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Aqueous Leaf Extracts of *Schefflera venulosa* and *S. Wallichiana* (Araliaceae) Protects the Liver Against Carbon Tetrachloride (CCl_4) - Induced Hepatic Damage in Albino Rats

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ABSTRACT

The hepatoprotective activity of the aqueous extracts of *S. venulosa* (AESV) and *S. wallichiana* (AESW) leaves on carbon tetrachloride (CCl_4)-induced liver damage in albino rats was investigated. Animals were pretreated with the AESV (250 and 500 mg/kg body weight) and AESW (250 and 500 mg/kg body weight) for 15 days and then challenged with CCl_4 (1 ml/kg body weight) in olive oil (1:1 v/v) on the 15th day. The degree of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein and total bilirubin. Further the effects of both the extracts on catalase (CAT), peroxidase (Px) and superoxide dismutase (SOD) were estimated in both CCl_4 treated and extract treated groups. Oral administration of AESV and AESW (500 mg/kg body weight) significantly protected from CCl_4-induced elevation in SGOT, SGPT, ALP, total bilirubin, total protein and decrease in the activities of hepatic antioxidant enzymes namely SOD, CAT and Px. The extracts (AESV and AESW at 500 mg/kg body weight) also protected against histopathological damage induced by CCl_4 such as distorted hepatocytes and necrosis. The present study suggests that AESV has potent antioxidant and hepatoprotective activity when compared to AESW.

Key words: *Schefflera* species, hepatoprotective, CCl_4, antioxidant enzymes.

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INTRODUCTION

The liver is the second largest glandular organ of the body and plays a vital role in metabolizing the carbohydrate, lipids, proteins and detoxifying xenobiotics and drugs[1]. The blood flow to the liver is around 20 to 25% of the total cardiac output. About 20,000 deaths are found every year due to liver disorders[2]. Liver diseases represent a major health burden worldwide, with liver cirrhosis being the ninth leading cause of death in western countries[3]. Therefore, treating the liver diseases with plant derived products which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. Exposure to various organic compounds, including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species (ROS). Thus, the identification of antioxidants, which can retard the process of lipid peroxidation by blocking the generation of free radical chain reaction, has gained importance in recent years. In living systems, varieties of antioxidant mechanisms play an important role in combating ROS[4,5]. The antioxidants act by raising the levels of endogenous defenses by up-regulating the expression of genes encoding the enzymes such as catalase (CAT), peroxidase or superoxide dismutase (SOD)[4,6]. Carbon tetrachloride (CCl_4) is a well-known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals[7]. Damage to the liver cell membrane leads to the leakage of the cytosolic enzymes into the blood stream[8]. The elevation of these cytosolic enzymes in the blood stream is a needful quantitative marker of the extent of hepatic damage. Furthermore, in spite of the advances in conventional medicine in the last decades, professionals and the lay public of developed countries pay increasing attention to phytomedicine[9,10]. About 600 commercial herbal formulations with claimed hepatoprotective activity are being sold all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess hepatoprotective activity. In India more than 93 medicinal plants are used in different combinations in the preparations of 40 patented herbal formulations[11]. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy[12]. The genus *Schefflera* is an epiphyte in the family Araliaceae. *Schefflera venulosa* (W. & A.) Harms. commonly called *Schefflera* vine is a large climbing shrub, with digitate compound leaves. The phytoconstituents are mainly caffeoyl acids, quercetin glycoside and oleanolic acid glycoside which helps in blood circulation and prevents cardiac and cerebral vascular diseases[13]. *Schefflera wallichiana* (W. & A.) Harms. is also an epiphyte with digitately compound leaves.

Phytochemical and *in vitro* antioxidant studies on *S. venulosa* and *S. wallichiana* have revealed positive results for the presence of phytochemicals such as saponins, tannins, flavonoids, alkaloids and reducing sugars with high antioxidant activity in aqueous extracts[14]. Hence, the present study has been undertaken with an aim to determine the protective effect of feeding the aqueous extract of *S. venulosa* and *S. wallichiana* to albino rats of the Wistar strain against the toxic effects of CCl_4. The level of various ROS-combating enzymes in the liver, namely, catalase, peroxidase and superoxide dismutase and also serum enzymes namely SGOT, SGPT, ALP, total protein and total bilirubin were estimated in liver homogenates to assess the protection provided by the aqueous extracts of *S. venulosa* and *S. wallichiana* against CCl_4 toxicity.

