Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (5):146-152
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Hepatoprotective and antioxidant activity of *Tragia involucrata* root extracts against CCl_4 induced hepatotoxicity in rats

Abdulrahman S. ALAnazi[1], Md. Jamir Anwar[2]* and Md. Afroz Ahmad[3]

[1]*Department of Clinical Pharmacy, Unaizah College of Pharmacy, Qassim University, Kingdom of Saudi Arabia*
[2]*Department of Pharmacology and Toxicology, Unaizah College of Pharmacy, Qassim University, Kingdom of Saudi Arabia*
[3]*Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India*

ABSTRACT

Tragia involucrata (TI) Linn is a shrub belongs to family Ephorbiaceae widely distributed in the Indian subcontinent. It has been used as a traditional remedy since ancient days but its hepatoprotective effect is not yet well characterized. The aim of this study was to assess the effect of T. involucrata extract in carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Wistar rats of either sex were treated with normal saline (0.5 ml/kg p.o.), T. involucrata extracts (100, 200 and 300 mg/kg p.o.), and silymarin (100 mg/kg p.o.) respectively. The treatment duration lasted for 7 days and on 8th day, 12 hrs overnight fasted animals except control group were treated with 1 ml of CCl_4 in liquid paraffin (1:1). Blood sample was collected 24 hrs after CCl_4 administration for estimation of biochemical markers in serum then the animals were sacrificed under ether anaesthesia for histopathology and biochemical estimations in hepatic tissue. CCl_4-induced hepatotoxicity was characterised by a significant ($p<0.001$) increase in SGOT, SGPT, alkaline phosphatase (ALKP), and thiobarbituric acid reactive substance (TBARS) as well as a significant ($p<0.001$) decrease in total protein (TP), total albumin (TA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). Treatment with T. involucrata extracts significantly ($p<0.05$ and $p<0.01$) attenuated CCl_4-induced hepatotoxicity. However in T. involucrata alone (per se group) there was no significant alteration as compared to control group. Silymarin is used as standard drug in this study. Thus, the result shows the dose dependent hepatoprotective and antioxidant activity of TI extract against CCl4 induced hepatotoxicity in rats.

Keywords: Tragia involucrata, Carbon tetrachloride, hepatotoxicity, oxidative stress.

INTRODUCTION

Liver is a pivotal inflammatory organ that, involved in metabolism, storage, and excretion of metabolites. It has considerable capacity to detoxify toxic substances and synthesise useful principles. There are considerable numbers of hepatotoxins that have been reported to cause a liver damage such as ethanol, paracetamol, and carbon tetrachloride (CCl_4) [1,2]. Acute liver failure is a rare disorder with high mortality and resource cost. In the developing world, viral causes predominate. However the incidence of virally induced disease has declined substantially in the past few years, with most cases now arising from drug-induced liver injury, often from paracetamol. However, a large proportion of cases are of unknown origin. Acute liver failure can be associated with rapidly progressive multiorgan failure and devastating complications [3]. Numerous experimental and clinical evidences demonstrate that hepatotoxicity occurs due to increase in lipid peroxidation and generation of free radicals (superoxide, hydrogen peroxide, and hydroxyl radical) or reduction in antioxidant capacity [4].

Tragia involucrata Linn (Euphorbiaceae) commonly known as Indian stinging nettle has been widely used in Ayurveda (Indian traditional system of medicine) as medicine for treating various disorders such as roots for pruritc skin eruptions, venreal diseases, diabetes, guinea worms and leaves for cephalagia. Interestingly the methanolic extract of the root possessed significant antiinflammatory and analgesic activity [5]. Interestingly the juice of T. involucrate leave is being used for treatment of Jaundice by peoples of Chakma ethnic group of Rangamati area in Bangladesh [6].

