

## Original Research

# Hepatoprotective effects of *Quercus infectoria* gall extract against carbon tetrachloride treated liver injury in rats

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**Summary.** In the present study, galls of *Quercus infectoria* possessing potent antioxidant and antiinflammatory properties were evaluated for their hepatoprotective effect against carbon tetrachloride (CCl<sub>4</sub>) induced hepatotoxicity in rats. Subcutaneous injection of CCl<sub>4</sub>, administered twice a week, produced a marked elevation in the serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and tumor necrosis factor alpha (TNF- $\alpha$ ). Histological analysis of the liver of these rats revealed marked necro-inflammatory changes that were associated with increase in the levels of TBARS, PGE<sub>2</sub> and catalase and decrease in the levels of glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Daily oral administration of aqueous ethanolic extract of *Quercus infectoria* galls at 200, 400 and 600 mg/kg doses produced a dose dependent reduction in the serum levels of liver enzymes and inflammatory mediators and attenuated the necroinflammatory changes in the liver. The QIE treatment also normalized various biochemical parameters of oxidative stress. Our study shows that the hepatoprotective effects of QIE and silymarin were comparable and suggests that QIE could be used as a hepatoprotective agent.

**Industrial relevance.** Research in traditional medicine has lead to the development of many modern medicines. In recent times, focus on plants research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. It is very interesting to note that there is no drug available in the modern system of medicine for treating hepatic disorders; only certain herbal preparations are available to treat this quite vulnerable disease. The situation/background thus explained above warrants for developing a safe, effective and scientifically validated hepatoprotective agent taking lead from traditional medicine, which is affordable for the rural poor population. The results of the present study clearly demonstrate the hepatoprotective potential of natural products. The scientific information generated would certainly help the industry in developing new, potent hepatoprotective drugs with no or least adverse effects.

**Keywords.** *Quercus infectoria*; galls; Carbon tetrachloride; Hepatoprotective

### Introduction

*Quercus infectoria* Olivier (Family: Fagaceae) is a small tree widely distributed in Greece, Asia Minor and Iran. The tree bears galls that emerge on its shoots as a consequence of attack of gall wasp, *Cynips gallae-tincotoriae* (Samuelson, 1992). The decoction of galls is usually employed as an astringent, gargle and enema (Nadkarni, 1982). In Asian countries, the galls of *Quercus infectoria* have been used for centuries in oriental traditional medicines for treating inflammatory diseases (Anonymous, 1995).

Gargle of hot water extract of galls is very effective against inflamed tonsils, while direct application of boiled and bruised galls on skin

effectively cures any swelling or inflammation (Chopra et al., 1956).



**Figure 1.:** *Quercus infectoria* Galls

The application of powdered galls in the form of ointment cures hemorrhoids (Anonymous, 1995).

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The constituents of galls comprise a large amount of tannins, gallic acid, syringic acid, ellagic acid,  $\beta$ -sitosterol, amentoflavone hexamethyl ether, isocryptomerin, methyl betulate, methyl oleanate and hexagalloyl glucose (Dar and Ikram, 1979; Dar et al., 1976; Hwang et al., 2000; Ikram and Nowshad, 1977; Kaur et al., 2004).

Pharmacological evaluation of *Quercus infectoria* galls has proved them to be astringent, antiparkinsonian, antitremorine, antiinflammatory, antidiabetic and antioxidant (Dar et al., 1976; Dar and Ikram, 1979; Hwang et al., 2000; Kaur et al., 2004; 2008). However hepatoprotective activity of galls has never been evaluated.

The role of oxidative stress in hepatic disorders has been well established (Cesaratto et al., 2004). In view of the potent antioxidant and antiinflammatory properties (Kaur et al., 2004; 2008) of the galls of *Quercus infectoria*, the present study was carried out to evaluate the protective effect of the aqueous ethanolic extract of its galls against carbon tetrachloride ( $\text{CCl}_4$ ) induced hepatotoxicity in rats.

