Protective role of bark extract of Bridelia retusa Spreng on CCl₄ induced histological toxicity in mice.

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ABSTRACT

The present investigation was undertaken to evaluate the protective role of bark extracts of Bridelia retusa S. on the liver and kidney after CCl₄ intoxication. The bark of Bridelia retusa S. was powdered and subjected to sequential extraction based on polarity using solvents such as petroleum ether, chloroform, ethanol and distilled water. The extract was filtered, concentrated, dried and stored in refrigerator until further use. Oral suspensions containing 25 and 50 mg/kg body weight of ethanolic and aqueous extracts were administered for 5 days to carbon tetrachloride treated female mice. The protective role of ethanolic and aqueous stem bark extracts of Bridelia retusa S. were investigated on histological profiles of liver and kidney. The effect of 50 mg of ethanolic extract was visible by reduced CCL₄ induced toxicity on the histology of liver and kidney which were restored to that of normal better than aqueous extract wherein steatosis and dilation of central vein caused by CCl₄ was reduced and normalized hepatocytes. Hence, the present study indicates that both the extracts possessed amelioration of histological changes in the liver and kidney of CCl₄ intoxicated mice though ethanolic extract showed better recovery in dose dependent manner.

Keywords: Bridelia retusa Spreng, sequential extraction, liver and kidney histology.

1. Introduction

India is well known for a plethora of medicinal plants. The traditional Indian medicinal plants act as antiradicals and DNA cleavage protectors[1]. Phytochemical 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. Epidemiological, clinical and laboratory research on antioxidants suggest their use in the prevention and treatment of a number of these disorders[2]. Carbon tetrachloride (CCl₄), an industrial solvent, a well-established hepatotoxin, it was demonstrated that liver is not the only target organ of CCl₄ but it causes free radical generation in other tissues also such as kidneys, heart, lung, testis, brain and blood in various studies by researchers [2,3,5,4].

Bridelia retusa S. is a moderate sized tree or a shrub belonging to Euphorbiaceae family found growing throughout India[7]. The chemical constituents found in fruit pulp and seeds include gallic acid, β-sitosterol, and ellagic acid[8]. The bark contains triterpenoid ketone and 16-40% tannins. Detail study of the bark for feed and fodder characteristics revealed the presence of 12.76% condensed tannins, 12.49% total phenols, 12.20% tannic acid, 9.03% tannads, 10.38% crude proteins, 69.31% Neutral Detergent Fibre (NDF), 28.34% Acid Detergent Fibres (ADF) and minerals like calcium, copper, iron, manganese, magnesium, phosphorus, zinc, etc[9,10].

Decoction of stem bark with country liquor is used for diarrhea, ear ache and prevents pregnancy. Pounded bark is mixed with gum of Sterculia urens 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]. Therefore, the present investigation was undertaken to evaluate the protective role of bark extracts of Bridelia retusa S. on the liver and kidney after CCl₄ intoxication.
2. Materials and Methods
2.1 Plant materials
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 mechanically and stored in air tight containers at room temperature for future use.

2.2 Preparation of bark extracts
The powder (50 gm) was subjected to extraction in soxhlet apparatus using various solvents of petroleum ether (40-60 °C), chloroform (60-62 °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 concentrated and filtered using Whatman filter paper no. 1. The filtered organic extract was evaporated while the aqueous extract was dried in a desiccator. The colour and percentage yield (% yield w/w, on dry plant material basis) of each extract was recorded after performing at least three replicates. The dried extracts obtained from each of the solvents were labeled, weighed and stored at 4 °C in air tight containers.

2.3 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. Department of Studies in Zoology, Karnatak University, Dharwad. Mice breed 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±2 °C.

2.4 Drug Formulation
*Bridelia retusa* S. bark extracts were administered at doses below acute LD50 level of intoxication according to the body weight of the mice. Oral suspension containing 25 mg/gm and 50 mg/gm (body weight) of ethanolic and aqueous extracts were prepared in distilled water.

2.5 Chemicals and Treatment
Carbon tetrachloride (Qualigens fine chemicals, Mumbai) toxicity was induced by subcutaneous (sc) injections of 2 ml/gm body weight diluted in Olive oil (1:1) just before administration.

2.6 Experimental Schedule
CCL4 induced toxicity was evaluated using acute injury model [11, 12]. The mice were divided into six groups of ten mice each as follows. Olive oil (0.5 ml) was gavaged orally to group I with normal food and water (negative control) and group II, CCL4 was administered to mice (positive control) for 3 days. The groups III, IV, were given two doses 25 and 50 mg/kg of ethanolic and V and IV were administered with aqueous extract to CCL4 intoxicated mice for 5 days. Extracts were administered 30 min after CCL4 treatment. All the mice were autopsied by mild ether anesthesia after 5 days. The mice were monitored for change in body weight and food consumption during the experiment. Liver and kidney were dissected out and subjected to histological studies.