The plant has been reported to have diverse pharmacologically active constituents such as alkaloids, terpenoids, quinines, sterols, flavonoids, lipids, phenolic compounds, proteins, saponins, sterols and triterpenoids [7]. Flavonoids are a large group of polyphenolic compounds are markedly found in fruits, vegetables and medicinal plants that play an important role in detoxification of free radicals [8]. It has been reported that natural terpenes have antioxidant and hepatoprotective activity [9]. Further, ample evidence suggests that the major mechanism involved in the hepatotoxicity of CCl_4 is lipid peroxidation by free radical derivatives of CCl_4 and has been extensively used in the experimental models to evaluate the therapeutic potential of drugs [10]. *T. involucrata* conatains various bioactive constituents such as terpenoid, phenolic and flavonoids which are well reported to have antioxidant and hepatoprotective activity [11]. Therefore, it is quite interesting to investigate the hepatoprotective effect of *T. involucrata* extracts against CCl_4 induced hepatotoxicity to supports its ethnopharmacological uses.

Silymarin is a natural compound that is present in species of Silybum marianum commonly known as Milk thistle. It is extracted from the seeds and fruits of Silybum marianum and in reality are a mixture of three structural components: Silibinin, Silydianine and Silychristine [12]. Silymarin is a flavonolignan that has been used worldwide for many years as a complementary alternative medicine because of the beneficial effects associated with the treatment of hepatic diseases [13]. In addition several clinical trials have shown that Silymarin exerts hepatoprotective effects in acute viral hepatitis, poisoning by Amanita phalloides, ethanol, paracetamol, and carbon tetrachloride [14, 15]. Many studies have demonstrated the beneficial hepatoprotective effects when treating with Silymarin [16].

Although *T. involucrata* has been a traditional remedy since antiquity days, its effect in hepatotoxicity is not yet well characterized. Thus, the aim of the present study was to assess the effect of *T. involucrata* leaf extract on CCl_4 -induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant material: The roots of *T. involucrata* (Euphorbiaceae) collected from Belpahari, Midnapore, West Bengal, India, in June 2014, were authenticated and supplied by M/S, United Chemical and Allied Products, Calcutta, India. A voucher specimen (P-713) has been kept for further reference.

Preparation of the root extract: The air-dried powdered roots of *T. involucrata* were defatted with petroleum ether (60°–80°C) in a soxhlet extractor and then with chloroform. The defatted material was further extracted three times with methanol at ambient temperature. The methanol extract (MTI) was dried in vacuum to yield a brownish-black residue, which was stored at 4°C. Just prior to use, the dried extract was dissolved in a mixture of propylene glycol and water (1:4). The vehicle was used as a control throughout the study.

Animals: Wistar albino rats weighing 150-200 g were raised from animal facility of KIET School of pharmacy. They were housed in polypropylene cages (6 animals per cage) under natural light-dark cycle. The animals were fed with standard pellet diet (Amrut rat and mice feed, Pune, India) and water ad libitum. The animals were treated in the most humane and ethically acceptable manner with maximum care to ensure that the animals were treated. The study was undertaken with prior approval from the Institutional Animals Ethics Committee.

Drugs and Chemicals: Silymarin was obtained as gift from Unicure, Noida, India. SGOT, SGPT, ALP was purchased from Span Diagnostics, Surat, India. All others reagents and chemicals used in this study were of analytical grade.

Experimental protocol:
The animals received pre-treatment with *T. involucrata* root extract and standard drug (Silymarin) for consecutive 7 days followed by CCl_4 administration. The animals were divided into 7 groups of six each. Group I animals served as normal control (received normal saline only). Group II as toxic control, treated with CCl_4 (1.5 ml/kg p.o.) diluted with liquid paraffin (1:1 ratio). The animals of group-III, IV and V were treated with graded doses (100, 200 and 300 mg/kg respectively) of methanolic extract of *T. involucrata* root. Silymarin was administered at dose of 100 mg/kg to positive control animals (Group VI). Animals of group VII were treated with MTI (300 mg/kg) only and

served as per se control. At the end of experimental protocol, blood sample was collected for estimation of biochemical markers in serum then the animals were sacrificed under ether anaesthesia for collection of liver for estimations in tissues and histopathological analysis.

