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Research Article
Hepatoprotective Impact of Geraniol Against CCl_4-Induced Liver Fibrosis in Rats

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Abstract

Background and Objective: Numerous experimental studies have shown various pharmacological activities including geraniol's cancer prevention agent and antioxidant capacity. The goal of this investigation is to mark the prospective defensive role of geraniol in rat's carbon tetrachloride (CCl_4) instigated in liver fibrosis. **Materials and Methods:** Liver fibrosis was prompted by subcutaneous injections of CCl_4, twice week by week and for about a month. Simultaneously, geraniol (200 mg kg^{-1}) was orally regulated every day. Post-Hoc-Test were carried out where $p<0.05$ has been established as a significant value. **Results:** The biochemical results showed that geraniol reduced liver damage just as manifestations of liver fibrosis. The administration of geraniol diminished the CCl_4-initiated the elevation in serum aminotransferase activities and alkaline phosphatase activity. Geraniol diminished the levels of TNF-α, NO and myeloperoxidase activity which were prompted by the CCl_4 treatment. The rise of serum hyaluronidase activity and hepatic hydroxyproline content was also curtailed by geraniol treatment. Besides, geraniol fundamentally declined hepatic malondialdehyde (MDA) formation and increased reduced glutathione (GSH) in CCl_4-treated rats. Geraniol has also increased the activity of hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) in the rats treated with CCl_4. Finally, the histological analysis of the liver bolstered the biochemical results. **Conclusion:** Our study has demonstrated that geraniol has a hepatoprotective upshot on liver fibrosis caused by CCl_4, supposedly due to its free radical scavenging, antioxidant and anti-inflammatory characteristics.

Key words: Carbon tetrachloride, liver fibrosis, Geraniol, antioxidant, antiinflammatory

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sundry studies have up-to-date that the plant kingdom parades a varied range of natural antioxidant molecules containing phenolic acids, flavonoids and other secondary metabolites and they can be appreciated in the cure of various diseases[1]. Geraniol (E-3, 7-dimethyl-2, 6-octadien-1-ol) is an acyclic monoterpene, produced from many species of plants, including roses. It is claimed that geraniol is present in over 160 essential oils. It is an important component of essential oil of ginger, lime, lemon, nutmeg, orange, lavender, rose, etc is an acyclic monoterpenoid and is the principal component of palmarosa oil and orange flower oil[2].

Geraniol has a wide range of pharmacological influence including anti-inflammatory, antioxidant and anticancer actions[3]. Also, geraniol has anti-tumor action against diethylnitrosamine (DENA) induced liver carcinoma in rats[4], murine leukemia, colon tumors and melanoma cells against multiple cancer cells[5,6]. In addition, geraniol exhibited potent insecticidal, antimicrobial, neuroprotective and nephroprotective effects[7].

Cellular toxicity has been focused in current years, the biotransformation of chemicals to particularly reactive metabolites that is the main reason for cellular toxicity. Carbon tetrachloride (CCl_4) has probably been studied more extensively both biochemically and pathologically than any other hepatotoxin[8]. CCl_4 is a typically utilized model for the induction of liver fibrosis in animals[9].

CCl_4's metabolism resulted in highly reactive radical trichloromethyl-free ($CCl_3\cdot$) in the endoplasmic reticulum by the action of a mixed-function oxidase organization. $CCl_3\cdot$ is the dominant reason for liver injury and reacts with molecular oxygen to beget the trichloromethyl peroxy radical[10]. Both trichloromethyl and its peroxy radical are vulnerable to binding to lipids or proteins, or of abstracting a hydrogen molecule from an unsaturated lipid, instigating lipid peroxidation and liver damage and consequently playing a significant role in disease pathogenesis[11]. $CCl_3\cdot$ is the crucial explanation behind liver injury and responds with sub-atomic oxygen to produce the trichloromethyl peroxy radical[10].

Furthermore, CCl_4 metabolism activates kupffer cells by elevating intracellular calcium thus causing them to release harmful cytokines which may promote the death of hepatocytes as confirmed by Wang *et al.*[12].

In light of the foregoing from previous reports; this study was planned to estimate geraniol's possible protective role against $CCl_4\cdot$ induced liver fibrosis in rats.

MATERIALS AND METHODS

Study site: The research was carried out at the Faculty of Science, Alexandria University, Egypt between March 2019 and March 2020.

Chemicals: Geraniol (99.9% pure), tetra methoxypropane, superoxide dismutase, hyaluronic acid, N-acetylglucosamine, hydroxyproline, Elman's reagent [5,5`-dithiobis-(2-nitrobenzoic acid],1-chloro-2,4-dinitrobenzene] and reduced glutathione were purchased from Sigma Chemical Company, St. Louis, MO, USA. Thiobarbituric acid, diethylene triaminopentaacetic, 3,3',5,5'-tetramethylbenzidine and pyrogallol were purchased from Sigma-Aldrich Company, Germany. Chloramine-T was bought from Aldrich Corporation, USA. Perchloric acid was purchased from Diamond Company, Germany. Bovine egg albumin, dithiothreitol (DTT) and picrylsulfonic acid (2.4.6-trinitrobenzenesulfonic acid, TNBS) were purchased from Oxford, India. We also bought all other chemicals from the El Nasr Corporation for Pharmaceutical Chemicals, Egypt.