MATERIALS AND METHOD

Plant materials and preparation of aqueous extracts

The leaves of *S. venulosa* and *S. wallichiana* were collected from the forests of Kodagu in Western Ghats (N12°20′14.97″and E75°48′24.86″). The leaves were washed thoroughly in tap water, shade dried and powdered. The powder (1000 g) was soaked in (1×10) ml of luke warm distilled water and was allowed to macerate for 48 hours at room temperature with constant stirring. The extract was filtered and the residue was pressed out through clean linen and added to the filtrate. The filtrate was evaporated to dryness in a shaker water bath at 42ºC. The yield was found to be 20.0 g. It was reconstituted in 0.25% olive oil, to desired concentrations and used for experiments.

Chemicals

Nitro blue tetrazolium (NBT) was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. CCl_4 was purchased from Qualigens Fine Chemicals, Mumbai, India. Curcumin was obtained from Sigma Aldrich Chemicals, Bangalore, India. All the other chemicals used were of analytical grade.

Animals

Wistar albino rats of both sexes weighing 110-190 g maintained in the Animal house of Department of studies in Zoology, Manasagangotri, Mysore were procured and used for the animal study with the CPCSEA Reg. No. 122/1999/CPCSEA, order no: UOM/IAEC/06/2013). Rats were bred normally and housed in separate polypropylene cages. Standard rat pellet diet was provided along with water *ad libitum*. The rats were maintained at temperature of 25±2ºC, a relative humidity of 50-55% and maintained under normal day/night schedule (12L: 12D).

Experimental design

Animals were randomly divided into nine groups of six animals each, and treated as mentioned below. The CCl_4 (1 ml/kg P.O.) diluted with olive oil (1:1) was administered on the 15th day. During the experiment, all the animals were fed with normal diet and water *ad libitum* for 15 days.

Group 1: Served as normal, healthy group and received olive oil (1ml/kg, P.O).

Group 2: Served as toxic control group and received olive oil (1ml/kg, P.O). 1:1 (v/v) mixture of CCl_4 in olive oil at a dose of 1 ml/kg, P.O. was administered on the 15th day.

Group 3: Served as extract control (for plant 1: *S. venulosa*) and was treated with aqueous leaf extract of *S. venulosa* (500 mg/kg, P.O.).

Group 4: Served as extract control (for plant 2: *S. wallichiana*) and was treated with aqueous leaf extract of *S. wallichiana* (500 mg/kg, P.O.).

Group 5: Treated with the aqueous leaf extract of *S. venulosa* (250 mg/kg, P.O). 1:1 (v/v) mixture of CCl_4 in olive oil at a dose of 1 ml/kg, P.O. was administered on the 15th day.

Group 6: Treated with aqueous leaf extract of *S. wallichiana* (250 mg/kg, P.O.). 1:1 (v/v) mixture of CCl_4 in olive oil at a dose of 1 ml/kg, P.O. was administered on the 15th day.

Group 7: Treated with the aqueous leaf extract of *S. venulosa* (500 mg/kg, P.O.). 1:1 (v/v) mixture of CCl_4 in olive oil at a dose of 1 ml/kg, P.O. was administered on the 15th day.

Group 8: Treated with the aqueous leaf extract of *S. wallichiana* (500 mg/kg, P.O.). 1:1 (v/v) mixture of CCl_4 in olive oil at a dose of 1 ml/kg, P.O. was administered on the 15th day.

Group 9: Served as standard group and received Curcumin (5 mg/kg). 1:1 (v/v) mixture of CCl_4 in olive oil at a dose of 1 ml/kg, P.O. was administered on the 15th day.

Biochemical Parameters

The animals were monitored for change in body weight and food consumption during the experiment. At the end of the experiment, all the animals were necropsied by mild Diethylether anesthesia and were sacrificed by cardiac puncture. Blood samples were collected from each group and the serum was separated at 2500 RPM for 15 min and biochemical investigations used for the assay of marker enzymes such as SGPT, SGOT, ALP, serum bilirubin and total protein were carried out using standard kits procured from Span Diagnostics® (Surat, India). Livers were dissected out and a portion was immediately preserved in 10% (v/v) formaldehyde solution for histopathological study. The remaining liver tissue was homogenized in ice cold buffer, centrifuged and utilized to examine the antioxidant status. Catalase (CAT) was tested spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi[15]. The peroxidase (Px) was tested by the method of Reddy *et al.*[16], the tissue

superoxide dismutase (SOD) was measured by the Nitro blue tetrazolium (NBT) decrease method of Kakkar *et al*.[17]

Histopathological studies

Histopathological studies of the livers of the different groups of rats were carried out to determine the effect of protection offered by feeding the aqueous leaf extracts of *S. venulosa* and *S. wallichiana* against the toxic effects of CCl_4.

