gm were used in the experiments. The mice were maintained in P.G. Deparment of Studies in Zoology, Karnatak University, Dharwad. Mice bred normally, almost throughout the year and permitted by local ethical committee. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. Standard mice pellet diet "Gold Mohar' (Hindustan Lever company, Mumbai) was provided along with water *ad libitum*. The mice were maintained under normal day/night schedule (12L:12D) at room temperature 25$\pm$2^{0}C.

Drug Formulation

Bridelia retusa S. bark extracts were administered at doses below acute LD_{50} level of intoxication according to the body weight of the mice. Oral suspensions containing 25mg/km and 50 mg/kg (body weight) of ethanolic and aqueous extracts were prepared in distilled water.

Chemicals and Treatment

Carbon tertrachloride (Qualigenes fine chemicals, Mumbai) toxicity was induced by subcutaneous (sc) injections of 2ml/gm body weight diluted in Olive oil (1:1) just before administration.

Experimental Schedule

CCL_4 induced toxicity was evaluated using acute injury model [10-11]. Mice were divided into six groups of six mice each.

Group I	1ml/kg Olive oil with normal food and water for 3days. (negative control)
Group II	2ml/kg CCL_4: Olive oil (1:1), sc for 3 days. (positive control)
Group III	2ml/kg CCL_4: Olive oil (1:1), sc for 3days + 25mg/kg ethanol extract for 5 days.
Group IV	2ml/kg CCL_4: Olive oil (1:1), sc for 3days + 50mg/kg ethanol extract for 5days.
Group V	2ml/kg CCL_4: Olive oil (1:1), sc for 3days + 25mg/kg aqueous extract for 5days.
Group VI	2ml/kg CCL_4: Olive oil (1:1), sc for 3days + 50mg/kg aqueous extract for 5days.

The extracts were administered 30 min after CCL_4 treatment. The mice were monitored for change in body weight and food consumption during the experiment. All the mice were necropsied by mild ether anesthesia after 5 days. Liver and kidney were dissected out to evaluate hepatoprotective and nephroprotective activity. The biochemical study such as estimation of DNA [12-13], proteins [14], glycogen [15], cholesterol [16] and activities of enzymes such as LDH [17] , ASAT and ALAT [18] , ACP and AKP [19] , Na^{+1}-K^{+1} ATPase, Ca^{+2} ATPase and Mg^{+2} ATPase [20] were assayed. The data was subjected to statistical analysis by using SPSS package and the significance ($p<0.05$) was determined.

Results

The toxic effect of CCL_4 was observed by decrease in the weight of ovary, liver and kidney while an increase in the weights of uterus, pancreas and adrenal glands. The protective activity of the extracts was justified by the significant ($p<0.05$) decrease in the weights of adrenal glands when compared to CCL_4 treated group (Table 2, Figure 1). Weight loss and decrease in food consumption observed in CCL_4 treated group was rectified in extract treated groups.

The ethanolic and aqueous extracts of the stem bark of *Bridelia retusa* were studied for their hepatoprotective activity in CCL_4 treated female mice. The enzyme activity of ALAT, ASAT, LDH and AKP increased significantly ($p<0.05$) whereas significant ($p<0.05$) decrease in the activity of ACP and ATPases in CCL_4 treated group when compared with the corresponding parameters of normal control group. The activity of ALAT and LDH in the liver were significantly lowered ($p<0.05$) in all

the groups treated with extracts when compared to CCL_4 treated group and were found to be brought almost to normal control. When compared to mice treated with CCL_4, group IV and V showed significantly ($p<0.05$) lowered ASAT activity while group III and V showed significantly ($p<0.05$) lowered AKP enzyme activity and the values were normalized to that of group I. The enzyme activity of ACP in the liver was significantly increased ($p<0.05$) in all the groups treated with extracts when compared to CCL_4 treated group and were found to be brought almost to normal control.

The ATPase enzyme activity was significantly ($p<0.05$) increased with respect to that of CCL_4 treated group and the values of Mg^{+2} ATPase approached normal control (Figure 2). The biochemical content (carbohydrate, DNA, RNA and protein) were estimated to be significantly ($p<0.05$) decreased in CCL_4 treated group when compared to that of negative control group and the activity in extract treated group was almost equivalent to that of normal group. The cholesterol content was significantly ($p<0.05$) increased when compared to CCL_4 exposed group which was significantly ($p<0.05$) reduced in extract treated groups (Figure 3 and 4).