Kits: BioAssay Systems, USA, has acquired kits for the analysis of liver functions, for example, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin and total protein. Likewise, from BioAssay Systems, USA, we bought kidney functions as urea, creatinine and lactate dehydrogenase (LDH). In addition, Rat Tumor necrosis factor-alpha (TNF-α) kit was purchased from Sigma-Aldrich, USA (Catalog No. RAB0480). Finally, the total antioxidant capacity kit (Catalog Number KA0802) and total nitric oxide (NO) kits from Abnova Corporation, Taiwan.

Animals: A total of 40 adult Sprague Dawley male rats (100±20 g) were gotten from the Animal House, Faculty of Medicine, Alexandria University, Egypt. In normal condition, the rats were lodged at room temperature in a 12 h light/dark cycle, with ordinary diet and water then acclimatized for 7 days and then split 10 rats in each cage to form 4 groups.

Experimental design:

Group I: Untreated rats (Control)

Group II: Rats given an only oral dose of geraniol (200 mg kg^{-1} b.wt., dissolved in corn oil)

Group III: Rats were given subcutaneous injections of CCl_4/corn oil (50% v/v) at a dose of 1 mg kg^{-1} b.wt. for about a month (4 weeks), Phenobarbital was

applied to the drinking water (350 mg L^{-1}) two weeks before the beginning of the CCl_4 injection and all through the study[9]

Group IV: (geraniol+CCl_4) rats given 200 mg kg^{-1} b.wt. geraniol in daily oral doses along with CCl_4 and phenobarbital for 4 weeks. Geraniol was also administered a week before the injection of CCl_4. The experimental design was approved by the Egyptian regional committee and the protocol adopted guidelines for the National Institutes of Health

Biological samples: At the end of the experiment design, the rats were sacrificed and the blood was pulled out by cardiac puncture from all groups and centrifuged for 10 min at 3800 rpm, subsequently the serum was stockpiled at -20°C until the assays were complete. The livers from all groups had been instantly taken away and washed by using ice-cold saline then sliced and without delay fixed in 10% formalin for histological examination and the residual liver tissues frozen at -20°C for biochemical analysis.

Biochemical estimation in blood and tissues: The assessment of various biochemical parameters for the analysis of liver function specifically AST and ALT according to Bergmeyer *et al.*[13], ALP by the reference of Moss[14], albumin according to the method of Doumas and Biggs[15] and protein content was evaluated according to Henry[16]. Also, Buttery *et al.*[17] method used to test the serum LDH activity. For studying kidney function, it was established by the concentration of serum urea concentration according to Gutmann and Bergmeyer[18] and creatinine by Heinegård *et al.*[19] methods. Also, hepatic malondialdehyde (MDA) was estimated by using Ohkawa *et al.*[20] method.

In addition, Serum total antioxidant activity was also determined using the Salim *et al.*[21] method. The antioxidant markers of the liver tissue calculated by measuring reduced glutathione (GSH) level and activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT)), were valued by the procedures of Ellman[22], Marklund and Marklund[23], Habig *et al.*[24], Smith *et al.*[25], Rotruck *et al.*[26] and Aebi[27], respectively.

For the determination of anti-inflammatory markers, NO was measured using the Giustarini *et al.*[28] method. As defined by Hillegass *et al.*[29], myeloperoxidase (MPO) activity was conducted as a sign of neutrophil aggregation and activation of inflammation. So, MPO activity in the hepatic homogenate tissue was also determined. In addition, TNF-α was measured using commercial ELISA kits according to Bonavida[30].

Finally, the biochemical liver fibrosis markers were calculated as the activity of Serum hyaluronidase (HAase) using the Reissig *et al.*[31] method and the Patiyal and Katoch[32] method used to assess the content of hepatic hydroxyproline.

Histological analysis: According to Griffith and Farris[33], hematoxylin and eosin (H and E) stained the sections of the liver and recorded changes in histology under the light microscope.

Statistical analysis: Data articulated as mean±standard deviation (SD). The discrepancy between the groups was calculated using Post-Hoc-Test (LSD) (one way ANOVA variance analysis). A $p<0.05$ value has been affirmed as being significant.

RESULTS

Impact of geraniol on lipid peroxide (MDA) and antioxidant system: Hepatic MDA was significantly increased in the CCl_4 group (36.243±1.351 nmol/mL/mg protein) compared to control group was 6.24±1.024 nmol/mL/mg protein ($p<0.05$) (Table 1). Protection with geraniol showed significant ($p<0.05$) reduction in LPO to 6.56±1.411 nmol/mL/mg protein. Also, the level of total serum antioxidant capacity in serum was significantly lessened in the CCl_4 group (0.31±0.106 mmol L^{-1}) compared to the control group was 0.58±0.202 mmol L^{-1} ($p<0.05$). Protection with geraniol showed significant ($p<0.05$) elevation in total antioxidant capacity to 0.57±0.121 mmol L^{-1}.