Statistical analysis

All values were expressed as Mean± Standard Error of the Mean (SEM). Statistical analysis was done using Statistical Analysis System (SAS) (16.0 version). The biochemical parameters were analyzed statistically using one-way analysis of variance (ANOVA), followed by a Duncan's multiple range test. The P value of <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Effect of AESV and AESW on serum hepatic enzyme levels

In the 15 days of hepatotoxicity study, there was no death record, nor signs of changes in the rats in any treated groups. The effects of aqueous leaf extracts of *S. venulosa* and *S. wallichiana* on serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) levels and change in weight of the liver in CCl_4 induced liver damage in rats are summarized in Table 1. Administration of CCl_4 (1 ml/kg p.o) on the 15^{th} day resulted a significant (P<0.05) elevation of hepatospecific serum marker enzymes SGOT, SGPT, ALP, total protein and total bilirubin in CCl_4 treated groups in comparison with the normal group. Administration of aqueous leaf extracts of *S. venulosa* and *S. wallichiana* (Group 7 and Group 8, Table 1) and Curcumin at the dose of 5 mg/kg (Group 9, Table 1), the level of these enzymes were found retrieving towards normalcy. With CCl_4 treatment, the mean liver weight increased to 4.87±0.18 g/100g b.w. from that of normal controls 2.66±0.10g/100g b.w., which was found to be statistically significant. The low dose pretreatment (250 mg/kg, P.O.) with aqueous extract of both the plants also resulted in an increase in the mean liver weight as compared to normal, which was statistically significant in Group 5 and 6 (Table 1). However, pretreatment with high dose (500 mg/kg, P.O.) showed the mean liver weight to return to near normalcy. Our results indicate the elevation of serum marker enzymes concentration SGOT, SGPT and ALP beside histological study. CCl_4 as many halogenated alkanes has been widely used in our daily life, but has been banned or restricted because of their distinct toxicity. Its production has steeply declined since 1980's; it was proved to be one of the most powerful

hepatotoxins capable of forming trichlomethyl and trichloromethylperoxyl radicals[18]. Yet CCl_4 continues to provide an important service in scientific research as a model substance to demonstrate the mechanisms of action of hepatotoxic effects and carcinogenicity or to evaluate hepatoprotective agents[19]. In living systems, the liver is considered to be highly sensitive to toxic agents. *Schefflera*, gains its importance in the present work because of its potent antioxidant activity. The present study is the first report on hepatotoxicity studies of *Schefflera* species. The most important member of the same family, commonly called *Panax ginseng* is one of the most valuable medicinal plants of the Araliaceae particularly used in Korea, China and Japan[20]. Ginseng and its principal components, ginsenosides, have shown a wide array of pharmacological activities including beneficial role in the regulation of liver functions and the treatment of liver disorders of acute/chronic hepatotoxicity, hepatitis, hepatic fibrosis/ cirrhosis, liver hepatectomy, liver transplantation and even liver failure and HCC[21]. To diagnose hepatotoxicity, serum enzymes SGOT, SGPT and ALP are the most sensitive markers employed[22]. SGOT is normally found in different tissues as liver, kidney, heart, muscle and brain. It enters into systemic circulation when any one of these tissues is damaged. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to keto acids[23]. SGPT is most concentrated in the liver. It leaks out into the bloodstream as the result of liver injury. Therefore, it is considered as a specific indicator of liver status[24]. Alkaline phosphatase is the key enzyme used test to detect obstruction in the biliary system. It is found both in the liver and the bile and it leaks into the blood stream in a manner similar to that of the SGOT and SGPT[25]. It is also found in other organs such as bone, placenta and intestine. The intoxication by CCl_4 and its metabolites (CCl_3 radical) causes acute hepatic failure. This radical, which is formed by a metabolic enzyme (Cytochrome P450), induce peroxidation of the unsaturated fatty acids of cell membrane and leads to membrane injury and leakage of sensitive markers of hepatocellular injury, such as serum SGOT and SGPT[26]. In the present study, it was observed that the animals treated with CCl_4 resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in hepatic structural integrity. The pretreatment with AESV and AESW, at the dose of 500 mg/kg, significantly attenuated the elevated levels of serum markers. The normalization of serum markers by AESV and AESW suggests that they are able to condition hepatocytes so as to protect the membrane integrity against CCl_4 induced leakage of marker enzymes into the circulation. This elevation results from damage to mitochondrial, cell membrane damage in hepatocytes and the liver biliary obstruction (cholestasis) respectively[27]. The