Biochemical estimation in serum:
Blood sample was collected and the serum was separated by centrifugation at 3000 rpm for 10 min and frozen at -20ºC for estimation of biochemical parameters in serum. The activity of serum SGOT, SGPT was determined by the method of Reitman and Frankel [17], ALP and TP were determined by reported methods of Kind and King [18] and Wooton [19] respectively.

Biochemical estimations in liver tissue:
The livers were excised by sacrificing the animals immediately after blood collection. The liver tissues were washed in ice-cold normal saline and weighed. Subsequently, liver tissue pieces of all the groups were weighed, homogenized (10% w/v) in chilled phosphate buffer (50 mM and 0.1 M, pH 7.4) and/or potassium chloride (1.17%), and centrifuged at 10,000 g for 20 min in high-speed cooling centrifuge (-4ºC). The clear supernatants were used for assaying the levels of thiobarbituric acid reactive substance (TBARS) [20], glutathione (GSH) [21], superoxide dismutase (SOD) [22], catalase (CAT) [23], and proteins [24].

Histopathological studies:
The livers were fixed with 10% formalin solution and embedded in paraffin. Sections of dehydrated hepatic tissue, 3–5 m thickness, were prepared and stained with hematoxylin and eosin (H & E) for histological examination. The histological examination of the liver sections was carried out by a pathologist in a blinded fashion [25].

Statistical analysis:
All data were represented as mean ± SEM. The results were analysed by ANOVA followed by Dunnett's t test. Statistical analyses were performed using Graphpad Prism 3.0 (San Diego, CA, USA). P values <0.05 were considered as statistically significant.

RESULTS

Effect of methanolic TI extracts on serum SGOT, SGPT, ALP, total protein and albumin level:
As shown in table 1, administration of CCl_4 induced significant hepatic damage manifested by significant ($p<0.01$) increase in the level of serum SGOT, SGPT, ALP, total protein, and albumin as compared to normal control. Pre-treatment with methanolic *T. involucrata* extracts (100, 200 and 300 mg/kg respectively) significantly reversed these changes dose dependently. Silymarin (100 mg/kg) pre-treatment also preserve these biochemical changes in serum markers of hepatic function induced by CCl_4 administration. However, MTI (300 mg/kg; per se) did not produce any significant alteration in serum hepatic biochemical markers as compared to control.

Effect of methanolic TI extracts on MDA, SOD, GSH and catalase activity in liver tissues:
Administration of CCl_4 resulted in oxidative stress manifested by significant ($p<0.01$) increase in lipid peroxidation measured as TBARS and reduction in glutathione ($p<0.01$), superoxide dismutase (SOD) and catalase (CAT) activity in hepatic tissues (Table 2) as compared to control. Pre-treatment with methanolic extract of *T. involucrata* root at 100, 200 and 300 mg/kg produced significant antioxidant effect by reversing the increased TBARS and altered GSH, SOD and CAT activity as compared to pathogenic control group. Moreover, Silymarin also produced significant antioxidant effect by restoring the oxidative stress parameters. However, T. involucrate (300 mg/kg, *per se*) did not produce any significant alteration in biochemical markers of oxidative stress (TBARS, GSH, SOD and CAT) as compared to control group.

Effect of methanolic TI extracts on histopathological studies of liver tissue:
Histopathological analysis of CCl_4-treated hepatic tissues (Fig. 1b) showed prominent centrilobular fatty change with prominent and enlarged central vein as compared with the control (Fig. 1a), where no such structural alterations were observed. Treatment with *T. involucrata* 100 mg/kg (Fig. 1c), 200 mg/kg (Fig. 1d), 300 mg/kg (Fig. 1e), and silymarin (Fig. 1f) demonstrated marked improvement in the CCl_4-induced micro-architectural changes hepatic tissues. However, *T. involucrata* per se (Fig. 1g) alone-treatment did not show any structural changes in hepatic tissues.