CCl_4 administration significantly ($p<0.05$) reduced the level of hepatic GSH, specific activities of SOD, GPx, CAT, GST and GR (4.316±1.451 units/mg protein, 7.457±1.907 μmoles of GSH oxidized/min/mg protein, 59.478±3.354 nmol of H_2O_2 released/min/mg protein, 0.489±0.104 μmoles of CDNB utilized/min/mg protein and 20.27±2.66 μg GSSG utilized/min/mg protein, respectively) when compared to the corresponding control values (16.24±1.104 units/mg protein, 30.387±1.917 μmoles of GSH oxidized/min/mg protein, 162.351±3.156 nmol of H_2O_2 released/min/mg protein, 2.081±0.169 μmoles of CDNB utilized/min/mg protein and 56.45±2.09 μg GSSG utilized/min/mg protein , respectively). Protection with geraniol exhibited a significant increase ($p<0.05$) in the hepatic GSH levels and in the hepatic specific activities of SOD, CAT, GSH-Px, GST and GR (Table 1 and 2).

Table 1: Impact of geraniol on levels of hepatic MDA, hepatic GSH and serum total antioxidant capacity of CCl_4 treated-groups

Animal groups	Lipid peroxidation (nmol/mL/mg protein)	GSH (ng g^{-1} protein)	Total antioxidant capacity (mmol L^{-1})
Group I	6.24±1.024	50.260±2.451	0.58±0.202
Group II	6.376±1.137	50.247±2.092	0.60±0.141
Group III	36.243±1.351[a]	8.265±1.637[a]	0.31±0.106[a]
Group IV	6.56±1.411[b]	49.017±2.125[b]	0.57±0.121[b]

Values are expressed in Mean±SD, each group contains 10 animals (n = 10), [a]Significant with control, [b]Significant with CCl_4 group, ($p<0.05$), I: Normal control, II: geraniol group, III: CCl_4, IV: CCl_4+geraniol

Table 2: Impact of geraniol on activities of SOD, CAT, GSH-Px, GST and GR in liver of CCl_4 treated-groups

Animal groups	SOD specific activity (units/ mg protein)	Gpx specific activity (µmoles of GSH oxidized /min/mg protein)	CAT specific activity (nmol of H_2O_2 released /min/mg protein)	GST specific activity (µmoles of CDNB utilized /min/mg protein)	GR (µg GSSG utilized/min/mg protein)
Group I	16.24±1.104	30.387±1.917	162.351±3.156	2.081±0.169	56.45±2.09
Group II	16.166±1.126	30.449±2.032	162.487±4.012	2.101±0.147	56.92±1.89
Group III	4.316±1.451[a]	7.457±1.907[a]	59.478±3.354[a]	0.489±0.104[a]	20.27±2.66[a]
Group IV	16.42±1.233[b]	28.812±2.417[b]	160.411±4.072[b]	1.929±0.184[b]	54.94±1.89[b]

Values are expressed in Mean±SD, each group contains 10 animals (n = 10), [a]Significant with control, [b]Significant with CCl_4 group, ($p < 0.05$), I: Normal control, II: geraniol group, III: CCl_4, IV: CCl_4+geraniol

Table 3: Impact of geraniol on hepatic MPO activity and level serum NO in CCl_4 treated-groups

Animal groups	Hepatic myeloperoxidase activity (µ g^{-1} protein)	No concentration (µmol L^{-1})	TNF-α (pg mL^{-1})
Group I	4.215±1.812	25.368±2.592	8.665±0.478
Group II	4.119±1.990	25.603±3.259	8.701±0.412
Group III	16.678±2.492[a]	297.685±3.743[a]	24.821±0.657[a]
Group IV	4.405±1.546[b]	29.976±2.554[b]	9.015±0.653[b]

Values are expressed in Mean±SD, each group contains 10 animals (n = 10), [a]Significant with control, [b]Significant with CCl_4 group, ($p<0.05$), I: Normal control, II: geraniol group, III: CCl_4, IV: CCl_4

Table 4: Impact of geraniol on hepatic hydroxyproline levels and level serum HAase activity in CCl_4 treated-groups

Animal groups	Hepatic hydroxyproline levels (nmol/mg of liver tissue)	Serum HAase activity (U L^{-1})
Group I	249.020±6.084	232.114±3.965
Group II	250.801±5.051	230.181±4.327
Group III	818.740±9.140[a]	105.542±4.319[a]
Group IV	256.240±7.905[b]	228.256±5.354[b]

Values are expressed in Mean±SD, each group contains 10 animals (n = 10), [a]Significant with control, [b]Significant with CCl_4 group, ($p<0.05$), I: Normal control, II: geraniol group, III: CCl_4, IV: CCl_4+geraniol

Impact of geraniol on inflammation markers: The CCl_4-intoxicated group exhibited significant elevation 16.678±2.492 µ g^{-1} protein ($p<0.05$) in MPO activity, compared to the control group (4.215±1.812 µ g^{-1} protein). Protection with geraniol significantly attenuated the increase in MPO activity (4.405±1.546 µ g^{-1} protein) in comparison with the CCl_4-treated group (Table 3).