above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells.

Table 1: Effect of aqueous leaf extract of S. venulosa (AESV) and S. wallichiana (AESW) on liver marker enzymes in the serum of experimental groups.

Groups	SGOT U/L	SGPT U/L	ALP U/L	TB mg/dl	TP gm/dl	Liver Weight (g/100g body weight)
Group I (Normal healthy group)	80.3±2.9^{a}	49.0±2.1^{a}	92.6±3.1^{a}	0.58±0.02^{a}	7.8±0.23^{e}	2.66±0.10^{a}
Group II (Toxic control group)	201.1±5.5^{f}	185.0±2.6^{e}	214.8±4.6^{e}	2.04±0.04^{f}	3.86±0.65^{a}	4.87±0.18^{e}
Group III (Extract control for *S. venulosa*)	82.0±1.8^{a}	51.0±2.4^{ab}	89.1±2.3^{a}	0.59±0.02^{ab}	7.75±0.21^{e}	2.92±0.27^{ab}
Group IV (Extract control for *S. wallichiana*)	84.1±3.7^{a}	48.5±2.6^{a}	91.5±2.6^{a}	0.69±0.02^{ab}	7.33±0.18^{de}	3.03±0.20^{ab}
Group V (Treated with AESV 250 mg/kg)	176.1±3.7^{d}	107.8±3.2^{d}	159.5±3.8^{c}	1.13±0.03^{d}	5.60±0.23^{bc}	3.84±0.21^{cd}
Group VI (Treated with AESW 250 mg/kg)	188.0±3.2^{e}	116.8±4.5^{d}	176.0±3.1^{d}	1.77±0.04^{e}	4.28±0.17^{a}	4.01±0.16^{d}
Group VII (Treated with AESV 500 mg/kg)	96.3±2.7^{b}	68.1±3.7^{c}	97.0±3.4^{a}	0.99±0.05^{c}	6.20±0.25^{c}	2.96±0.24^{ab}
Group VIII (Treated with AESW 500 mg/kg)	120.3±4.8^{c}	70.3±3.6^{c}	113.0±5.0^{b}	1.06±0.04^{cd}	5.13±0.20^{b}	3.31±0.15^{bc}
Group IX (Standard group)	90.6±2.8^{ab}	58.6±2.9^{b}	94.0±2.6^{a}	0.70±0.02^{b}	7.05±0.26^{d}	2.68±0.10^{a}

All values are mean of three replicates and are expressed as mean± SEM (*n=06* in each group). Numbers in the same column followed by the same letter are not significantly different at P<0.05.

U/L = Units per liter; mg/dl = milligram per deciliter; g/dl = gram per deciliter

Effect of AESV and AESW on antioxidant enzymes of liver tissue

The effects of AESV and AESW on the enzymatic antioxidants, namely superoxide dismutase (SOD), catalase (CAT) and peroxidase (Px) are shown in Table 2. Administration of AESV and AESW (Group 7 and 8, Table 2) increased the activities of SOD, CAT and Px in CCl_4 induced liver damage in rats to prevent the accumulation of excessive free radicals and protect the liver from CCl_4 intoxication. A significant decrease in the activities of enzymatic antioxidants (SOD, CAT, Px) were noted after single administration of CCl_4 (Group 2, Table 2). Upon administration

of AESV and AESW (Group 7 and 8, Table 2) the enzymatic antioxidants were significantly ($P<0.05$) reversed to near normal. The effects of AESV and AESW were comparable with that of standard reference drug Curcumin. The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, CAT, Px, etc, which are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent the generation of hydroxyl radical and protect the cellular constituents from the oxidative damage. It is known that SOD converts superoxide anion into H_2O_2 and O_2, whereas CAT reduces H_2O_2 to H_2O, resulting in the detoxification of free radicals[28, 29]. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to accumulation of superoxide radicals and hydrogen peroxide. In the present study, it was observed that the AESV and AESW significantly increased the hepatic SOD activity in CCl_4 - induced liver damage in rats. AESV and AESW have reduced the reactive free radicals that might lessen oxidative damage to the tissue and improve the activities of the hepatic antioxidant enzymes. Hence it may be opined that the mechanism of hepatoprotection by AESV and AESW is due to its antioxidant effect.