## MATERIALS AND METHODS

**Plant Material.** The galls of *Quercus infectoria* were purchased locally and were identified and authenticated at National Botanical Research Institute, Lucknow. The dried galls were powdered and extracted (250 g) successively with 1 l of ethyl alcohol (50% v/v) in a soxhlet extractor for 18–20 h. It was concentrated under reduced pressure and controlled temperature (40–50 °C) (yield 68.3% w/w). For the pharmacological tests the extract was suspended in double distilled water containing carboxymethyl cellulose (1%, w/v, CMC). A preliminary phytochemical screening was carried out on extract to assess the presence of alkaloids, glycosides, saponins, flavanoids and steroids.

**Drugs and chemicals.** All the drugs and chemicals used in the study were of analytical grade. Carbon tetrachloride was obtained from Merck Limited, India. Silymarin was obtained from Ranbaxy Laboratories Limited, India. The chemicals used for evaluation of oxidative stress parameters were obtained from Sisco Research Laboratories, India. Kits used for the estimation of serum aspartate and alanine transaminase (AST and ALT) levels were purchased from Centronic GmbH, Germany. Folin-Ciocalteu reagent was purchased from Sigma Chemicals, St. Louis.

**Determination of total phenolics.** One hundred milligrams of the *Quercus infectoria* extract was extracted with 250 ml of methanol/water (60:40, v/v; 0.3% HCl) and filtered through a 0.45- $\mu\text{m}$  Millipore filter. To 100  $\mu\text{l}$  of filtrate, 100  $\mu\text{l}$  of Folin-Ciocalteu reagent (50%, v/v) and 2.0 ml sodium carbonate (2%, w/v) were added and mixed completely. After 2 hours, the absorbance of the solution was measured at 750 nm. Quantization

was based on the standard curve of gallic acid (0–1.0 mg/ml), dissolved in methanol/water (60:40, v/v; 0.3% HCl). Phenolic content was expressed as milligrams gallic acid equivalent (GAE) per gram sample.

**Experimental Animals.** Studies were carried out using male wistar albino rats (180–220 g) which were purchased from the animal house of CDRI, Lucknow and were kept in departmental animal house in a cross ventilated room at  $25 \pm 2^\circ\text{C}$  and relative humidity 44–56%. The animals were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. All procedures described were reviewed and approved by the Institutional animal ethical committee.

**Hepatoprotective Study.** The rats were divided into six different groups comprising of six animals each. Group I served as normal control was given (1%, w/v, CMC) orally, once daily. Groups II through V received subcutaneous injections of  $\text{CCl}_4$  in olive oil (1:1) at a dose of 2 ml/kg twice a week to induce hepatotoxicity. Group II served as  $\text{CCl}_4$  control, groups III, IV and V were administered *Quercus infectoria* extract at doses of 200, 400 and 600 mg/kg b.wt. Group VI received silymarin at a dose of 100 mg/kg. In the treatment groups *Quercus infectoria* extract and silymarin were administered orally once daily, starting 2 h before injection of  $\text{CCl}_4$ . After 1 week of treatment, the rats were sacrificed and blood was collected, serum separated and stored.

**Estimation of serum AST, ALT, TNF- $\alpha$  level and hepatic PGE<sub>2</sub> level.** The AST and ALT levels in the serum were estimated using commercially available kits. Serum TNF- $\alpha$  level and hepatic PGE<sub>2</sub> level were measured by commercially available ELISA kits.

**Estimation of hepatic antioxidant level and protein.** The reduced glutathione (GSH) level in the liver was determined according to the method of Ellman (1959). Hepatic superoxide dismutase (SOD) activity was estimated by the method of Kakkar et al. (1984). The Glutathione peroxidase (GPx) activity was estimated by the method of Paglia and Valentine (1967). The catalase activity was estimated by the method of Aebi (1984).

**Estimation of hepatic thiobarbituric acid reactive substances (TBARS).** The hepatic TBARS level, an index of malonyldialdehyde (MDA) production was determined by the method of Okhawa et al. (1979).