The NO level often considered a fundamental indicator of inflammation. As shown in Table 1, the level of NO in serum was significantly increased in the CCl_4 group (297.685±3.743 µmol L^{-1}) compared to control group was 25.368±2.592 µmol L^{-1} ($p<0.05$). Protection with geraniol showed a significant ($p<0.05$) reduction in the level of NO to 29.976±2.554 µmol L^{-1} (Table 3).

As well, CCl_4 injection amplified serum TNF-α level to three times compared to the normal group ($p<0.05$). While, protection with geraniol caused a significant reduction in TNF-α when compared to TNF-α levels in the CCl_4 group (Table 3).

Impact of geraniol on the markers for liver fibrosis: CCl_4 caused a significant increase in hepatic hydroxyproline (818.740±9.140 nmol mg^{-1}) with liver fibrosis ($p<0.05$) (Table 4). Meanwhile, protection with geraniol caused a significant reduction in the content of hydroxyproline in liver tissue to 256.240±7.905 nmol mg^{-1}.

The activity of serum HAase in the CCl_4 group was significantly diminished to 105.542±4.319 U L^{-1} compared to the control group (232.114±3.965 U L^{-1}), as shown in Table 4. Geraniol protection significantly improved HAase activity compared with the CCl_4 group to 228.256±5.354 U L^{-1}.

Impact of geraniol on markers of liver function: The specific activities of serum ALT, AST and ALP of animals showed a significant ($p<0.05$) increase by the CCl_4 treatment in group III compared to the control group, while the specific activities of these enzymes significantly ($p<0.05$) declined by protection with geraniol (Table 5). Table 5 also revealed that the serum total protein and albumin decreased significantly ($p<0.05$) in CCl_4 group compared to the control, while this upsurge was switched by the impact of geraniol.

Table 5: Impact of geraniol on the levels of serum ALT, AST, ALP, albumin and total protein concentrations in examined rats

Animal groups	ALT (U mL^{-1})	AST (U mL^{-1})	ALP (IU L^{-1})	Albumin (g dL^{-1})	Serum protein (g dL^{-1})
Group I	12.49±3.425	22.89±4.491	54.716±4.351	4.042±0.210	6.98±0.115
Group II	24.73±3.704	23.59±4.023	55.160±3.259	3.909±0.179	6.97±0.108
Group III	175.26±4.541[a]	256.28±3.312[a]	130.013±6.147[a]	2.001±0.103[a]	6.41±0.109[a]
Group IV	14.08±4.912[b]	25.02±4.316[b]	56.755±3.305[b]	3.93±0.096[b]	6.87±0.217[b]

Values are expressed in Mean±SD, each group contains 10 animals (n = 10), [a]Significant with control, [b]Significant with CCl_4 group, ($p<0.05$), I: Normal control, II: geraniol group, III: CCl_4, IV: CCl_4+geraniol

Table 6: Impact of geraniol on the levels of serum urea, creatinine and LDH activity concentrations in examined rats

Animal groups	Serum urea (mg dL^{-1})	Serum creatinine (mg dL^{-1})	LDH activity (U L^{-1})
Group I	22.145±2.409	0.531±0.057	241.545±6.923
Group II	22.429±2.412	0.530±0.061	241.462±5.807
Group III	188.405±4.454[a]	2.564±0.134[a]	1195.319±7.785[a]
Group IV	24.247±3.273[b]	0.536±0.031[b]	246.718±6.402[b]

Values are expressed in Mean±SD, each group contains 10 animals (n = 10), [a]Significant with control, [b]Significant with CCl_4 group, ($p<0.05$), I: Normal control, II: geraniol group, III: CCl_4, IV: CCl_4+geraniol

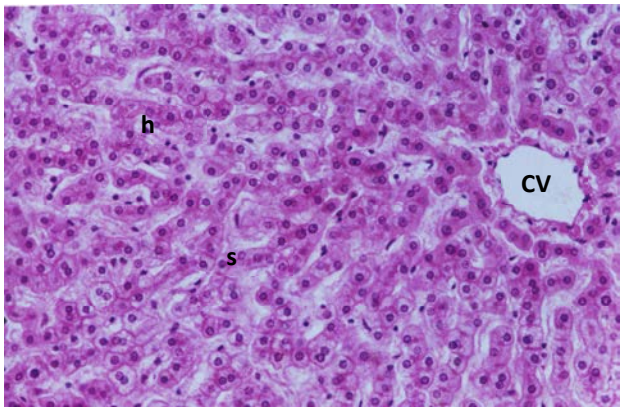

Fig. 1: A photomicrograph of a section in the liver of control group (I) showing the acidophilic cords of hepatocytes (h) radiating from centrilobular venules (CV) and separated by blood sinusoids (s), The hepatocytes have granular cytoplasm and vesicular nuclei (H and E X400)