3. Result
The ethanolic and aqueous stem bark extracts of *Bridelia retusa* S. were investigated protective role in liver and kidney of CCL4 treated female mice. The histologic observations of the liver in control mouse revealed normal structure of the liver. The hepatic lobules possess the rows of cuboidal or polyhedral hepatic cells forms hepatic cords from the center to periphery of the lobule. Hepatic cords arranged radially around the central vein. Between hepatic cords however, narrow irregular spaces the lacunae through which hepatic sinusoids runs (Fig.1). In the mice treated with CCL4, histologic study of the liver exhibited steatosis of hepatic cells with loss of radial arrangement and dilated central vein (Fig. 2). In the mice treated with 25 and 50 mg ethanolic extract for 5 days after CCL4 exposure, histologic study of the liver revealed steatosis and dilation of central vein was reduced and normalized hepatocytes. The recovery of CCL4 induced toxicity by decreased steatosis and radial arrangement of hepatocytes was achieved by all the extracts and 50 mg of ethanolic extract showed better results.

Effect of extracts of stem bark of *Bridelia retusa* (ESBB) on liver of mice exposed to CCL4

![Image of histological observation](image.png)

Fig 1: T. S. of the liver of the normal mouse (negative control) showing radially arranged hepatic cords around the central vein and hepatocytes with centrally located nuclei.
Fig 2: T. S. of the liver of the mouse (positive control) treated with CCL₄ for 3 days, histologic study of the liver exhibited severe liver degenerated with steatosis in the hepatocytes.

Fig 3: T. S. of the liver of the mouse treated with CCL₄ for 3 days and 25 mg/kg ethanol extract for 5 days, histologic study of the liver exhibited decreased steatosis.

Fig 4: T. S. of the liver of the mouse treated CCL₄ for 3 days and 50 mg/kg ethanol extract for 5 days, histologic study of the liver showed moderately brought central vein, hepatic cell with preserved cytoplasm and prominent nucleus.

Fig 5: T. S. of the liver of the mouse treated with CCL₄ for 3 days and 25 mg/kg aqueous extract for 5 days, histologic study of the liver revealed moderate steatosis degeneration.
Fig 6: T. S. of the liver of the mouse treated CCL₄ for 3 days and 50 mg/kg aqueous extract for 5 days, histologic observations revealed moderate regeneration.

Photographs original exposures at 100 X
CV - Central vein
H - Hepatocytes
St - Steatosis
B - Ballooned Hepatocytes

The histologic observation of the kidney of control mouse revealed that the cortex consists of numerous Bowman’s capsule which are double layered cup like structure inside highly anatomizing network of afferent and efferent arterioles called glomerulus. The cortical tubules were well organized with connective tissue and inter tubular spaces. Tubular walls are made up of thick epithelial cells (Fig. 7). Histologic examination of the kidney of mice treated with CCL₄, showed small and atrophied glomeruli, loosely arranged in Bowman’s capsule (Fig. 8). Histologic study of kidney of mice treated with 25 mg ethanol extract for 5 days after CCL₄ exposure, displayed small glomeruli were moderately atrophied and loosely arranged in Bowman’s capsule (Fig. 9). Histologic observation of the kidney of mice treated with 50 mg ethanol extract for 5 days after CCL₄ exposure, showed loosely arranged glomeruli in Bowman’s capsule with minimum atrophy (Fig. 10). Histologic observations of the kidney of mice treated with 25 and 50 mg aqueous extract for 5 days after CCL₄ exposure, revealed glomeruli were less atrophied and less loosely arranged in Bowman’s capsule (Figs. 11 and 12). The effect of 50 mg of ethanolic extract was visible by reduced CCL₄ induced toxicity on the histology of kidney.

Effect of extracts of stem bark of *Bridelia retusa* (ESBB) on kidney of mice mice exposed to CCL₄

Fig 7: T. S. of the kidney of the normal mouse (negative control) showing cortical tubules with normal arrangement. Thick epithelial cells with prominent glomerulus in Bowman’s capsule.

Fig 8: T. S. of the kidney of the mouse (positive control) treated with CCL₄ for 3 days showed glomeruli were small and atrophied, loosely arranged in Bowman’s capsule. Histologic study of kidney of mouse displayed disorganized glomerulus.
**Fig 9**: T. S. of the kidney of the mouse treated CCL₄ for 3 days and 25 mg/kg ethanol extract for 5 days. The small glomeruli were moderately atrophied and loosely arranged in Bowman’s capsule.

**Fig 10**: T. S. of the kidney of the mouse treated with CCL₄ for 3 days and 50 mg/kg ethanol extract for 5 days showed glomeruli were loosely arranged in Bowman’s capsule with minimum atrophy.

**Fig 11**: T. S. of the kidney of the mouse treated with 25 mg/kg aqueous extract for 5 days revealed that the glomeruli were less atrophied and loosely attached to Bowman’s capsule.

**Fig 12**: T. S. of the kidney of the mouse treated with CCL₄ for 3 days and 50 mg/kg aqueous extract for 5 days showed glomeruli were less atrophied and less loosely arranged in Bowman’s capsule.