**Histopathological analysis of liver.** For histopathological studies, the liver tissues were fixed with 10 % phosphate-buffered neutral formalin, dehydrated in graded (50–100%) alcohol and embedded in paraffin. Thin sections were cut

and stained with routine hematoxylin and eosin (H&E) stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue (Amresh et al., 2007).

**Statistical analysis.** The values are expressed as mean  $\pm$  S.E.M. of six observations. The results obtained were statistically analyzed by student's T-test.

## RESULTS

**Phytochemical screening and total phenolics.** The preliminary phytochemical screening of *Quercus infectoria* extract indicated the presence of Tannins, carbohydrate, alkaloids, terpenoids, flavanoids and glycosides. The *Quercus infectoria*

extract was found to contain  $438.17 \pm 14.80$  mg/g total polyphenolics expressed as milligrams gallic acid equivalent (GAE) per gram sample.

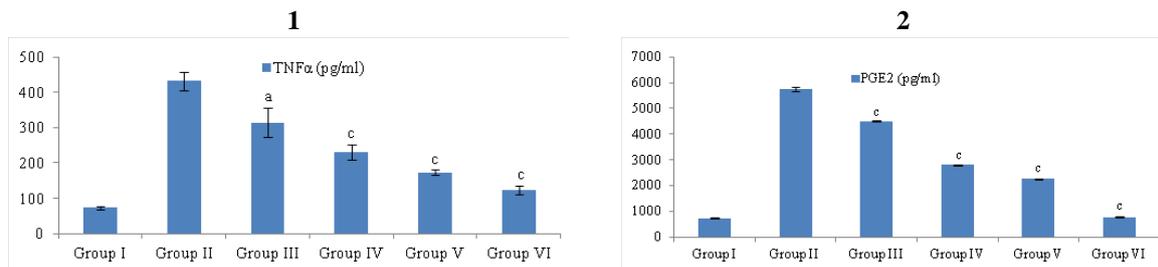
**Effect of *Quercus infectoria* extract on serum AST and ALT levels.** Subcutaneous injection of  $\text{CCl}_4$  produced a significant increase in the serum levels of AST and ALT ( $317.00 \pm 5.12$  and  $181.83 \pm 6.13$  U/L) as compared to the levels in the normal rats ( $180.60 \pm 7.81$  and  $90.10 \pm 3.97$  U/L). Oral administration of *Quercus infectoria* extract at 200, 400 and 600 mg/kg b.wt, daily over a period of 1 week produced a significant ( $p < 0.01$ ,  $p < 0.001$ ) dose dependent decrease in the levels of AST (8.14, 32.73 and 42.06%) and ALT (33.14, 40.07 and 60.50%) (Table 1).

**Table 1.** Effect of *Quercus infectoria* extract on serum AST and ALT levels in rats with  $\text{CCl}_4$  induced hepatotoxicity.

Treatment	AST (U/L)	ALT (U/L)
Group I	$180.60 \pm 7.81$	$90.10 \pm 3.97$
Group II	$317.00 \pm 5.12$	$181.13 \pm 6.13$
Group III	$291.19 \pm 5.13^b$	$121.10 \pm 1.20^c$
Group IV	$213.23 \pm 7.12^c$	$108.54 \pm 2.13^c$
Group V	$183.66 \pm 5.97^c$	$71.53 \pm 2.28^c$
Group VI	$171.33 \pm 7.71^c$	$66.81 \pm 2.11^c$

Values represent mean  $\pm$  S.E.M of six animals in each group. Group I: 1% w/v, CMC, Orally; Group II:  $\text{CCl}_4$ , 2 ml/kg twice a week, SC; Group III: *Quercus infectoria* extract 200 mg/kg b.wt, orally +  $\text{CCl}_4$ , 2 ml/kg twice a week, SC; Group IV: *Quercus infectoria* extract 400 mg/kg b.wt orally +  $\text{CCl}_4$ , 2 ml/kg twice a week, SC; Group V: *Quercus infectoria* extract 400 mg/kg b.wt orally +  $\text{CCl}_4$ , 2 ml/kg twice a week, SC; Group VI: Silymarin 100 mg/kg b.wt orally +  $\text{CCl}_4$ , 2 ml/kg twice a week, SC

