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The Hepatoprotective Activity of the Methanolic Extract of *Teucorium oliverianum* Ging Ex Benth Against Carbon tetrachloride-induced Hepatotoxicity in Rats.

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ABSTRACT

The present study was designed to investigate the influence of the methanol extract of *Teucorium oliverianum* on the hepatic toxicity induced by carbon tetrachloride (CCl_4) in rats. Rats were randomly divided into 5 equal groups as follows: Group I (control): Animals received vehicles only. Group II (CCl_4): Rats received CCl_4 30% in olive oil (by *intraperitoneal injection*) once every 72 hrs for 15 days. In addition to CCl_4 treatment, groups III, IV and V received respectively the methanolic extract of *Teucorium oliverianum*200, 400 and 800 mg/kg orally daily for 15 days. The liver marker enzymes, malondialdehyde (MDA), glutathione-S-transferase (GST), reduced glutathione (GSH) and total antioxidant capacity were determined. The histopathological findings of the liver tissue and phytoconstituents of the methanol extract of *Teucorium oliverianum* were also investigated. The administration of CCl_4induced a significant increase in the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), MDA, GST and decreased total antioxidant capacity and GSH. However, treatment with the methanol extract of *Teucorium oliverianum* in a dose dependent manner significantly normalizes the liver enzyme marker and restores the antioxidative defense and the liver tissue damage. It could be concluded that the hepatoprotection conferred by the *Teucorium oliverianum* might be due to its high content of total polyphenols and flavonoids which act as a powerful free radical scavengers and antioxidants.

Keywords: *Teucorium oliverianum*, CCl_4, hepatotoxicity, liver enzyme markers, antioxidant enzymes, rats

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INTRODUCTION

Carbon tetrachloride(CCl_4) is a toxic compound, frequently used to provoke hepatic damage in experimental animals [1-3]. It induces lipid peroxidation within a few minutes of administration in laboratory animals, and is well-known to cause severe tissues injury and increase lysosomal enzymes activities[4]. The toxicity of $CCl_4$is caused by its reactive free radical form (CCl3) generated through a reductive reaction by the hepatic cytochrome P450s. One of the potential hepatoprotective agents are plants, due to their contents of a combination of different phytochemicals which are synergistic in their action [5-7]. *Teucrium* species (family Lamiaceae) are recognized for their medicinal application and show interesting biological properties such as, hypolipidemic, hypoglycemic, hepatoprotective, anti-inflammatory, antipyretic, antitumor, antiulcer, antibacterial and insect antifeedant activities [8-13]. The genus *Teucrium* is also well-known for its rich resources of diterpenes including neoclerodane skeleton along with more than 220 diterpenes as previously described by Piozzi, *et al.*[14]. Additionally, these plants are rich in essential oils as found in the aerial parts of several *Teucrium* spp., and the proportion of the main chemical constituents (especially oxygenated sesquiterpenes and monoterpene/sesquiterpenes hydrocarbons) was reported to differ remarkably from species to species [15]. *Teucrium polium* has been shown to possess hepatoprotective effect [16] antimicrobial, antispasmodic and anti-inflammatory [17], and analgesic properties [18]. Furthermore, *Teucrium chamaedrys* has been used for gastric pain, kidney disorders and heart diseases due to its as antimalarial, antispasmodic effect [19, 20]. Despite the wide distribution of *Teucrium oliverianum* in dry and stony places of hills and deserts over the Mediterranean countries, South Western Asia, North Africa and Europe a few researches have been done on it. Several compounds with a biologic significance has been isolated from *Teucrium oliverianum*[21-23], and the alcoholic extracts of the plantwas reported as a potent inducer of the antinociceptive activity [24]. Ajabnoor*et al.*[25] recorded its antidiabetic, and hypoglycemic potentials and more, while its activity against hepatocellular carcinoma was recently discovered by Shahat *et al.*[26].