Figure 1: Effect of T. involucrate extracts on CCl4-induced hepatotoxicity in rats

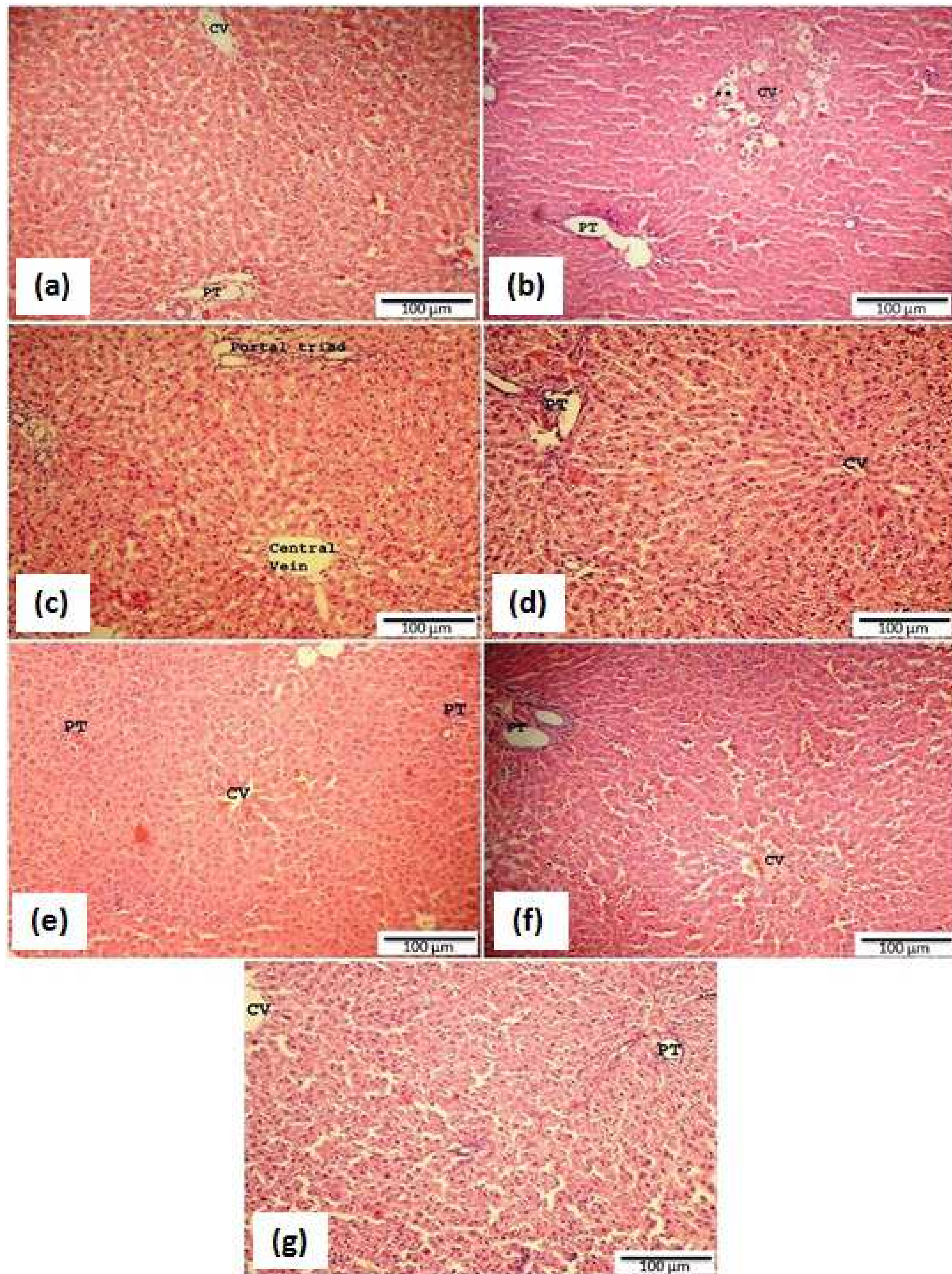

Fig. 1 Histology of liver (1 a-g): CCl_4 treatment (fig. 1b) showed evidence of centrilobular fatty change with prominent and enlarged central vein as compared to control (fig. 1a). Tragia involucrata root extract at dose of 100 mg/kg, 200 mg/kg and 300 mg/kg (fig. 1c, 1d and 1e) showed reversal of these histopathological changes in dose dependent manner. Silymarin also restored the changes in hepatic tissue produced by CCl_4 (fig. 1f). Tragia involucrata per se (fig. 1g) did not produce any histopathological alteration in hepatic tissue. (magnification, 40X).