The nephroprotective activity of ethanolic and aqueous extracts of *B. retusa* was investigated in CCL_4 induced toxicity in mice. The enzyme activity of ALAT, ASAT, LDH and AKP increased significantly ($p<0.05$) whereas significant ($p<0.05$) decrease in the activity of ACP and ATPases in CCL_4 treated group when compared with the corresponding parameters of normal control group. The enzyme activity of ALAT, ASAT, LDH and AKP in the kidney were significantly lowered ($p<0.05$) in all the groups treated with extracts when compared to CCL_4 treated group and were found to be equivalent to that of the normal control. The enzyme activity of ACP in the liver was significantly increased ($p<0.05$) in all the groups treated with extracts when compared to CCL_4 treated group and were found to be brought almost to that of group I. The ATPase enzyme activity was significantly ($p<0.05$) increased when compared to that of CCL_4 treated group and the values almost approached the normal control (Figure 5).

The biochemical content (carbohydrate, DNA, RNA and protein) were estimated to be significantly ($p<0.05$) decreased while cholesterol concentration was significantly ($p<0.05$) increased in CCL_4 treated group when compared to that of negative control group. The concentration of protein, carbohydrate, DNA and RNA in extract treated group were found to be significantly ($p<0.05$) increased while cholesterol showed a non significant decrease when compared to that of CCL_4 treated group and the values were almost that of normal group (Figure 6 and 7).

Discussion

CCL_4 is a manufactured chemical that does not occur naturally [22]. Mostly, CCL_4 is used in the production of chloroflurocarbons (CFC) and other chlorinated compounds which has been banned as an environmental pollutant. It is used as a solvent in coal tar, resins and raw material of flurocarbons, applicable as coolant and as fire extinguishers. CCL_4 is a well known hepatoxin and nephrotoxin. Short and long term exposure to CCL_4 also causes damage to skin, brain and blood, and in some cases causes death as well [23]. Carbon tetrachloride is thought to be metabolized by the hepatic cytochrome P-450 enzymes with the production of the highly toxic trichloromethyl radical, which binds to macromolecules, initiating lipid peroxidation and destroying cell membranes. CCL_4 breaks down to highly toxic trichloromethyl (CCL_3*) and trichloromethyl peroxyl (CCL_3O_2*) radicals by Cytochrome P_{450} enzymes, an apoprotein located in endoplasmic reticullum of cells cleaves C-CL bond. The mechanism involves CCL_4 getting metabolized to CCL_3*, which further reacts with molecular oxygen resulting in the formation of CCL_3O_2*. Trichloromethyl (CCL_3*) and trichloromethyl peroxyl (CCL_3O_2) combine with cellular lipids and proteins [24-27].The toxic effect of CCL_4 in the present study was observed by decrease in the weight of ovaries, liver and kidney. The metabolic dysfunction could be the cause for weight loss and the significant weight gain in extract group may be due to its crucial role in synthesizing newer cytoplasm, enabling regeneration of cells, thus improving the functioning as reported in earlier studies [28].

The enzyme activity of ALAT, ASAT, LDH and AKP in the present study, increased significantly ($p<0.05$) whereas significant ($p<0.05$) decrease in the activity of ACP and ATPases in CCL_4 treated group when compared with the corresponding parameters of normal control group of liver and kidney. Increased ALAT was a better index of liver injury, as its activity represents 90% of the total enzyme activity. The reported decrease in transaminase activity due to extract indicated that stabilization of plasma membrane and protection against CCL_4 toxicity. The increase activity of AKP by extract was due to its increased synthesis in presence of biliary pressure. Earlier reports have indicated toxic effects of compounds increases activity of marker enzymes like ASAT, ALAT, AKP and LDH as a result of tissue damage including liver and kidney. Increase in the LDH and AKP activities indicated cellular damage due to loss in functional integrity of cell membrane [29]. Increase urinary LDH suggests renal tubular injury which was reduced by grape seed extract as reported earlier. Uncoupling of oxidative phosphorylation by CCl_4 causes a fall in the activity of ATPase [30].