MATERIALS AND METHODS

Animals The study was performed using 50 Wistar albino mature male rats (180-200 g) obtained from the College of Pharmacy, Al-Qassim University, KSA. Rats were kept under standard conditions of temperature (22-26^{0}C), relative humidity (55-60%), and fed a standard pellet diet with water *ad libitum*. The animals care and handling were in accordance with the world accepted standard guidelines. All animal procedures were approved by an Institutional Review Board of Agricultural and Veterinary Medicine, Qassim University, KSA.

Chemicals Analytical grade chemicals were purchased from Sigma Aldrich, St. Louis, MO, USA and were used for the bioassays in the laboratory.

Methanol extract preparation The collected plant was air dried powdered (300 g) and soaked in methanol (3000 ml) with continuous shaking for 72 h. the methanol extract was filtered, and the residues were re-percolated for three times. The extract was concentrated under reduced pressure. The obtained methanol extract was used for assaying its bioactivity and phytoconstituents. The Voucher specimen was deposited in the Department of Botany, for further reference.

Experimental design:

Rats were randomly divided into 5 equal groups (6 rats each) as follows: Group I (control): Animals received vehicles only [4 ml/kg (0.5 % carboxy methyl cellulose) orally for 15 days and olive oil (3 ml/kg body weight *i.p.*) once every 72 hrs]. Group II (CCl_4): Rats received 30% CCl_4 in olive oil (3 ml/kg body weight *i.p.*) once every 72 hrs. (The hepatotoxic group) [7]. Groups III, IV and V (extract +CCl_4): rats received the plant extract 200, 400 and 800 mg/kg, respectively, orally for 15 days in addition to the CCl_4 injection as previously mentioned. The animals were maintained in their respective groups for 15 days. Twenty-four hours after the last administration period, animals were anesthetized by diethyl ether. Blood samples were taken from the retro-orbital venous plexus using a glass capillary tube after a fast of 12 hrs, centrifuged at 3000 rpm for 10 min and stored at -20°C.

Phytochemical analysis:

Total phenolic and tannin contents

Total phenolic [27] and total tannins [28] of the methanol extracts of *Teucrium oliverianum* were carried out using the Folin-Ciocalteu reagent. The values were stated as gallic acid equivalents per g of extract.

Total flavonoids

Total flavonoid content of the methanol extracts of *Teucrium oliverianum* was determined by a colorimetric method of Zhishen *et al*. [29]. and calculated using a quercetin calibration curve. The results were expressed as quercetin equivalents per g of extract.

Biochemical analysis:

Determination of malondialdehyde (MDA)

The lipid peroxidation in serum was determined as described by Iqbal et al., [30].

Determination of glutathione –*S*- transferase (GST) activity

The activity of GST was determined according to Habig et al.,[31].

Determination of reduced glutathione(GSH)

Reduced glutathione was determined as described by Jollow et al., [32].

Determination of total antioxidant capacity:

The method of Koracevic *et al*. [33] was used to determine total antioxidant capacity.

Evaluation of liver function

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel, [34]. Alkaline phosphatase (ALP) was determined according to the method of Tietz [35].

Histopathological studies

A routine paraffin embedding technique for tissue processing (dehydration in ethanol, clearing in xylene and impregnation in melted wax) was carried out on liver specimens. Sections (5µm thick) were prepared and stained with hematoxylene and eosin (H&E) as described by Suvarna et al., [36], and examined for pathological findings of hepatotoxicity.

Statistical analysis:

Data were analyzed using a One-way analysis of variance (ANOVA) with $p \leq 0.05$. When a significant difference was determined, a comparison of the means using Duncan, multiple range test was carried out [37, 38].

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RESULTS

Phytochemical analysis:

The quantitative phytochemical investigation of *Teucrium oliverianum* illustrated the occurrence of total phenolics, tannins and flavonoids in a different quantity respectively, 29.25±3.10 mg/g, 14.24±0.31 mg/g, and 16.51±0.24 mg/g of methanolic residues (*n*= 5).

Table 1: Quantitative analysis of the methanolic extract of Teucorium oliverianum (mg/g)

Total phenolics	Tannins	Non-tannins phenolics	Total flavonoids
29.25±3.10	14.24±0.31	15.01±0.25	16.51±0.24

Data are expressed as means±SEM.