Table 1: Effect of *T. involucrata* root extracts on CCl_4-induced changes in SGOT, SGPT, ALP, Total Protein and Total Albumin levels

Group No.	Treatment (mg/kg p.o.)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Protein (g/dl)	Total Albumin (g/dl)
I	Normal (Control)	33.33 ± 1.45	21.83 ± 1.01	20.50 ± 1.17	11.66 ± 0.84	6.66 ± 0.55
II	CCl_4 (Pathogenic)	135 ± 3.08$^{\#\#}$	117.66 ± 1.97$^{\#\#}$	62.0 ± 1.82$^{\#\#}$	5.16 ± 0.47$^{\#\#}$	2.5 ± 0.42$^{\#\#}$
III	Silymarin (100)	36.83 ± 1.01**	27.66 ± 1.14**	23.33 ± 2.10**	11.0 ± 0.57**	6.16 ± 0.70**
IV	TIE 100	88.83 ± 3.27**	76.16 ± 2.75**	47.0 ± 1.65**	7.66 ± 0.49$^{\#,}$*	3.83 ± 0.47$^{\#}$
V	TIE 200	51.33 ± 2.29**	61.16 ± 1.07**	34.0 ± 1.52**	8.66 ± 0.49*	4.5 ± 0.42
VI	TIE 300	38.16 ± 1.49**	28.33 ± 0.88**	25.33 ± 1.68**	9.5 ± 1.72**	5.66 ± 0.71**
VII	TIE 300 *per se*	37.16 ± 1.30**	23.83 ± 2.19**	22.83 ± 1.35**	11.0 ± 0.96**	6.16 ± 0.94**

Data are represented as mean ± SEM (n = 6)

*All values are expressed as mean ± SEM. Control, (Carboxymethylcellulose, 10ml/kg); CCl_4 (Carbon tetrachloride 35 mg/kg); TIE 100, 200 and 300 (Tragia involucrate extract 100, 200 and 300 mg/kg p.o.); SGOT (Serum glutamic oxaloacetic transaminase); SGPT (Serum glutamic-pyruvic transaminase); ALP (Alkaline phosphatise).$^{\#}$P < 0.05 and $^{\#\#}$P < 0.01, vs. Group 1 (control); * P<0.05, **P<0.01, vs. Group 2 (Pathogenic control), significant by ANOVA followed by Dunnett's t-tests*

Table 2: Effect of *T. involucrata* root extracts on the biochemical markers of oxidative stress in hepatic tissue

Group No.	Treatment (mg/kg p.o.)	TBARS (µM/L)	GSH (µM/L)	SOD (U/mg protein)	CAT (U/mg protein)
I	Normal (Control)	6.33 ± 0.66	54.5 ± 1.78	4.83 ± 0.60	34.0 ± 1.41
II	CCl_4 (Pathogenic)	9.66 ± 0.49$^{\#\#}$	28.66 ± 1.68$^{\#\#}$	1.66 ± 0.33$^{\#\#}$	16.16 ± 1.15$^{\#\#}$
III	Silymarin (100)	6.66 ± 0.66**	53.16 ± 1.24**	4.66 ± 0.76**	33.66 ± 1.25**
IV	TIE 100	9.16 ± 0.30$^{\#\#}$	34.16 ± 1.92$^{\#\#}$	3.83 ± 0.60*	19.16 ± 1.44$^{\#\#}$
V	TIE 200	8.0 ± 0.57	42.5 ± 1.72$^{\#\#,}$**	4.16 ± 0.60*	24.33 ± 1.33$^{\#\#,}$ **
VI	TIE 300	6.5 ± 0.42**	51.5 ± 1.11**	4.5 ± 0.42**	32.0 ± 1.41**
VII	TIE 300 *per se*	6.83 ± 0.74**	52.83 ± 1.62**	4.66 ± 0.49**	33.0 ± 1.15**