The biochemical content (carbohydrate, DNA, RNA and protein) were estimated to be significantly

($p<0.05$) decreased cholesterol concentration was significantly ($p<0.05$) increased in CCL_4 treated group when compared to that of negative control group and the activity in extract treated group was almost equivalent to that of normal group in the present study. The CCl_3 radical reacts with cellular proteins and other macromolecules like DNA and RNA with a simultaneous attack on polyunsaturated fatty acids leading to damage. Alterations in RNA metabolism after CCL_4 administration followed by a decrease in the tissue RNA levels, subsequently changing the nucleo-cytoplasmic ratio in the affected the tissue.

The metabolism of CCL_4 yields products that cause fragmentation of endoplasmic reticulum and disruption of ribosomes into subunits. The capacity of liver microsomes to incorporate amino acids is depressed, causing a generalized inhibition of protein synthesis. Previous studies have shown that *Phyllanthus amarus* extract increased DNA and RNA contents in the liver tissue [30]. In the present study, 50mg/kg showed better result when compared to 25mg/kg of extract in restoring normal activity of enzymes and biochemical contents. Similar results of effective mitigation of toxic effects of carbon tetrachloride in a dose-dependent manner and thus offered significant protection as seen in the present study [31].

The activity of enzymes and concentration of biochemicals were equalized more to that of normal by ethanolic extract rather than aqueous extract. Similar to the present study, ethanolic extract showed better protective effect which may be attributed to the individual or combined effects of phytoconstituents additional to that of aqueous extract [32].

Conclusion

The change in enzyme activity and biochemicals caused by CCl_4 in liver and kidney was brought to normal values by both ethanolic and aqueous extracts, though ethanolic extract showed better recovery from toxic effects in a dose dependent manner. Such plants may help to discover new chemical classes of drugs that could serve as selective chemotherapeutic agents for the maintenance of health.

The promising results obtained indicate extensive study on this plant will enable to exploit its potentials. Thus, this study justifies the folklore medicinal and therapeutic value of the plant.

Acknowledgment

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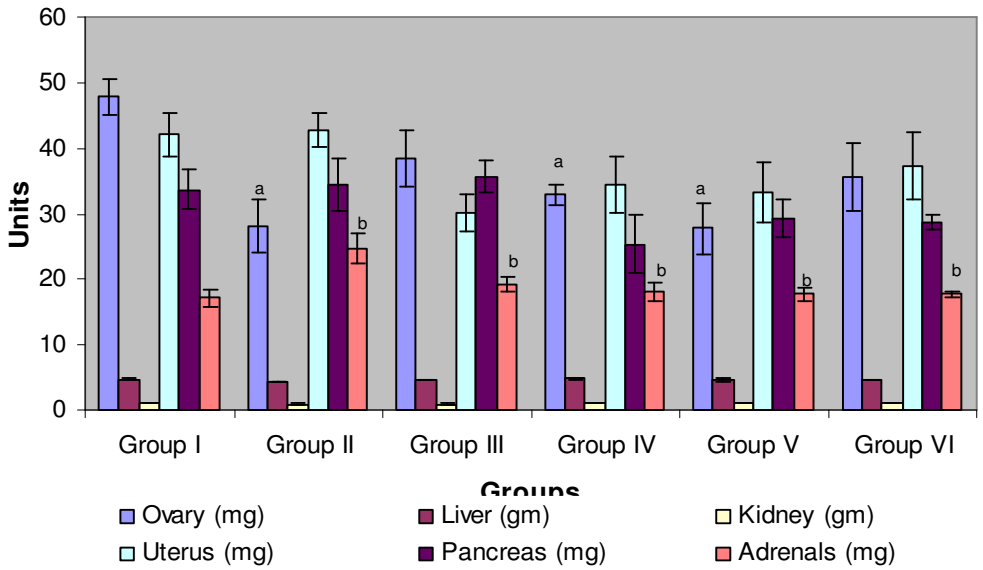
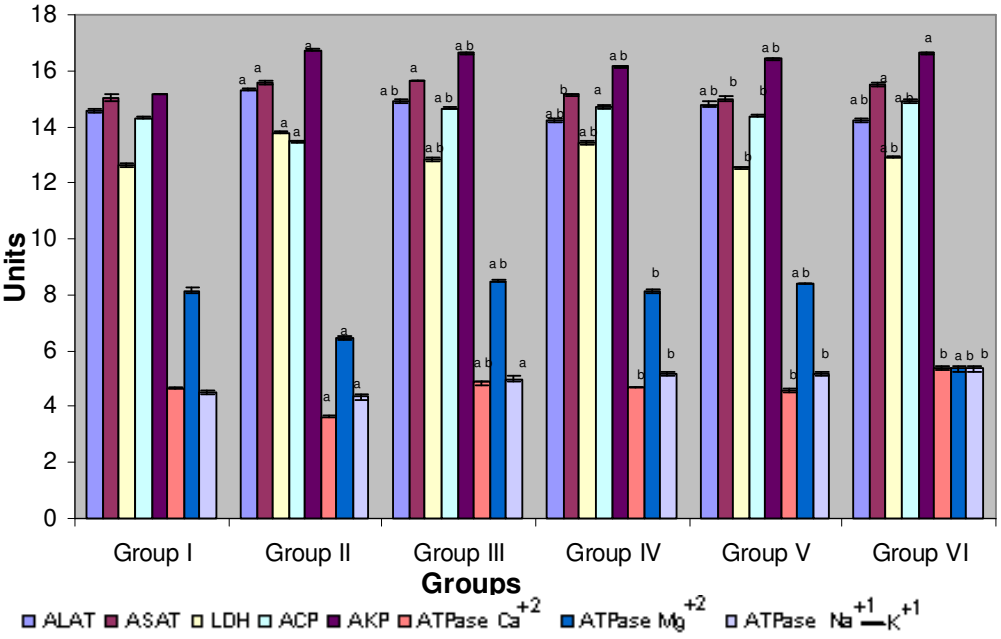
Figure 1: Effect of *Bridelia retusa S.* stem bark extract on organs weight in mice exposed to CCl_4.