Liver marker enzymes

The effect of the methanolic extract of *Teucorium oliverianum* in the serum of CCl_4 treated rats is presented in table 2. The administration of CCl_4 30% in olive oil (1 ml/kg body weight *i.p*) every 72 hrs for 15 days significantly induced the serum activities of AST,ALT and ALP. However, the treatment of hepatotoxic rats with the methanolic extract of *Teucorium oliverianum* induced a dose- dependent decrease in the activities of the previously mentioned enzymes.

Table 2: Effect of the methanolic extract of Teucorium oliverianum (T.O) on liver enzymes in rats treated with CCl4

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Control (vehicle)	45.33 ± 2.09^{e}	80.33 ± 1.84^{e}	58.00 ± 1.87^{e}
CCl_4	142.3 ± 1.43^{a}	163 ± 1.47^{a}	157.3 ± 4.40^{a}
T.O (200 mg/kg b.wt. + CCl_4)	120.3 ± 2.24^{b}	135.3 ± 2.01^{b}	134.3 ± 3.11^{b}
T.O (400 mg/kg b.wt.+ CCl_4)	69.6 ± 1.67^{c}	124 ± 3.48^{c}	111.6 ± 3.68^{c}
T.O (800 mg/kg b.wt.+ CCl_4)	59.0 ± 1.22^{d}	88.3 ± 3.68^{d}	80.06 ± 0.94^{d}

Values are expressed as means±SEM.*n*= 6 rats. Means in the same column with different letters are significantly different p≤ 0.05. ALT = alanine aminotransferase, AST= aspartate aminotransferase, ALP= alkaline phosphatase.

Table 3: Effect of the methanolic extract of Teucorium oliverianum (T.O) on serum oxidative stress parameters in rats treated with CCl_4.

Groups	MDA (umol/L)	GSH (umol/L)	GST (U/L)	Total antioxidant capacity (mMol/L)
Control (vehicle)	1.74±0.09c	3.99±0.11a	35.6±2.32c	0.29±0.03a
CCl_4	6.67±0.17a	1.6±0.08d	69±0.70a	0.11±0.01c
T.O (200 mg/kg b.wt. + CCl_4)	5.53±0.18a	2.64±0.16c	63.0±2.94a	0.16±0.01b
T.O (400 mg/kg b.wt.+ CCl_4)	3.93±0.11b	2.78±0.11c	50.6±1.59b	0.18±0.01b
T.O (800 mg/kg b.wt.+ CCl_4)	1.81±0.11c	3.69±0.15a	38.3±0.88c	0.25±0.01a

Values are expressed as means±SEM.*n*= 6 rats. Means in the same column with different letters are significantly different p≤ 0.05. MDA= malondialdehyde, GSH= Reduced glutathione, GST= Glutathione-S-transferase.

Oxidative stress parameters

Table 3 shows the effect of the methanolic extract of *Teucorium oliverianum* on the oxidative stress parameters in rats treated with CCl_4. It was found that administration of CCl_4 significantly elevates the level of MDA and the GST activity, and significantly decreased the level of the reduced glutathione as well as the

antioxidant capacity. Treatment with the methanolic extract of *Teucorium oliverianum* resulted in a significant improvement in these parameters in a dose dependent manner.

Histopathological findings

The microscopic examination of livers from rats given CCl_4 alone showed areas of centrolobular lymphocytic infiltrations near the congested central vein, multifocal areas of necrosis and excess of mononuclear cell infiltrations and aggregations at the portal area (Fig.1). Administration of the methanolic extract of *Teucurium oliverianum* induced a pronounced improvement in the histopathologic lesions in a dose-dependent manner also.

