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Research Article

HEPATOPROTECTIVE EFFECT OF THE METHANOLIC EXTRACT OF WHOLE PLANT OF *BORRERIA ARTICULARIS* ON CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

The hepatoprotective activity of methanolic extract of *Borreria articularis* (*L.F) F.N. Willams:* (Rubiaceae) at doses of 250 mg/kg and 500 mg/kg were evaluated by carbon tetrachloride (CCl_4) intoxication in rats. The toxic group which received 25%CCl_4inolive oil (1 ml/kg) per oral (p.o), alone exhibited significant increase in serum ALT, AST, ALP, TBlevels. It also exhibited significant ($P<0.001$) decrease in TP and ALB levels. The groups received pretreatment of *Borreria articularis* at a dose of 250 and 500 mg/kg b.w.p.o. had reduced the AST, ALT, ALP and TB levels and the effects were compared withstanderddrug(Silymarin100mg/kgb.w.p.o).Thetotal protein (TP) and albumin (ALB) levels were significantly increased in the animalsreceived pretreatment of the extract at the moderate and higher dose levels and the histopathological studies also supported the protective effect of the extract.

Keywords: Carbon tetrachloride, Silymarin, *Borreria articularis*, biochemical parameters and histological study.

INTRODUCTION

The largest organ in the human body, liver, plays a very important role in the metabolism of foreign compounds entering the body. The exposure to the foreign compounds may be through consumption of alien/contaminated foods, from exposure to chemical substances in the occupation environment or through synthetic drugs consumed for various pathological conditions. These compounds have many toxic manifestations on the human liver. [1] The liver gets injured also by viruses, chemicals, alcohol and autoimmune diseases. Liver diseases remained one of the serious health problems, and medicinal plants and herbs have been in use for treating these in the Indian traditional systems of medicine, especially Ayurveda. The present modern age demands proof on a scientific basis to justify the various medicinal uses of herbs. [2] India is well-known for a plethora of medicinal plants. The medicinal use of many plants (as hepatoprotectants) like *Andrographis paniculata, Azardiracta indica, Cassia fustula, Elephantopus scaber, Hibiscus rosasinensis, Phyllanthus debilis, Picrorrhiza kurroa, Glycyrrhiza glabra* linn has been reported in the literature. [1, 3, 4] *Borreria articularis* (*L.F) F.N. Willams:* (Rubiaceae) *(syn: Spermacoce articularis L.F and Spermacoce hispida L.* is an important medicinal plant used widely in Indian folk medicine. [5] Leaf extract of the plant is in use against hemorrhoids, galls tones, jaundice**,** and conjunctivitis, roots are used to mouthwash to relieve toothache, decoction of the herb used to relieve headache, [5, 6,] while seeds are demulcent in diarrhea, dysentery.[7,8,9,10] and anti-bacterial activity. [11] The previous phytochemical studies, has reported the presence of a triterpenoidal constituents, from this plant were reported earlier [7, 8] they have been characterized as beta-amyrin and 3-acetoxy-oleana-12-en-29-oic acid. [12] It also contains alkaloids, glycosides, steroids, flavonoids and tannins. Liver diseases are a major public health problem all over the world. Now-a-days, the prevention of liver cirrhosis

and fibrosis is the major and vital concern of the therapy.

This plant used in folk medicine in many parts of Native to tropical Asia from northern India, southwards and eastwards to southern China, the Philippines, Malaysia and Srilanka and parts of India. Hence the present study is focused to evaluate the hepatoprotective potentials of the whole plant against carbon tetrachloride-induced liver injury in albino rats and the analyzed parameters included total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), tot-al protein (TP) and albumin (ALB) and histopathology of liver damage.

MATERIALS AND METHODS

2.1. Collection of Plant Material

Whole plant of the *Borreria articularis (L.F) F.N. Willams:* (Rubeaceae) were collected from pallivelpulla, Warangal District, Andhra Pradesh (India). The plant was authenticated by Prof. Raju S. Vastavaya Department of Botany, Kakatiya University, Warangal, Andhra Pradesh (India) and a specimen voucher (C.No.1029/Param and V.S. Raju) was deposited for future reference.