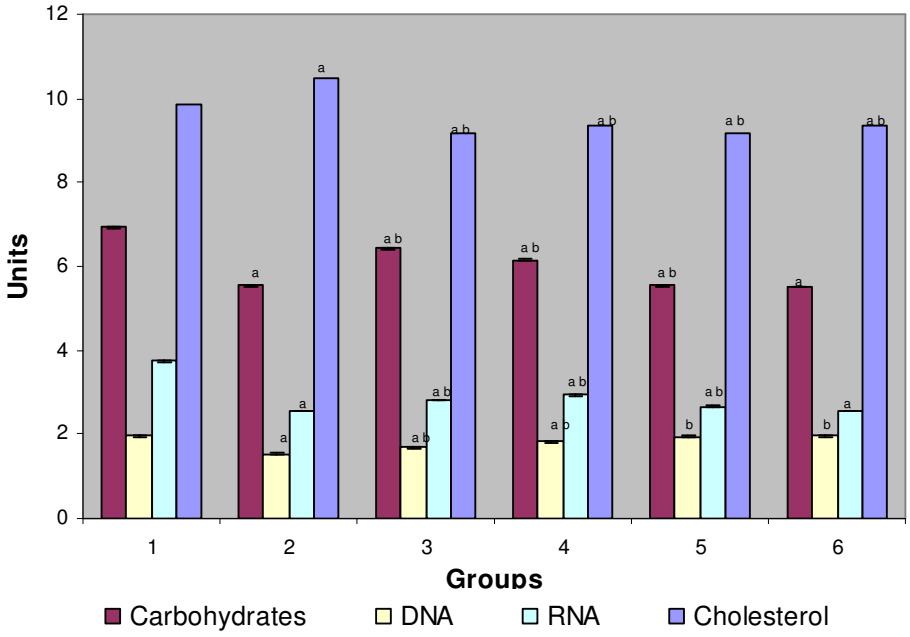
[a] Significant (<0.05) when compared to group I. [b] Significant (<0.05) when compared to group II.

Figure 2: Hepatoprotective effect of *Bridelia retusa S* .stem bark extract on enzyme activity of Transaminase, dehydrogenase and phosphotase in CCl_4 treated mice.