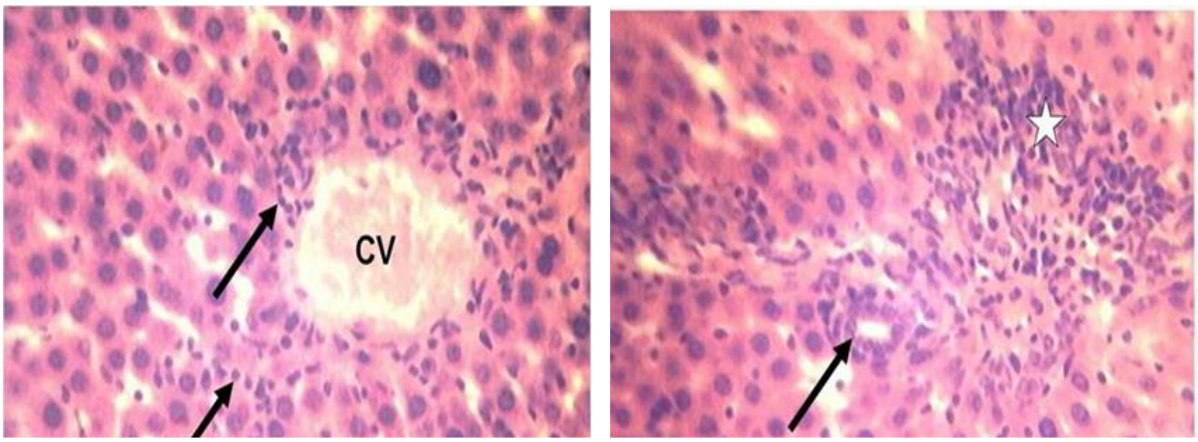

Fig 1: Liver of rats treated with CCl_4 showing areas of centrolobular lymphocytic infiltrations (arrows) near the congested central vein (CV) and excess of mononuclear cell infiltrations and aggregations (white asterisk) at the portal area (arrow). H&E. X 400.

DISCUSSION

The present experiment aimed to investigate the impact of the methanolic extract of *Teucorium oliverianum* on the hepatic toxicity induced by CCl_4 in rats. Our data showed that the administration of CCl_4once every 72 hrs for 15 days significantly induced the serum activities of AST, ALT and ALP indicating liver damage through altering membrane integrity and escaping of these enzymes into circulation. It is well known that CCl_4 toxicity is due to its metabolic activation by cytochrome P450 leading to the generation of trichloromethyl radical ($\cdot CCl_3$) and peroxy trichloromethyl radical ($\cdot OOCCl_3$), which in turn induce lipid peroxidation, responsible for injuries in the liver [5]. The combination of these free radicals with polyunsaturated fatty acids of hepatic cell membrane leads to elevation of thiobarbituric acid reactive substances (TBARSs) concentration with subsequent necrosis [6], and increasing the activity of lysosomal enzymes [7]. These findings were also reported by several authors [39, 40]. Conversely, after administration of the methanolic extract of *Teucorium oliverianum,* there was a significant decrease in the serum activities of AST,ALT and ALP indicating that *Teucorium oliverianum* protects the liver damage induced by CCl_4. Lipid peroxidation is the primary cause of liver damage. In the present experiment, indications of lipid peroxidations included elevation in the levels of MDA and the GST activity and reduced the levels of the antioxidant capacity and diminished GSH. MDA is a marker of lipid peroxidation and a well-known marker of oxidative stress. An increase in free radicals induces the production of MDA remarkably [41]. GSH is a strong scavenger of free radical oxygen species. The declined concentration of GSH gives a sign of GSH utilization in the neutralization of free radicals [42]. Administration of the methanolic extract of *Teucorium oliverianum* restored both GSH and MDA levels. The present study revealed that the methanolic extract of *Teucorium oliverianum* is rich in phenolic compounds, particularly flavonoids and tannins. Flavonoids and tannins represent a major group of antioxidants, which acts efficiently as scavengers conferred of free radicals [43]. A consumption of flavonoids, natural products with variable phenolic structures, was found to associate with a reduced risk of several types of diseases [44]. Also, various flavonoids have shown a hepatoprotective effects, which were linked to their antioxidant activities and obviously reduction of lipid peroxidation[45, 46]. At the same direction, from the pathologic findings, it

was clear that the methanolic extract of *Teucorium oliverianum* alleviated the toxic potentials of CCl_4in a dose-dependent manner.

CONCLUSION

It could be concluded that the hepato-protection conferred by the *Teucorium oliverianum* might be due to its high content of total polyphenols and flavonoids, which act as a powerful free radical scavengers and antioxidants.

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