2.2. Preparation of plant extract

The whole plant of *Borreria articularis (L.F) F.N. Willams:* (Rubeaceae) were made into a coarse powder and extracted with methanol by maceration. The crude extract was evaporated by using Rotavapour (BUCHI, Germany) under reduced pressure.

2.3. Phytochemical Screening [13].

The extract was subjected to preliminary phytochemical screening of whole plant of *Borreria articularis.* Methanolic extract showed the presence of various phytoconstituents i.e. Alkaloids, Steroids, Triterpenoids, Flavonoids and Tannins. TLC study: Precoated TLC plate of Silica gel 60F254 (MERCK, India) of 0.2mm thickness was used. TLC pattern of BAME was developed using Toluen:Ethylacetate:Diethylenamine (70:20:10), Chloroform:Methanol (90:10), Chloroform:Water (90:10) and Ethylacetate: Methanol: Water (81:11:8) as solvent system and sprayed with Vanillin-Sulphuric acid (VS) reagent. Then, the plates were scanned in CAMAG-TLC scanner, the spots were observed were bluish-black, purple spots characteristic for steroidal and triterpenoidal compounds recorded and the R_f values were determined. The earlier reported chloroform extract of the aerial parts and roots of *Borreria articularis* yielded a new triterpene, 3-acetoxy-oleana-12-en-29-oic acid along with beta-amyrin. The structures were established by means of spectral as well as chemical studies. [12]

2.4. Animals

Female albino wistar rats (100-150 g) procured from M/S Mahaveera Enterprises, Hyderabad (India) and were used for the studies. The animals were housed in large polypropylene cages in a temperature controlled room ($20^0C \pm 2^0C$) provided with standardized pellet feed and clean drinking water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee.

2.4. Hepatoprotective Studies [14]

The animals were divided into 5 groups of six animals each and subjected to the following treatments. Group-I served as the control and received 2% gum acacia (1ml/kg p.o) daily for 7days. Group-II served as the toxic and received 25% CCl_4 in olive oil (1ml/kg.p.o) daily for 7 days. Group-III served as the standard and received (100 mg/kg.p.o) daily for 7 days. Group-IV and V were treated with extract of BAME at 250 and 500mg /kg.p.o respectively for 7 days. All administrations were by oral route. On the 7^{th} day of the experiment, the animals in Groups II-V have received 25% CCl_4 in olive oil at dose of (1ml/kg.p.o) after 30 Silymarin.Blood samples were

collected from the animals,36 hours after CCl4 administration through common carotid artery.

2.5. Biochemical estimation

All the animals were anaesthetized with thiopentone sodium (60 mg/kg.i.p) and scarified on 7th day, 36 hours after administration of CCl_4 and blood was collected through the common carotid artery by carefully opening the neck region of the rat. The blood samples were allowed to coagulate at room temperature and the serum was separated by centrifuge at 3000 rpm for 15 min and was then used f or the analysis of biochemical hep-
atic markers i.e. total bilirubin (TB), alanine aminotran sferase (ALT), aspartate aminotransferase (AST), alka line phosphatase (ALP), total protein (TP) and albumi n (ALB) were estimated by their specific methods.

2.6. Histopathological examination

A portion of liver tissue from each group was carefully dissected out, washed with 0.9% normal saline solution and preserved in a 10% Formaldehyde solution for histopathological studies. Sections (4-5mm thick) were prepared and stained with hemotoxylin and Eosin dye for photomicorscopic observation. The microscopic slides of the liver cells were photographed at a magnification of x100. The toxic group showed excessive formation of connective tissue with nodules and scarred tissue, cell-necrosis, fatty changes, hyaline degeneration, ballooning degeneration and infiltration of Kupffer cells and lymphocytes. Most effective pretreated group *Borreria articularis* 500 and Silymarin group had most effective hepatic cytoprotective action with near-normal histology.