Data are represented as mean ± SEM (n = 6)

*All values are expressed as mean ± SEM. Control, (Carboxymethylcellulose, 10ml/kg); CCl_4 (Carbon tetrachloride 35 mg/kg); TIE 100, 200 and 300 (Tragia involucrate extract 100, 200 and 300 mg/kg p.o.); TBARS (Thiobarbituric acid reactive substances); GSH (Glutathione); SOD (Superoxide dismutase); CAT (Catalase). $^{\#}$P < 0.05 and $^{\#\#}$P < 0.01, vs. Group 1 (control); * P<0.05, **P<0.01, vs. Group 2 (Pathogenic control), significant by ANOVA followed by Dunnett's t-tests*

DISCUSSION

Liver is one of the vital organ plays important role in the metabolism of drugs and nutrients, but it is the most vulnerable tissue to toxic effects of these agents. The injury and dysfunction of liver caused by CCl_4 in experimental animals stimulates conversion to highly reactive toxic free radical CCl_3O^- by cytochrome P_{450}. In addition, it has been shown that electrophilic chlorine is generated from CCl_4 by rat liver microsomes in the presence of NADPH and molecular oxygen. The current view envisages an initial reductive dehalogenation of CCl_4 to trichloromethyl radical, followed immediately by reaction with oxygen to form $^-OOCCl_3$. Through various possible pathways, trichloromethylperoxyl radical would react further to yield phosgene and a chlorine radical [26]. These organic peroxides formed after reaction with oxygen leads to swelling of smooth endoplasmic reticulum and dissociation of ribosomes from the rough endoplasmic reticulum. Accumulation of lipids ensues due to inability of the cells to synthesize lipoprotein from triglycerides and lipid acceptor proteins leading to the fatty liver [27].

In CCl_4-induced hepatotoxic groups, CCl_4 upon administration to experimental animals undergoes enzymatic activation primarily by CYP2E1 to trichloromethyl free radical within the endoplasmic reticulum (ER) membrane. This is followed by chloromethylation, saturation, peroxidation and progressive destruction of the unsaturated fatty acid of the ER membrane phospholipids leading both structural and functional damage to hepatocytes [28]. The peroxidative breakdown of polyunsaturated fatty acid affects the permeability of mitochondrial, endoplasmic reticulum and plasma membranes resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage [29] and thus leakage of liver cytosolic enzymes.

Liver function can be assessed by estimating the activity of serum enzymes such as SGOT, SGPT and ALP. These cytosolic enzymes are specifically present in liver in high concentration and serves as marker of hepatotoxicity. The present study revealed that carbon tetrachloride significantly increased the SGOT, SGPT and ALP where as decreased total protein and albumin corresponded to the extensive liver damage. CCl_4 causes acute hepatocyte injuries, altered membrane integrity and as a result enzymes in hepatocytes leak out [30]. Pre-treatment with *T. involucrata* root extract (100, 200 and 300 mg/kg p.o.) as well as silymarin (100 mg/kg p.o.) significantly (p<0.01) preserved these enzymatic changes (Table 1). These results indicate that *T. involucrata* extracts has the ability to protect hepatocyte against CCl4-induced injury, which is consistent with a previous study [10] wherein they reported the protective effect of rutin, a flavonoid against CCl_4-induced hepatotoxicity.

Lipid peroxidation is an indication of the severity of CCl_4-induced hepatic damage and thus has been implicated in the alteration of membrane structure and inactivation of antioxidant enzymes [10]. Malondialdehyde is a major lipid peroxidation end product measured as thiobarbituric acid reactive substances (TBARS) are considered as a late biomarker of oxidative stress. Previous studies demonstrated that CCl_4-induced hepatotoxicity could be due to induction of free radical mediated lipid peroxidation in rats [31]. Our results revealed that pre-treatment with *T. involucrata* extract (100, 200 and 300 mg/kg) produced significant ($p<0.01$) reversal of elevated TBARS level in dose dependent manner (Table 2). However, per se treatment with *T. involucrata* extract (300 mg/kg; p.o.) has not produced any significant changes in TBARS level.