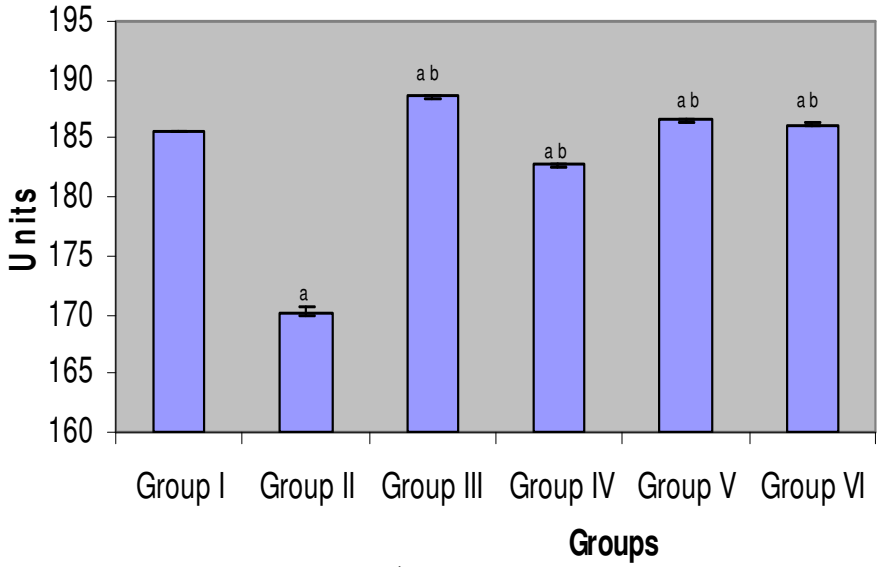
[a] Significant (<0.05) when compared to group I. [b] Significant (<0.05) when compared to group II.

Figure 3: Hepatoprotective effect of *Bridelia retusa S* .stem bark extract on biochemical content in CCl_4 treated mice.



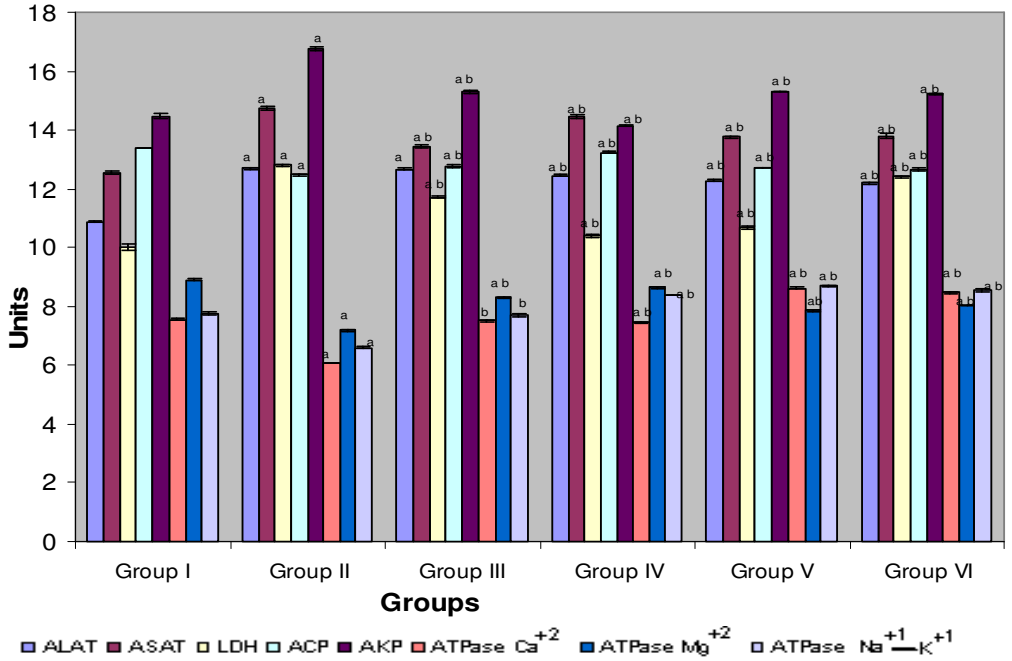
[a] Significant (<0.05) when compared to group I. [b] Significant (<0.05) when compared to group II.

Figure 4: Hepatoprotective effect of *Bridelia retusa S* .stem bark extract on proteins in CCl_4 treated mice.