Table 2: Effect of aqueous leaf extracts of *S. venulosa* (AESV) and *S. wallichiana* (AESW) on liver antioxidant enzymes in the serum of experimental groups.

Groups	SOD (U/min/mg protein)	CAT (U/min/mg protein)	Px (U/min/mg protein)
Group I (Normal healthy group)	48.43±0.73^{a}	26.51±0.90^{a}	0.99±0.03^{a}
Group II (Toxic control group)	18.96±0.98^{h}	8.78±0.56^{f}	0.27±0.01^{f}
Group III (Extract control for *S. venulosa*)	46.01±0.50^{b}	26.33±0.83^{a}	0.79±0.02^{c}
Group IV (Extract control for *S. wallichiana*)	45.08±0.33^{b}	25.18±0.88^{a}	0.68±0.02^{d}
Group V (Treated with AESV 250 mg/kg)	28.44±0.47^{f}	14.40±0.76^{de}	0.49±0.03^{e}
Group VI (Treated with AESW 250 mg/kg)	23.54±0.64^{g}	13.17±0.87^{e}	0.44±0.02^{e}
Group VII (Treated with AESV 500 mg/kg)	35.75±0.34^{d}	19.30±0.54^{c}	0.68±0.02^{d}
Group VIII (Treated with AESW 500 mg/kg)	31.98±0.63^{e}	16.05±0.41^{d}	0.64±0.03^{d}
Group IX (Standard group)	42.45±0.67^{c}	21.94±0.80^{b}	0.90±0.02^{b}

All values are mean of three replicates and are expressed as mean± SEM (n=06 in each group). Different letters within in a column indicate significant differences by Duncan's multiple range tests at $P<0.05$.

Histopathological studies

Histopathological studies of rat liver tissue from Group 1 animals show predominantly normal hepatic architecture with normal lobules and hepatocytes (Figure. 1A). In CCl_4-treated group (Figure. 1B), severe hepatotoxicity was observed by distorted hepatic lobular architecture with foci of necrotic hepatocytes and foci of mixed inflammatory exudates. Normal hepatic architecture with normal lobules and hepatocytes were observed in Figure. 1C and D. Severe necrosis and damage to hepatic cells was observed in sections E, F and H (Figure. 1). Minimal distortion of hepatic lobules with normal cells was observed in animals (Figure. 1G). The liver taken from animals treated with the standard drug Curcumin showed the mildly distorted and regenerating lobules (Figure. 1I).