<sup>b</sup> $p < 0.001$  vs. Group II, <sup>c</sup> $p < 0.001$  vs. Group II

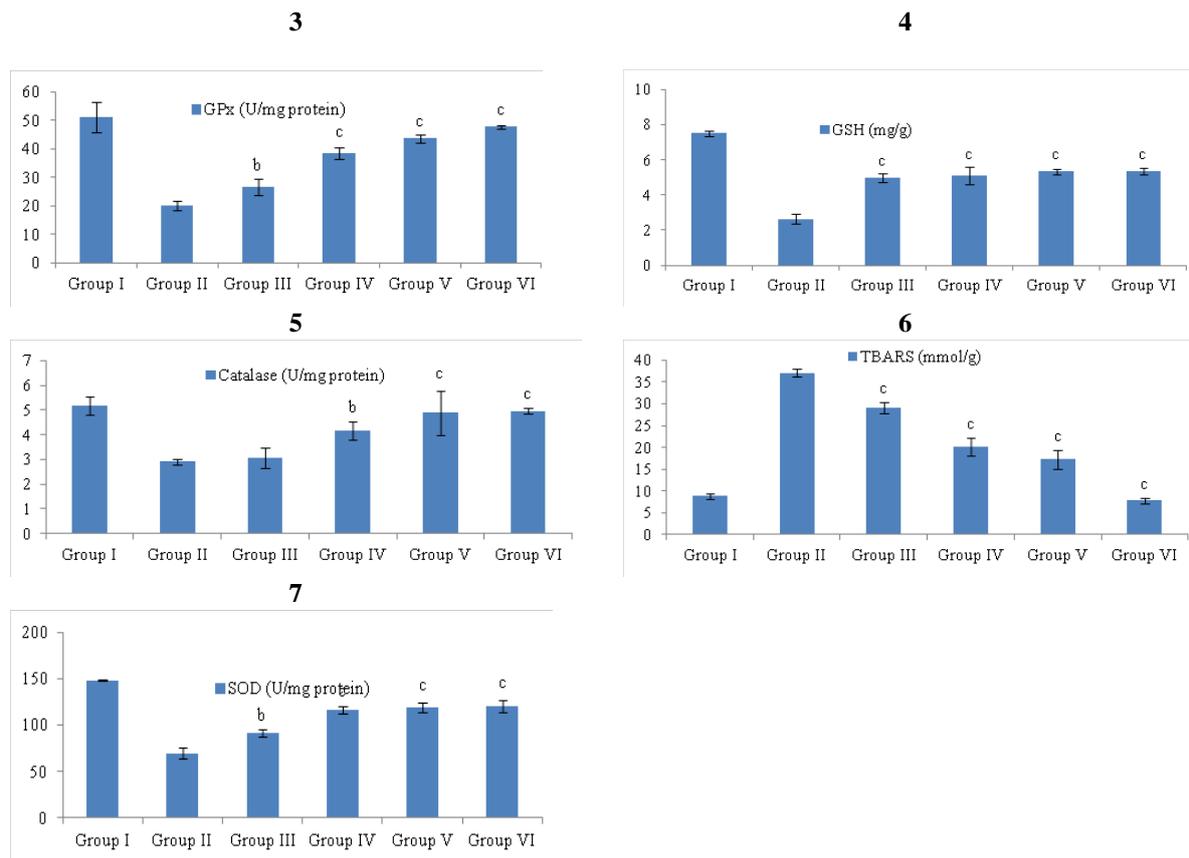


**Figures 2 & 3.** Effect of *Quercus infectoria* extract on TNF  $\alpha$  and PGE<sub>2</sub> levels in rats with  $\text{CCl}_4$  induced hepatotoxicity. Values represent mean  $\pm$  S.E.M of six animals in each group. Group I: 1% w/v, CMC, Orally; Group II:  $\text{CCl}_4$ , 2 ml/kg twice a week, SC; Group III: *Quercus infectoria* extract 200 mg/kg b.wt, orally +  $\text{CCl}_4$ , 2 ml/kg twice a week, SC; Group IV: *Quercus infectoria* extract 400 mg/kg b.wt orally +  $\text{CCl}_4$ , 2 ml/kg twice a week, SC; Group V: *Quercus infectoria* extract 400 mg/kg b.wt orally +  $\text{CCl}_4$ , 2 ml/kg twice a week, SC; Group VI: Silymarin 100 mg/kg b.wt orally +  $\text{CCl}_4$ , 2 ml/kg twice a week, SC. <sup>a</sup> $p < 0.05$  vs. Group II, <sup>b</sup> $p < 0.001$  vs. Group II, <sup>c</sup> $p < 0.001$  vs. Group II

**Effect of *Quercus infectoria* extract on serum TNF- $\alpha$  and liver PGE<sub>2</sub> levels.** The hepatotoxicity induced by CCl<sub>4</sub> was associated with marked increase in the levels of TNF- $\alpha$  and PGE<sub>2</sub>. There was a six-fold increase in the serum levels of TNF- $\alpha$  and PGE<sub>2</sub> following the effect of following CCl<sub>4</sub> injection ( $432.12 \pm 26.93$  pg/ml and  $5743.00 \pm 81.93$  pg/g respectively) when compared to normal ( $72.91 \pm 5.17$  pg/ml and  $729.13 \pm 11.61$  pg/g respectively). At a dose of 200 mg/kg, *Quercus infectoria* extract produced a moderate ( $314.51 \pm 41.48$  pg/ml,  $p < 0.05$ ) change in the TNF- $\alpha$  level while a significant ( $4503.00 \pm 13.97$  pg/g,  $p < 0.001$ ) decrease in the PGE<sub>2</sub> level was observed. Like silymarin, treatment with extract at 400 and 600 mg/kg b.wt over one week produced a significant ( $p < 0.001$ ) dose-dependent decrease in the levels of TNF- $\alpha$  and PGE<sub>2</sub> (Figures 1 & 2).

**Effect of *Quercus infectoria* extract on hepatic antioxidants and TBARS levels.