Furthermore, antioxidant enzymes such as GSH, SOD and CAT are the first line of defence against damage by oxidative stress. They alleviate oxidative stress by eliminating reactive oxygen radical such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), and preventing the formation of more reactive hydroxyl radical ($^{\circ}OH$). It has been well reported that acute administration of CCl_4 induces oxidative stress in rats [32]. The pathogenesis of oxidative stress induced hepatotoxicity could be due to either increased generation of reactive oxygen species and/or depletion of the antioxidants enzymes in the defence system [33]. In the present study, we found that there was significant ($p<0.01$) decreased in GSH, SOD and CAT activity following CCl_4 treatment. Pre-treatment with *T. involucrata* root extracts (100, 200 and 300 mg/kg; p.o.) significantly increased the GSH, SOD and CAT activity. These finding indicates that *T. involucrata* could considerably ameliorate the oxidative stress of cellular antioxidant defence system.

The histopathological study of hepatic tissue further confirmed the hepatic damage evidenced by swelling of hepatocytes and necrosis in CCl_4 treated groups. The mechanism underlying these histopathological changes are many more but perooxidative breakdown of polyunsaturated fatty acid is the most important in addition to oxidative stress (Figure 1). *T. involucrata* root extract preserved these CCl_4-induced histopathological changes in hepatocytes. In our study it was evidenced the changes in TBARS, and free radical scavenging enzymes such as GSH, SOD and CAT similar to previous reports of CCl_4-induced histopathological changes [33].

It has been well documented that *T. involucrata* root contains flavonoid and terpenoid [34]. Flavonoids have been reported to produce hepatoprotective and antioxidant activity [35], while terpenoids have antioxidant and free radical scavenging activity [36]. Thus it appears that the augmentation of endogenous antioxidants, maintenance of serum SGOT, SGPT, ALP level, hepatic and/or serum antioxidant status, and the reversal of altered histopathological changes by either combined or independent action of flavonoid and terpenoid compounds present in *T. involucrata* extracts may contribute to its hepatoprotective effect.

CONCLUSION

The present study provides experimental evidence for the hepatoprotective effect of *T. involucrata* against CCl_4-induced hepatotoxicity in rats appears to be related to its antioxidant property of its phytoconstituents present in the root. However additional investigations are required to ascertain the effects in other rodent models of hepatotoxicity and also to determine the active constituents responsible for hepatoprotective effects.

REFERENCES

[1] Lopez VRL, Razzeto GS, Giménez MS, Escudero NL: *Plant Foods for Human Nutrition* **2011**, 66(2):157–162.
[2] Huang GJ, Deng JS, Huang SS, Shao YY, Chen CC, Kuo YH: *Food Chemistry* **2012**, 132(2):709–716.
[3] Bernal W, Auzinger G, Dhawan A, Wendon J: *Lancet* **2010**, 376(9736): 190-201.
[4] Ganie SA, Dar TA, Zargar B, Hamid R, Zargar O, Dar PA, Abeer SU, Masood A, Amin S, Zargar MA: *J Environ Pathol Toxicol Oncol*. **2014**, 33(2):131-43.
[5] Perumal Samy R, Ignacimuthu S, Sen A: *J Ethnopharmacol* **1998**, 62:173.
[6] http://www.mpbd.info/plants/tragia-involucrata.php
[7] Chaterji A, Pakrashi SC. Publication and information Directorate, SCIR, New Delhi **1994**, 3: 58-59.
[8] Rice-Evans C, Packer L: Flavonoids in health and diseases, Marcel Decker Inc, New York, **1998**, 483-522.
[9] Gonzalez-Burgos E, Gomez-Serranillos MP: *Curr Med Chem* **2012**, 19(31):5319-41.
[10] Khan RA, Khan MR, Sahreen S: *BMC Complement Altern Med.* **2012**, 12:178.
[11] Naik SR, Panda VS: *Liver Int* **2007**, 27(3):393-9.
[12] Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam D, Sengupta P, Maiti BC, Maity TK: *Tropical Journal of Pharmaceutical Research* **2007**, 6(3): 755-765.
[13] Vargas-Mendoza N, Madrigal-Santillán E, Morales-González A, Esquivel-Soto J, Esquivel-Chirino C, García-Luna Y González-Rubio M, Gayosso-de-Lucio JA, Morales-González JA: *World J Hepatol.* **2014**, 6(3):144-9.
[14] Pradhan SC, Girish C: *Indian J Med Res.* **2006**,124(5):491-504.