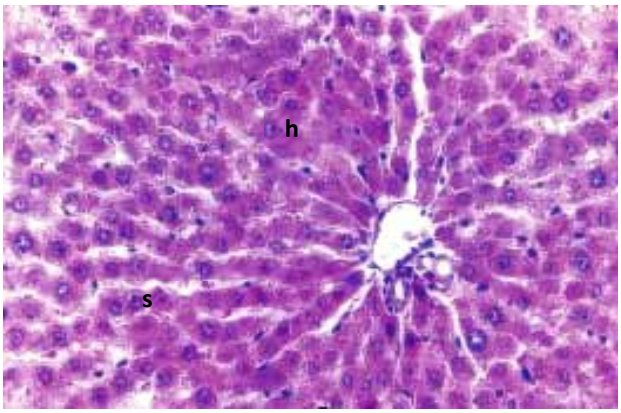

Fig. 2: A photomicrograph of a section in the liver of geraniol group (II) showing apparently normal hepatic architecture (h), The hepatocytes have vesicular nuclei and granular acidophilic cytoplasm, Note mild dilatation of blood sinusoids(s) (H and E X400)

Impact of geraniol on kidney function markers: Our data signposted that CCl_4 treatment caused a significant upswing ($p<0.05$) in serum urea and creatinine and LDH activity values compared to the corresponding control group values. Protection with geraniol induced a significant drooping in serum creatinine and urea and in LDH activity (Table 6).

Impact of geraniol on histological evaluation: In histological evaluation, Fig. 1 illustrated the normal lobular architecture structure of the liver of the control rat (Group I). Also, Fig. 2 illustrated that the hepatocytes have vesicular nuclei and granular acidophilic cytoplasm. Note mild dilatation of blood sinusoids and normal lobular architecture structure like the control group. While the CCl_4-treated group showing focal degenerative changes of the hepatocytes with vacuolated cytoplasm as shown in Fig. 3. Also, distorted hepatic architecture, congestion and thickening of the basal lamina of the central vein and most the hepatocytes have dense acidophilic cytoplasm and dense nuclei when compared with the control group ($p<0.05$) where normal lobular architecture was detected.

In contrast, in Fig. 4 protection with geraniol has markedly alleviated the degrees of liver necrosis and inflammatory cell infiltrations in comparison with the CCl_4-treated group. Furthermore, protection with geraniol showing hepatocytes appeared more or less similar to control apart from few cells with vacuolated cytoplasm.

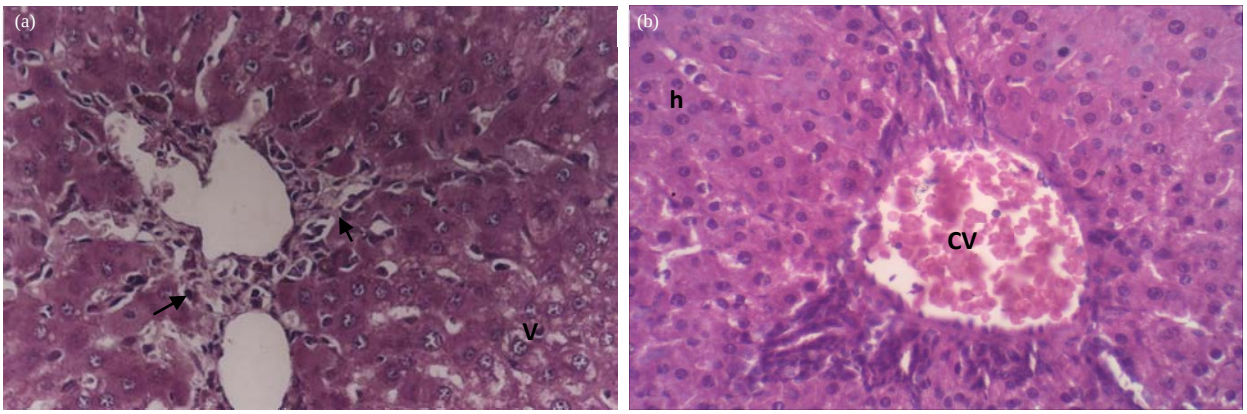

Fig. 3(a-b): Photomicrographs of a section in the liver of CCl_4 group (III). (a) Showing focal degenerative changes of the hepatocytes with vacuolated cytoplasm (V), Note the periportal mononuclear cell infiltration (arrow head) (b) showing; distorted hepatic architecture, congestion and thickening of the basal lamina of the central vein (CV), Most of the hepatocytes (h) have dense acidophilic cytoplasm and dense nuclei (H and E X400)