** In order to

evaluate the effect of *Quercus infectoria* extract on CCl<sub>4</sub> induced oxidative stress, tissue levels of GSH, TBARS and activities of antioxidant enzymes SOD, GPx and catalase were measured. Administration of CCl<sub>4</sub> by subcutaneous route produced a marked decrease in the liver GSH level and SOD and GPx activity. This was accompanied by an increase in the TBARS level and catalase activity in the liver. Daily treatment with *Quercus infectoria* extract at doses of 200, 400 and 600 mg/kg produced a significant ( $p < 0.01$ ,  $p < 0.001$ ) and dose-dependent increase in the GSH level and SOD and GPx activity. It also normalized the TBARS level and the effect was comparable to silymarin ( $p < 0.001$ ). The extract administration also produced a dose-dependent decrease in catalase activity but the effect was not statistically significant in case at 200 mg/kg b.wt but was found to be statistically significant at 400 and 600 mg/kg doses (Figures 3, 4, 5, 6 & 7).



**Figures 4, 5, 6, 7 & 8.** Effect of *Quercus infectoria* extract on GPx, Catalase, SOD, GSH and TBARS levels in rats with CCl<sub>4</sub> induced hepatotoxicity. Values represent mean  $\pm$  S.E.M of six animals in each group. Group I: 1% w/v, CMC, Orally; Group II: CCl<sub>4</sub>, 2 ml/kg twice a week, SC; Group III: *Quercus infectoria* extract 200 mg/kg b.wt, orally + CCl<sub>4</sub>, 2 ml/kg twice a week, SC; Group IV: *Quercus infectoria* extract 400 mg/kg b.wt orally + CCl<sub>4</sub>, 2 ml/kg twice a week, SC; Group V: *Quercus infectoria* extract 400 mg/kg b.wt orally + CCl<sub>4</sub>, 2 ml/kg twice a week, SC; Group VI: Silymarin 100 mg/kg b.wt orally + CCl<sub>4</sub>, 2 ml/kg twice a week, SC. <sup>a</sup> $p < 0.05$  vs. Group II, <sup>b</sup> $p < 0.001$  vs. Group II, <sup>c</sup> $p < 0.001$  vs. Group II

**Effect of *Quercus infectoria* extract on Liver histology.** Subcutaneous injection of CCl<sub>4</sub> brought about histological changes in the rat liver where marked leukocytic infiltration, centrilobular necrosis and vacuolation were observed. Treatment with *Quercus infectoria* extract produced significant attenuation of the inflammatory and necrotic changes and the cellular architecture of the liver was preserved.