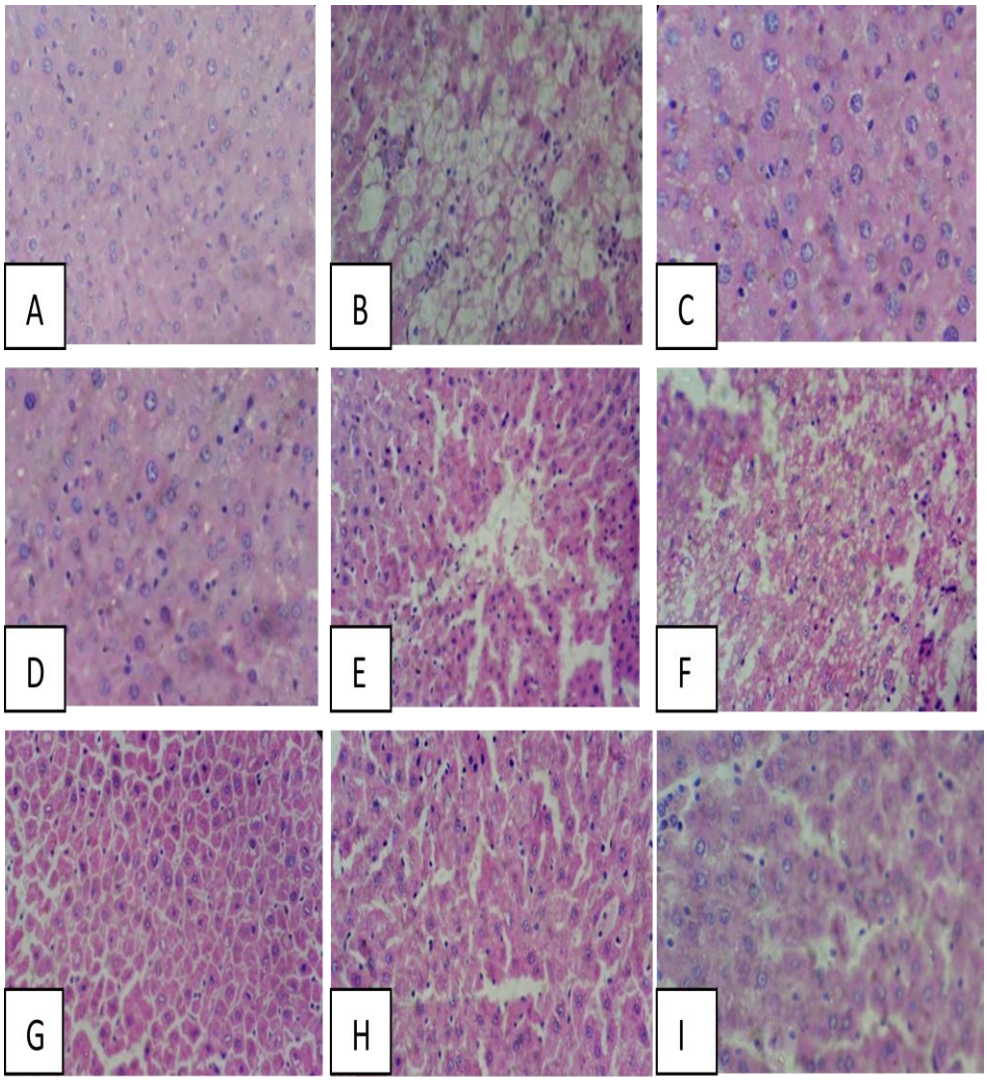

Figure 1: Histopatological sections of the liver

In the CCl_4-induced liver damage, the free radicals are generated in which the cytotoxic effect is not localized but can be propagated intercellularly, thereby increasing the interaction of these radicals with phospholipids structures and inducing peroxidation process that destroy the organ structure. Our studies indicated that in the case of control, the hepatocytes having a normal hepatic architecture were visible. However, in case of the CCl_4-treated rats, total loss of hepatic architecture and areas of hemorrhage and necrosis were seen. In case of the rats pretreated with the aqueous leaf extracts of *S. venulosa* and *S. wallichiana* [500 mg/kg, b.w.], followed by exposure to CCl_4, the liver showed to retain the normal hepatic architecture with few areas of hemorrhage between the columns of hepatocytes.

Normal healthy rats, showing normal hepatic cells with normal lobules (A). Distorted hepatic lobular architecture with foci of necrotic hepatocytes in CCl_4 treated rats (B). Normal hepatic architecture with normal lobules and hepatocytes in extract control groups (C and D). Liver sections of animals treated with 250 mg extract and CCl_4 showing distorted hepatic lobules, extensive chronic inflammatory foci with dilated central veins and stasis of blood (E, F and H). Animals treated with 500 mg of the extract and CCl_4 showing minimal distortion of hepatic lobules (G). Animals treated with standard drug Curcumin showing mildly distorted hepatic lobular architecture (I).

CONCLUSION

The data presented in our study suggest that the aqueous leaf extract of *S. venulosa* has potent antioxidant activity against free radicals, thus preventing oxidative damage to major biomolecules and afford significant protection against CCl_4 - induced oxidative stress and liver damage in rats when compared to the aqueous leaf extract of *S. wallichiana*. Further studies on the isolation and characterization of compounds responsible for the hepatoprotective activity are needed to be evaluated for their potential benefits.

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ETHICAL APPROVAL

All animals in this study follow the institutional Animal Ethical Committee, according to guidelines given by Committee for Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No. 122/1999/CPCSEA), order no: UOM/IAEC/06/2013.

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