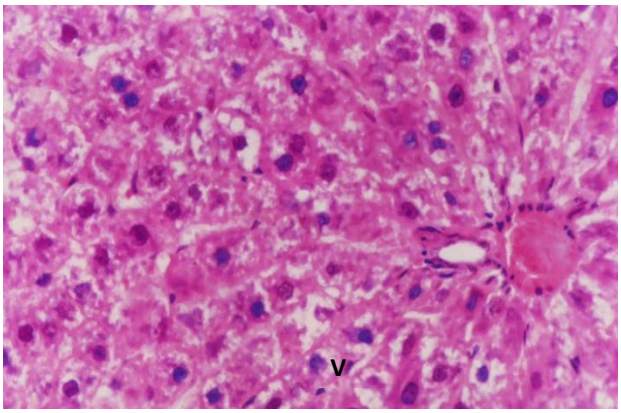

Fig. 4: A photomicrograph of a section in the liver of rat of CCl_4+geraniol group (IV) showing; hepatocytes appeared more or less similar to control apart from few cells with vacuolated cytoplasm (V) (H and E X400)

DISCUSSION

Liver fibrosis is a complicated and structured response to chronic hepatocellular damage that is a major medical concern with substantial morbidity and mortality. Patient mortality with liver fibrosis is progressively growing because it shows various pathologic conditions, sometimes consequential with the development of liver carcinoma[34].

This study provides proof of geraniol's protective function against the liver fibrosis caused by CCl_4 in rats. In this study, the treatment of rats with CCl_4 is changed into exceptionally highly reactive metabolite (trichloromethyl radical). These activated radicals covalently bind to macromolecules and prompt peroxidative destruction of endoplasmic reticulum membrane lipids that are affluent in polyunsaturated fatty acids. This results in the genesis of lipid peroxides triggering membrane damage[35].

Protection with geraniol stimulated hepatoprotective effects against hepatocellular injury that was proven by attenuating serum ALT, AST and ALP activities. In addition, geraniol activated hepatoprotective effects against the decrease in liver synthetic function as reflected by the upsurge in serum albumin and total protein levels.

This study revealed the high increase in activity of hepatic MPO (a neutrophil specific enzyme) signifying that the injury and fibrosis in tissue contain the influence of neutrophil infiltrations. As a consequence, geraniol's anti-inflammatory activity may be one of the pathways leading to anti-fibrotic action of liver fibrosis triggered by CCl_4.

Furthermore, patients with inflammatory diseases have been shown to have elevated serum TNF-α levels relative to healthy individuals[36] and as a result, TNF-α has been accepted as a biomarker for inflammatory status[37]. This is in line with the results achieved by Elguindy *et al.*[4], which revealed different anti-inflammatory properties of geraniol.

Reactive oxidative species (ROS) have a precarious role in activating HSCs during liver fibrogenesis and oxidative stress is a critical reason in chronic liver damage and fibrosis[38]. CCl_4's hepatotoxicity is based on its metabolism through cytochrome P-450, which produces highly reactive trichloromethyl free radicals, leading to lipid peroxidation and membrane damage[39]. Currently, protection with geraniol prevented the upsurge in MDA and upgraded the decline of SOD activity in the liver. This signposts that the antioxidant

property of geraniol may be the primary mechanism of protection against CCl_4-induced liver fibrosis in rats. CCl_4 also reduced GST, GR, GPx, catalase and GSH levels in the experimental animal's hepatic tissue.

Protection with geraniol significantly dropped MDA level, scavenge the decreased GSH and motivated the activities of both SOD and CAT toward normal values. Our results confirmed that geraniol has an antioxidant role in CCl_4 toxicity. These results were in accordance with Elguindy *et al.*[4] who confirmed that treatment with geraniol improved the activities of antioxidant enzymes by enhancing the activities of GST, GPx, CAT and SOD in addition to GSH level.

In addition, GSH is an intracellular thiol used to guard against free radicals and drug detoxification[40]. Because CCl_4 is a toxic electrophilic compound, it may attack the nucleophilic part of GSH and diminish its macromolecules binding effect[41]. Actually, during hepatic fibrosis, HSC is the fundamental source of collagen synthesis[42]. Hydroxyproline is the key distinguishing compound in collagen; the concentration of collagen can be mirrored by hydroxyproline estimation and can be utilized to express the degree of fibrosis[9]. CCl_4 activation of liver fibrosis increased the level of hydroxyproline in the liver significantly. Geraniol was useful for repairing hepatic fibrosis. Improvement of fibrotic changes in the liver and encouraging liver regeneration in fibrotic rats was confirmed by the noticeable drop of hydroxyproline deposition in hepatocytes[43].

As established by Rostami and Parsian[44], serum hyaluronic acid (HA) and hyaluronidase (HAase) are documented to be signs of toxic liver injury. Also, previous studies revealed that the HA concentration significantly amplified in chronic diseases of the liver of different etiology. This is due not only to increased hepatic growth but also to a decline in the activity of hyaluronidase enzyme leading to a decrease in its degradation[45]. The current results display that geraniol significantly reduced the content of hyaluronic acid by improving the activity of hyaluronidase. These results indicate that geraniol has antifibrotic influence.

CONCLUSION

The present study revealed that geraniol has beneficially hepatoprotective and antifibrotic impact against oxidative damage induced by CCl_4. Geraniol's protective effect on hepatic fibrosis may be due to its free radical scavenging, antioxidant and anti-inflammatory effects. These upshots may be useful in developing new hepatic fibrosis prevention strategies.