## DISCUSSION.

In the present study, we have evaluated the hepatoprotective effect of *Quercus infectoria* extract against CCl<sub>4</sub> induced acute hepatotoxicity in rats. Subcutaneous injection of CCl<sub>4</sub> induced liver damage that was revealed by significant increase in serum levels of AST and ALT. This was further substantiated by the altered levels of oxidative stress markers, pro-inflammatory mediators and the inflammatory changes revealed in liver histology. CCl<sub>4</sub> undergoes reductive metabolism by CYP2E1 into a highly reactive trichloromethyl radical that initiates lipid peroxidation, disrupts membrane integrity and causes death of hepatocytes (Basu, 2003). Daily treatment with *Quercus infectoria* extract over a week afforded hepatoprotection in a dose dependent manner. In our study, *Quercus infectoria* extract was found to be as potent as silymarin with regard to its hepatoprotective effect. Like silymarin the *Quercus infectoria* extract also exhibited antioxidant properties and augmented the levels of hepatic GSH and SOD and GPx activities. In addition, it also produced a marked reduction in the levels of TBARS, an index of lipid peroxidation. The antioxidant property can be attributed to the presence of high content of polyphenolics in the gall extract (Kaur et al., 2008). Several authors have observed a direct correlation between polyphenolic content and antioxidant activity (Rice-Evans et al., 1997).

Alcoholic extract of *Quercus infectoria* has earlier been shown to exhibit anti-inflammatory activity against various inflammagens (Kaur et al., 2008). Such an anti-inflammatory effect has also been shown with hepatoprotective agent silymarin (Fogden and Neuberger, 2003). The pro-inflammatory cytokine, TNF- $\alpha$  has been reported to play a key role in the pathogenesis of various liver diseases (Simeonova et al., 2001). *Quercus infectoria* extract markedly attenuated the inflammatory response and significantly decreased the TNF- $\alpha$  and PGE<sub>2</sub> levels. Following its release from activated Kupffer cells, TNF- $\alpha$  aggravates both oxidative stress and inflammatory response in the liver (Simeonova et al., 2001; Ghavami et al., 2005). The key role of TNF- $\alpha$  in CCl<sub>4</sub> induced liver damage has also been substantiated in an earlier study where treatment with soluble TNF- $\alpha$

receptor prevented liver injury and decreased mortality in rats (Czaja et al., 1995).

Further, the inhibition of TNF- $\alpha$  has also been shown to be associated with decreased mortality in patients with alcoholic hepatitis (Akriviadis et al., 2000). Besides, TNF- $\alpha$  also induces COX-2 expression leading to synthesis of prostaglandins that act as potent chemoattractant and participate in the vascular changes associated with inflammation (Feng et al., 1995). The efficacy of COX-2 inhibitors, celecoxib and NS-398 against alcohol-induced hepatotoxicity has been demonstrated in the rat model (Yi et al., 2003; Ganey et al., 2001). Besides inhibiting the release of inflammatory mediators, COX-2 inhibitors have been shown to preserve the liver histology. Based on the findings of our study, the hepatoprotective effect of *Quercus infectoria* extract could be attributed to its anti-inflammatory properties.

## CONCLUSIONS.

Our studies clearly demonstrate that by preventing the development of oxidative stress and inhibiting the release of TNF- $\alpha$  and PGE<sub>2</sub>, the galls of *Quercus infectoria* affords protection against CCl<sub>4</sub> induced hepatotoxicity and has the potential for use as a hepatoprotective agent.

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