[15] Wagoner J, Negash A, Kane OJ, Martinez LE, Nahmias Y, Bourne N, Owen DM, Grove J, Brimacombe C, McKeating JA, Pécheur EI, Graf TN, Oberlies NH, Lohmann V, Cao F, Tavis JE, Polyak SJ: *Hepatology* **2010**, 51(6):1912-21.
[16] Fraschini F, Demartini G, Esposti D: *Clin Drug Inv* **2002**, 22: 51-65.
[17] Reitman S, Frankel SA: *Am. J. Clin. Pathol.* **1957**, 28: 56–63.
[18] Kind PRN, King EJ: *J. Clin. Pathol.* **1954**, 7: 332.
[19] Wooton IDP, Microanalysis in Medical Biochemistry, 4th edn, Churchill, London, **1964**, 138-140.
[20] Ohkawa H, Oshishi N, Yagi K: *Anal Biochem* **1979**, 95: 351–358.
[21] Sedlack J, Lindsay RH: *Anal Biochem* **1968**, 25: 192–205.
[22] Marklund S, Marklund G: *European Journal of Biochemistry* **1974**, 47: 469–474.
[23] Clairbome, A. Assay of catalase. In R. A. Greenwald (Ed.), Hand book of methods of oxygen free radical research. Boca Raton, Florida: CRC Press, **1985**, pp. 283–284.
[24] Lowary OH, Rosebrough NJ, Farr AL, Randall RJ: *Journal of Biological Chemistry* **1951**, 193: 265–275.
[25] Belur B, Kandaswamy N, Mukherjee KL. Laboratory technology – a procedure manual for routine diagnostic tests. Tata Mc Graw Hill Co. Ltd, Delhi, India, **1990**, p.1124–118.
[26] Mica BA, Pohl LR: *Arch. Biochem. Biophys.* **1983**, 225: 596-609.
[27] Fullbert JC, Cals MJ: *Pathol Biol (Paris)* **1992**, 40: 66.
[28] Chen P, Li C, Pang W, Zhao Y, Dong W, Wang S, Zhang J: *Am J Pathol.* **2009**, 174(1):63-70.
[29] Poli G, Albano E, Dianzani MU: *Chem Phys Lipids.* **1987**, 45(2-4):117-42.
[30] Cheng HL, Hu YY, Wang RP, Liu C, Liu P, Zhu DY: *World J Gastroenterol* **2000**, 6:402–404.
[31] Adetoro KO, Bolanle JD, Abdullahi SB, Ahmed OA: *Asian Pac J Trop Biomed*. **2013**, 3(5):395-400.
[32] Alkreathy HM, Khan RA, Khan MR, Sahreen S: *BMC Complement Altern Med.* **2014**, 14:452.
[33] Kuriakose GC, Kurup GM: *Indian J Exp Biol.* **2008**, 46(1):52-9.
[34] Mothana RA, Abdo SA, Hasson S, Althawab FM, Alaghbari SA, Lindequist U: *Evid Based Complement Alternat Med.* **2010**, 7(3):323-30.
[35] Ai G, Liu Q, Hua W, Huang Z, Wang D: *J Ethnopharmacol.* **2013**, 146(3):794-802.
[36] Saleh MA, Clark S, Woodard B, Deolu-Sobogun SA: *Ethn Dis.* **2010** Winter, 20(1 Suppl 1):S1-78-82.