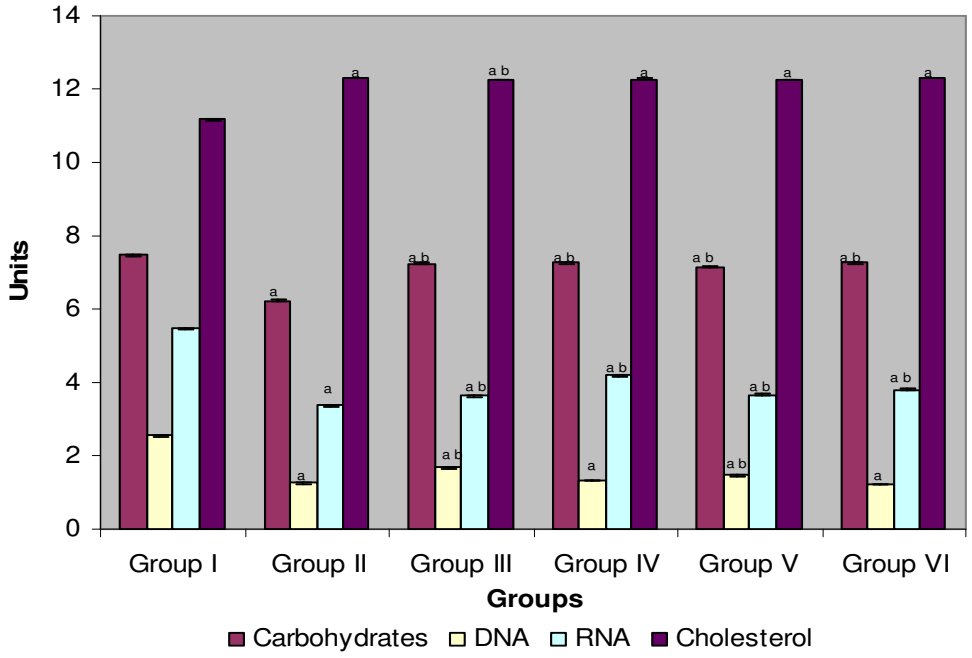
[a] Significant (<0.05) when compared to group I. [b] Significant (<0.05) when compared to group II.

Figure 5: Nephroprotective effect of *Bridelia retusa S.* stem bark extract on Transaminase, dehydrogenase and phosphotase enzyme activity in CCl4 treated mice.



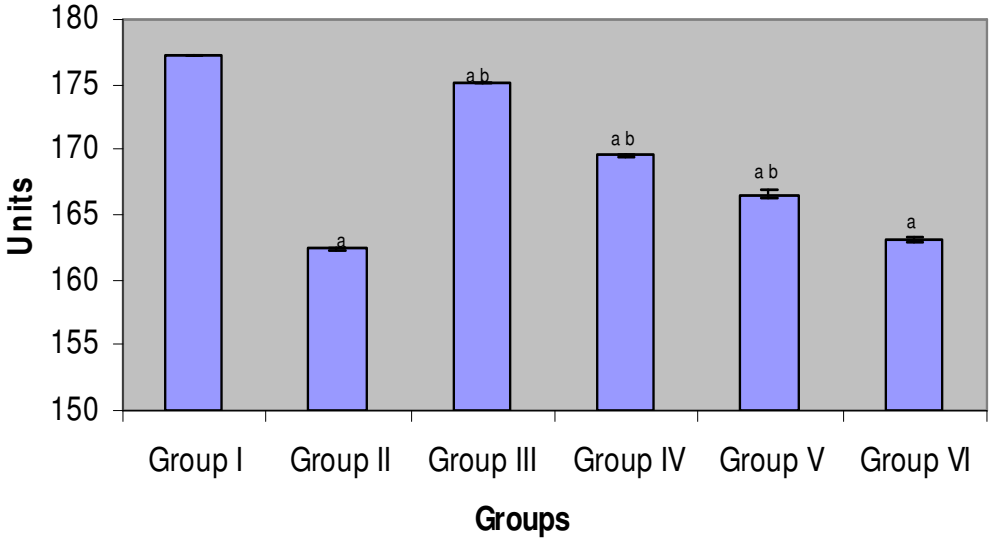
[a] Significant (<0.05) when compared to group I. [b] Significant (<0.05) when compared to group II.

Figure 6: Nephroprotective effect of *Bridelia retusa S.* stem bark extract on biochemical content in CCl_4 treated mice.



[a] Significant (<0.05) when compared to group I. [b] Significant (<0.05) when compared to group II.

Figure 7: Nephroprotective effect of *Bridelia retusa S.* stem bark extract on proteins in CCl_4 treated mice.



[a] Significant (<0.05) when compared to group I. [b] Significant (<0.05) when compared to group II.