2.8 Reduction in CCL_4-Induced Prolongation of Pentobarbitone Induced Sleeping Time [15]

This method is used to screen anti-ccl_4 toxicity of drugs in animals. Hepatotoxic chemicals like ccl_4 reduce the level of drug metabolizing enzymes in liver. Therefore metabolism of Pentobarbitone reduced resulting in prolongation of pentobarbitone induced sleeping time. If a plant drug reduces this ccl_4-indduced prolongation of sleeping time the drug can be considered hepatoprotective against ccl_4 toxicity. Pentobarbitone induced sleeping time was carried out in Swiss albino mice 50%v/v CCL_4 in olive oil at dose of 50µl/kg/p.o was used as the toxic substance for inducing liver damage. All the various groups of animals were given Pentobarbitone (60mg/kg.i.p.) 2h after CCL_4 (50%v/v in olive oil) administered. The time between loss of righting reflex and its recovery was recorded.

2.9. Statical analysis

The data were expressed as Mean ± SEM and statistically assessed by one-way analysis of variance (ANOVA) and subjected to Dennett's test. The $P<0.05$ was considered significant.

3. RESULTS

The preliminary phytochemical analysis of the crude extract of *Borreria articularis* indicated the presence of Alkaloids, Steroids, Flavonoids, Tannins and Terpenoids**.** The TLC studies carried out also exhibited the R_f values which coincide with the standards. The results of the hepatoprotective studies are given in Table-1. The administration of CCl_4 induced acute liver damage which was well indicated by increased ALT, AST, ALP and TBL when compared with the control group. The group received the toxicant alone also caused decrease in the total protein and albumin levels. The pretreatment of BAME at a dose of 250 and 500 mg/kg exhibited reduction in the serum levels of ALT ($P<0.001$), AST ($P<0.01$), ALP ($P<0.001$) and TBL ($P<0.001$). The TP ($P<0.001$) and ALB ($P<0.01$) levels were also increased and statistically significant when compared with the toxic group. The effect exhibited by Group V & VI (*Borreria articularis* 250&500mg/kg) was

comparable with the standard group treated with Silymarin (100 mg/kg b.w). The pretreatment of *Borreria articularis* at dose of 250 & 500 mg/kg although controlled the rise of ALP ($P<0.01$) and decrease in the ALB ($P<0.05$), it was not effective in controlling the rise of other hepatospecific enzymes as well as the TB and TP levels. The increase in dose levels of *Borreria articularis* had exhibited an increase in efficacy which was reflected in the values of biochemical parameter

4. DISCUSSION

It is well-established that CCl_4 induces hepatotoxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. The CCl_4 is bio-transformed by the cytochrome P450 system (CYP2E1) in the endoplasmic reticulum to produce trichloro methyl free radical (•CCl3) trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen form trichloromethyl peroxyl radical (•OOCCl3) which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxyl free radical leads to cell death. [16]

Assessment of liver damage can be made by estimating the activities of serum ALT, AST, ALP, TB, TP and ALB which are enzymes and proteins originally present in higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage. The elevated levels of these entire marker enzymes observed in the CCl_4 treated group II rats in this present study corresponded to the extensive liver damage induced by toxin, the tendency of these marker enzymes to return towards a near-normalcy in Group IV and V (*Borreria articularis* 250 and 500) treated rats was a clear manifestation of hepatoprotective effect of *Borreria articularis*. A high concentration ofbilirubin in serum is an indication for increased erythrocyte degeneration rate. It also reflects the necrotic conditions of hepatocytes. The oral administration of *Borreria articularis* at 500 mg/kg p.o reduced the serum TB levels.