SIGNIFICANCE STATEMENT

This study established that geraniol can be valuable as a hepatoprotective and an antifibrotic agent at the recommended dose (200 mg kg^{-1} b.wt.). As a consequence, this study will open an unprecedented approach for the researchers to discover a safer and more potent treatment for hepatic fibrosis.

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REFERENCES

1. Osungunna, M.O., 2020. Screening of medical plants for antimicrobial activity: pharmacognosy and microbiological perspectives. J. Microbiol., Biotechnol. Food Sci., 9: 727-735.
2. Elsharif, S.A. and A. Buettner, 2017. Structure–odor relationship study on geraniol, nerol and their synthesized oxygenated derivatives. J. Agric. Food Chem., 66: 2324-2333.
3. Zielińska, A., C. Martins-Gomes, N.R. Ferreira, A.M. Silva, I. Nowak and E.B. Souto, 2018. Anti-inflammatory and anti-cancer activity of citral: Optimization of citral-loaded solid lipid nanoparticles (SLN) using experimental factorial design and LUMiSizer®. Int. J. Pharm., 553: 428-440.
4. Elguindy N.M., G.A. Yacout, E.F. El Azab and H.K. Maghraby, 2016. Chemoprotective effect of *elettaria cardamomum* against chemically induced hepatocellular carcinoma in rats by inhibiting nf-κb, oxidative stress and activity of ornithine decarboxylase. South African Journal of Botany 105: 251-258.
5. Queiroz, T.B., G.F. Santos, S.C. Ventura, C.A. Hiruma-Lima, I.O.M. Gaivão and E.L. Maistro, 2017. Cytotoxic and genotoxic potential of geraniol in peripheral blood mononuclear cells and human hepatoma cell line (HepG2). Genet. Mol. Res., 10.4238/gmr16039777
6. Sharma, S.H., S. Thulasingam and S. Nagarajan, 2017. Terpenoids as anti-colon cancer agents – A comprehensive review on its mechanistic perspectives. Eur. J. Pharmacol., 795: 169-178.
7. Elguindy N.M., G.A. Yacout and E.F. El Azab, 2018. Amelioration of dena-induced oxidative stress in rat kidney and brain by the essential oil of *elettaria cardamomum*. Beni-Suef Univ. J. Basic Appl. Sci., 7: 299-305.
8. Neuman, M.G., 2020. Hepatotoxicity: mechanisms of liver Injury. In: Liver Diseases, Neuman, M.G., Springer International Publishing, Cham, Switzerland, pp: 75-84.