Photographs original exposure at 100 X

CT - Cortical tubules
G - Glomerulus
BC - Bowman’s capsule
TC - Tubular cell
4. Discussion

In the present study, the mice treated with CCL₄ exhibited steatosis of hepatic cells with loss of radial arrangement and dilated central vein. The recovery of CCL₄ induced toxicity in the present study was evident by decreased steatosis and radial arrangement of hepatocytes which was achieved by both the doses of the extracts though 50 mg of ethanolic extract showed better results. Carbon tetrachloride is considered as a direct hepatotoxin which produces centrilobular necrosis and steatosis. The mechanism of hepatic injury by CCl₄ involves lipid peroxidation of membrane bound fatty acids which result in destructing the cell membrane and the intracellular organelles of the hepatocyte. In the present study the mice treated with CCL₄ histologic study of the liver exhibited steatosis of hepatic cells with loss of radial arrangement and dilated central vein. CCl₄ administration elicited extensive changes in liver morphology, including marked fatty degeneration, necrosis and hepatocyte ballooning, similarly in present study ballooning and steatosis was observed. CCl₄ treated group showed that the liver cells are intoxicated with CCl₄ with the normal architecture of the liver was completely damaged. In the mice treated with extracts for 5 days after CCL₄ exposure, the histologic study of the liver revealed decreased steatosis and dilatation of central vein was reduced and normalized hepatocytes by ethanolic extract at 50 mg which was more than in aqueous extract. The extract treated group of earlier findings showed the normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations compared to the normal saline control group. The toxin mediated changes in livers of mice pretreated with Capparis spinosa root bark extracts in different doses one hour after the administration of CCl₄ showed less intensity than those observed in the livers of carbon tetrachloride treated mice. The liver sections of CCl₄ intoxicated mice also showed massive fatty changes, necrosis and ballooning degeneration as also seen in present findings. The findings in present study are in confirmation with those reported by Vidya and Krishna, that CCl₄ induced fatty liver in mice lead to necrosis, decrease in sinusoidal spaces, fatty degeneration and congestion of liver. However, such changes were almost negligible in Repchol and Superliv supplemented groups. Parenchyma showed occasional fat containing cells with dilated and congested central vein which was moderately improved by aqueous Solanum nigrum extract. On histological examinations, there were minimal lesions and fatty change was almost absent which revealed that extract may contain the active ingredient(s) which protects the liver. The liver sections showed almost complete recovery from necrosis and fatty infiltration due to methanol extract of Leucas hirta while aqueous showed moderate effects which coincides with the present study wherein the ethanol extract showed pronounced effects. Those livers that were treated with CCl₄ were reported with a high degree of fatty change, compression of sinusoid and distended hepatocytes. Hepatocytes of liver of CCl₄ treated rats showed intense centrilobular necrosis and fatty changes (steatosis) which includes intracellular accumulation of neutral fats. The hepatocytes were distended with fat vacuoles due to increase deposition of intracellular lipids. The liver damage was not observed in silymarin and normal arrangement of hepatocytes was achieved without necrosis in Clerodendrum serratum extract treated group in comparison to other extracts that showed moderate or insignificant effect as reported by Vidya and Krishna.

Vernonia amygdalina extract administered to liver damaged rats apparently accelerated the reversion of the liver damage as reported by Arhogro et al. in dose dependent manner as also seen in present study with 50 mg ethanolic extract showing the best effects. The protective effect on CCl₄ induced toxicity on liver weight was confirmed by histologic study in the present findings which revealed decreased steatosis and dilatation of central vein and normalized hepatocytes by 50 mg of the extract which was more than in lower dose of both the extracts.

In the present study, the histologic examination of the kidney of mice treated with CCl₄ showed glomeruli were small and atrophied, loosely arranged in Bowman’s capsule. Histologic observations of the kidney of mice treated with extracts for 5 days after CCl₄ exposure, revealed glomeruli were less atrophied and less loosely arranged in Bowman’s capsule, which was better in ethanolic extract when compared with that of aqueous extract. In earlier studies reported by Onyemaechi et al., distortion of renal architecture with tubular necrosis and glomerular degeneration on treatment with Kigelia africana fruit extract ameliorated the overall changes induced by cisplatin. Adhesion of glomerular tuft to Bowman’s capsule along with interstitial hemorrhages including vacuolar and granular degenerative changes was evident in the kidney of CCl₄ intoxicated birds, however, such changes were almost negligible in herbal Repchol and Superliv supplemented groups reported by Sonkusale et al. The chemical constituents obtained from plants may be pharmacologically screened for developing novel agents with biological activity. Thus both the changes in weight and histology due to CCl₄ were rectified by plant extract with ethanolic extract at 50 mg showing better protection.

5. Conclusion

The changes in histology caused by CCl₄ in liver and kidney when compared to normal control were rectified by both ethanolic and aqueous extracts, though ethanolic extract showed better effects.

6. Acknowledgment

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7. References

4. Ahmad FF, Cowan DL, Sun AY. Detection of free radical formation in various tissues after acute carbon tetrachloride administration in gerbil. Life Sci 1987;