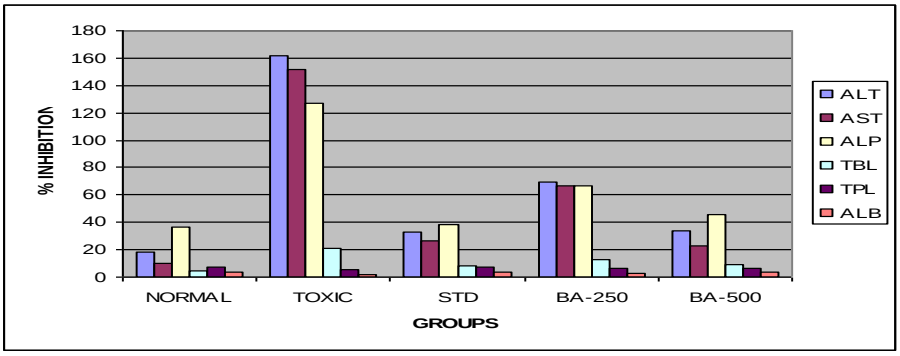
The TP and ALB levels were depressed in hepatotoxic conditions due to defective protein biosynthesis in liver. The CCl_4 intoxication causes disruption and dissociation of polyribosome on endoplasmic reticulum and thereby, reducing the biosynthesis of protein. The pretreatment of *Borreria articularis* might have reduced the polyribosome damage and this mechanism might have pro-tective effect. The histopathological studies are direct means for assessing the protective effect of the drug from liver injuries. The groups received CCl_4 alone, the damage of cells around the central vein were well evident, whereas, the intensity of damage was found lesser in the studies involved pretreatment of *Borreria articularis*. The results of the histopathological studies supported and well correlated with data obtained from evaluation of the biochemical parameters.

Table 1: Effect of the *Borreria articularis* on the Concentrations of Serum Total Bilirubin, Enzymes, Total Proteins and Albumin.

Groups	ALT (IU/L)	AST (IU/L)	ALP (KA units/dl)	TBL (mg%)	TPL (g%)	ALB (g %)
NORMAL	18.22±0.09	10.38±3.25	36.34±4.37	0.17±0.04	7.28±0.16	3.89±0.14
TOXIC (CCl_4 1ml/kg.b.wt)	162.02±5.87	151.5±45.3	126.78±6.86	0.98±0.53	5.59±0.25	1.47±0.25
STANDARD (Silymarin-100mg/kg.b.wt)	32.52±8.24***	26.69±11.6***	38.60±2.16***	0.33±0.03***	7.04±0.11***	3.84±0.12***
***BORRERIA ARTICULARIS* 250mg/kg.b.wt**	69.56±10.62	66.35±7.52	66.98±3.85**	0.54±0.04	6.28±0.19	2.93±0.08*
***BORRERIA ARTICULARIS* 500mg/kg.b.wt**	34.20±6.11***	23.03±7.24***	45.45±2.62***	0.36±0.04***	6.73±0.08***	3.57±0.04**

Values are expressed as Mean ± S.D, (n = 6) one-way ANOVA followed by Dunnett's test *P value < 0.05, **P value < 0.01, *** P value < 0.001 compared with toxic group.

TBL =Total Bilirubin, ALT= Alanine Transaminase, AST = Aspartate transaminase, ALP= Alkaline Phosphatase, TPL= Total Protein, ALB= Albumin serum levels.



BA- BORRERIA ARTICULARIS-250 & 500

Figure 1: Group I (Normal control)
Section of liver with normal cell structure

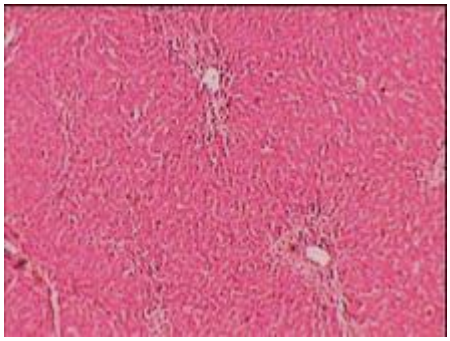


Figure 2: Group II (Toxic-CCL_4)
Section of liver showing
centriolobular necrosis