9. Yacout, G.A., N.M. Elguindy and E.F. El Azab, 2012. Hepatoprotective effect of basil (*Ocimum basilicum* L.) on CCl_4-induced liver fibrosis in rats. Afr. J. Biotechnol., 11: 15702-15711.
10. Chen, X., X. Ying, L. Chen, W. Zhang and Y. Zhang, 2015. Protective effects of sesamin on liver fibrosis through antioxidative and anti-inflammatory activities in rats. Immunopharmacol. and Immunotoxicol., 37: 465-472.
11. Ničković, V.P., T. Novaković, S. Lazarević, L. Šulović and Z. Živković *et al*., 2018. Pre- vs. post-treatment with melatonin in CCl 4-induced liver damage: Oxidative stress inferred from biochemical and pathohistological studies. Life Sci., 202: 28-34.
12. Wang, M., J. Niu, L. Ou, B. Deng, Y. Wang and S. Li, 2019. Zerumbone protects against carbon tetrachloride (CCl_4)-induced acute liver injury in mice via inhibiting oxidative stress and the inflammatory response: Involving the TLR4/NF-κB/COX-2 pathway. Molecules, Vol. 24, No. 10. 10.3390/molecules24101964
13. Bergmeyer H.U. and E. Bernt, 1974. Aminotransferases and related enzymes. In: Methods of Enzymatic Analysis, Bergmeyer, H.U., Academic Press, New York, pp. 735–763.
14. Moss, D.W., 1987. Diagnostic aspects of alkaline phosphatase and its isoenzymes. Clinical Biochem., 20: 225-230.
15. Doumas, B.T. and H.G. Biggs, 1972. Determination of Serum Albumin. In: Standard Methods of Clinical Chemistry, Cooper, G.A. (Ed.). Vol. 7, Academic Press, New York, USA., pp: 175-188.
16. Henry, K.M., 1965. A comparison of biological methods with rats for determining the nutritive value of proteins. Br. J. Nutr., 19: 125-135.
17. Buttery, J.E., B.R. Chamberlain, C.R. Milner and P.R. Pannall, 1985. Colorimetric measurement of plasma lactate. Am. J. Clinical Pathol., 84: 363-365.
18. Bergmeyer, H.U., 1974. Methods of Enzymatic Analysis. 2nd Edn., Academic Press, New York, ISBN: 0895732424, pp: 534.
19. Heinegard, D. and G. Tiderstrom, 1973. Determination of serum creatinine by a direct colorimetric method. Clin. Chim. Acta, 43: 305-310.
20. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
21. Salim, E.I., S.F. Harras, A.G. Abdalla and M.H. Mona, 2018. Syphacia muris infection in rats attenuates colorectal carcinogenesis through oxidative stress and gene expression alterations. Implications for modulatory effects by Bryostatin-1. Acta parasitologica, 63: 198-209.
22. Ellman G.I., 1959. Tissue sulphhydryl groups. Arch. Biochem. Biophys., 82: 70-77.
23. Marklund, S. and G. Marklund, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.
24. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
25. Smith, I.K., T.L. Vierheller and C.A. Thorne, 1988. Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). Anal. Biochem., 175: 408-413.
26. Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science, 179: 588-590.
27. Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.
28. Giustarini, D., R. Rossi, A. Milzani and I. Dalle-Donne, 2008. Nitrite and nitrate measurement by Griess reagent in human plasma: Evaluation of interferences and standardization. Methods Enzymol., 440: 361-380.
29. Hillefass, L.M., D.E. Griswold, B. Brickson and C. Albrightson-Winslow, 1990. Assessment of myeloperoxidase activity in whole rat kidney. J. Pharmacol. Methods, 24: 285-295.
30. Bonavida, B., 1991. Immunomodulatory effect of tumor necrosis factor. Biotherapy, 32: 127-133.
31. Reissig, J.L., J.L. Strominger and L.F. Leloir, 1955. A modified colorimetric method for the estimation of N-acetylaminosugar. J. Biol. Chem., 217: 959-966.
32. Patiyal, S.N. and S.S. Katoch, 2006. Tissue specific and variable collagen proliferation in Swiss albino mice treated with clenbuterol. Physiol. Res., Vol. 55, No. 1. Griffith. J.J.Q. and E.J. Farris, 1942. The rat in laboratory investigation. Journal Am. Pharm. Assoc. (Scientific ed.), 31: 287-288.
33. Targher, G. and C.D. Byrne, 2017. Non-alcoholic fatty liver disease: an emerging driving force in chronic kidney disease. Nat. Rev. Nephrol., 13: 297-310.
34. Naseri, L., M. Khazaei, E. Ghanbari and M.A. Bazm, 2019. *Rumex alveollatus* hydroalcoholic extract protects CCL_4-induced hepatotoxicity in mice. Comp. Clin. Pathol., 28: 557-565.
35. Zhao, X., J.L. Song, J.H. Kil and K.Y. Park, 2013. Bamboo salt attenuates CCl_4-induced hepatic damage in sprague-dawley rats. Nutr. Res. Pract., 7: 273-280.
36. Mohammadalipour, A., J. Karimi, I. Khodadadi, G. Solgi, M. Hashemnia, N. Sheikh and M. Bahabadi, 2017. Dasatinib prevent hepatic fibrosis induced by carbon tetrachloride (CCl4) via anti-inflammatory and antioxidant mechanism. Immunopharmacol. Immunotoxicol., 39: 19-27.
37. Bruno, S., M.B.H. Sanchez, C. Pasquino, M. Tapparo, M. Cedrino, C. Tetta and G. Camussi, 2019. Human liver-derived stem cells improve fibrosis and inflammation associated with nonalcoholic steatohepatitis. Stem Cells Int., 2019: 1-14.

38. Aslan, A., O. Gok, O. Erman and T. Kuloglu, 2018. Ellagic acid impedes carbontetrachloride-induced liver damage in rats through suppression of NF-kB, Bcl-2 and regulating Nrf-2 and caspase pathway. Biomed. Pharmacother., 105: 662-669.
39. Chahal, A., A.K. Saini, A.K. Chhillar and R.V. Saini, 2018. Natural antioxidants as defense system against cancer. Asian J. Pharm. Clin. Res., 11: 38-44.
40. Sokar, S.S., M. El-Sayad, M.E.S. Ghoneim and A.M. Shebl, 2017. Combination of Sitagliptin and Silymarin ameliorates liver fibrosis induced by carbon tetrachloride in rats. Biomed. Pharmacother., 89: 98-107.
41. Youngmin A.L. and S.L. Friedman, 2020. Stellate cells and fibrosis. In: The Liver: Biology and Pathobiology, Youngmin A.L. and S.L. Friedman, Wiley, New Jersey, United States, pp: 444-454.
42. Dewidar, B., J. Soukupova, I. Fabregat and S. Dooley, 2019. TGF-β in hepatic stellate cell activation and liver fibrogenesis: updated. Curr. Pathobiol. Rep., 3: 291-305.
43. Rostami, S. and H. Parsian, 2013. Hyaluronic acid: from biochemical characteristics to its clinical translation in assessment of liver fibrosis. Hepat. Mon., 10.5812/hepatmon.13787
44. Díaz-Juárez, J.A. and R. Hernández-Muñoz, 2017. Rat liver enzyme release depends on blood flow-bearing physical forces acting in endothelium glycocalyx rather than on liver damage. Oxid. Med. Cell. Longevity, 2017: 1-15.