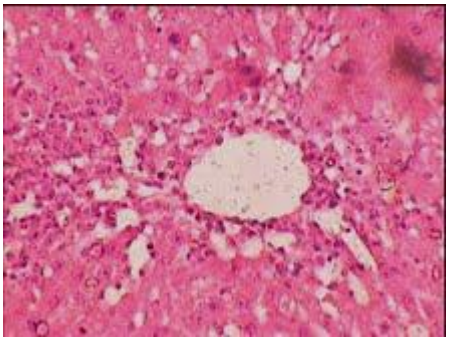


Figure 3: Group III (Standard-Silymarin)

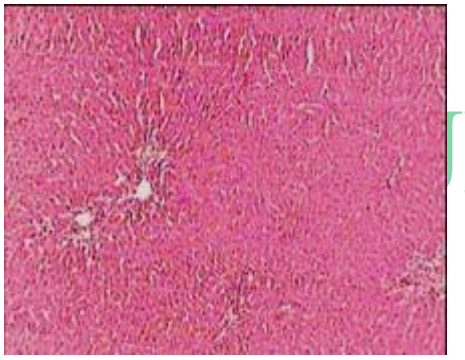


Figure 4: Group IV (*Borreria arti- cularis* -250)

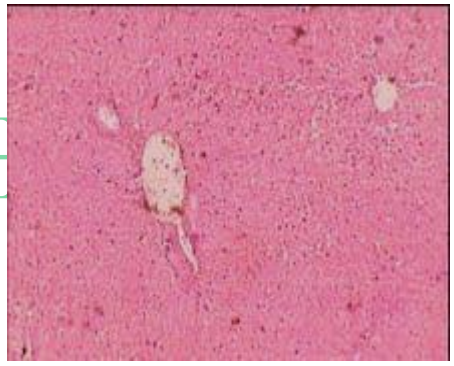
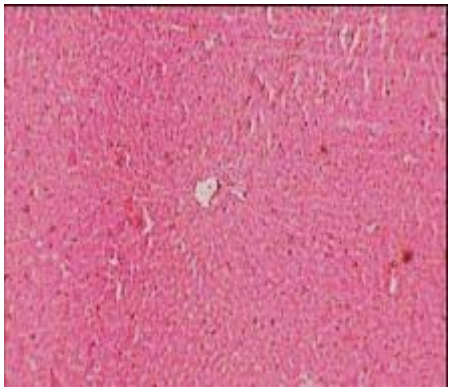


Figure 5: Group V (BAME-500) Section of liver showing significantly reduced
Necrotic area



CONCLUSION

In the present study, it is proved that the methanolic extract of *Borreria articularis* was found to be non-toxic to mice up to the dose of 2g/kg.p.o It exhibited significant hepatoprotective activities, it showed dose-dependant effects, the effect of *Borreria articularis* 500mg/kg.p.o was found to be almost like that of silymarin (100mg/kg.p.o). The hepatoprotective effects of *Borreria articularis* were evidenced by the amelioration of biochemical indicators of liver damage and pathological disturbances caused by Carbon tetrachloride. For the first time alkaloids, steroids/triterpenoidal compounds and their glycosides, flavonoids and tannins were detected in the plant. The pharmacological effects of the extracts my be attribute to the phytoconstituents present in them. The results showed that *Borreria articularis* are endowed with hepatoprotective effects. The finding also substantiates their traditional claim for the treatment of liver diseases. Since the findings are promising, further investigations on the plant are carrying out to prove their efficacy and develop them as herbal hepatoprotective agents. Pentobarbitone induced sleeping time study also supported the hepatoprotective potential of the extracts.

The sleeping time action of barbiturates is prolonged due to defective metabolism of damaged liver. The sleeping time due to administration of Pentobarbitone sodium was considerably reduced in animals received 500mg/kg body weight of *Borreria articularis* prior to Carbon tetrachloride intoxication. This parameter also supports the protective effect of the extracts.

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