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Research Article

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STUDY THE POSSIBLE HEPATOPROTECTIVE EFFECT OF DIFFERENT DOSES OF *Ammi majus* SEEDS' EXTRACT AGAINST CCl_4 INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

Liver is considered as the major organ responsible for conducting various metabolic processes and according to it's highly exposed to toxic effect of different xenobiotics predisposing to many types of diseases and disorders. The role of plant with antioxidant activity in the treatment and prevention of chemical-induced liver damage was extensively studies. *Ammi majus* show antioxidant effect their use in diabetic nephropathy and myocardial injury due to the presence of different active constituent such as quercetine, marmesinin, kempefrol and other compounds that inhibit cytochrome p450 such as xanthotoxin bergapten, imperatorin and isoimpinellin. Accordingly, this study was designed to evaluate the hepatoprotective effect of the aqueous solution of ethanolic extract of the *Ammi majus* against carbon tetrachloride (CCl_4) induced liver damage in rats. Eighty adult rats of both sex divided into four groups allocated as follows: Group I- (negative control), rats received D.W 2ml/kg for 14 days. Group II- rats treated with single oral daily dose *Ammi majus* seeds extract 16mg/rat/day alone for 14 days. The animals of groups I and II were sacrificed by anesthetic ether on the day 15. Group III- (positive control) rats received single oral daily dose of 2ml/kg/day D.W. for 14 days and at the day 15, the animals received single oral dose of CCl_4, the animals were sacrificed by anesthetic ether 24 hr after CCl_4 administration. Groups IV (A, B, C, D and E) received either (1mg or 2mg or 4mg or 8mg or 16mg/rat/day), respectively for 14 days of *Ammi majus* ethanolic extract then at the day 15 they received single dose of CCl_4 then sacrificed after 24 hours after CCl_4 administration. Rats' livers were obtained for preparation of tissue homogenate to evaluate MDA & GSH in the hepatic homogenate as indicator of lipid peroxidation. Blood samples were collected by intra-cardiac puncture, and utilized for evaluating serum enzymes activities manifested by aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in addition for assessing total serum Bilirubin (TSB). Analysis of data revealed significant amelioration of oxidative stress in rats pre-treated with different doses of *Ammi majus* extract (4mg,8mg and 16mg/rat/day for 14days) compared to group III of animals intoxicated by CCl_4 as evidenced by lowering MDA contents and elevation of GSH levels in liver tissue homogenate but the levels still significantly different compared to controls. Elevation of serum activities of ALT, AST and ALP, in addition to TSB levels as a results of treatment with toxic dose of CCl_4 was significantly reduced by pre-treatment with different doses of *Ammi majus* extract but the levels still significant different from control. *Ammi majus* extract also attenuated hyperbilirubemia caused by CCl_4 intoxication. From the data obtained from this work, we can conclude that the extract of *Ammi majus* showed protective effect against CCl_4 induced-hepatotoxcity.

Keywords: *Ammi majus*, CCl_4 induced-hepatotoxcity, hyperbilirubemia.

INTRODUCTION

The liver is a key organ in regulating the homeostasis of the body by carrying various essential functions like protein synthesis, storage, metabolism of fat and carbohydrate , detoxification of drugs and toxins, excretion of bilirubin, as well as playing a role in hormones metabolism.[1]

Many chemicals that are inhaled or swallowed can damage the liver among these, are drugs, industrials and pollutants. The severity of liver injury may vary from nonspecific structural and functional change to acute liver failure or chronic injury. Carbon tetrachloride (CCl_4) is a vehicle for many organic compounds, formerly used as fire extinguisher, dry cleaner, grain fumigant and anthelmentic however, its use for these purposes has now been abandoned because safer alternatives are available, but it is still used in fumigation of grain and insecticides.[2] It is activated by cytochrome (CYP 2E1, 2B1 or 1B2 and possibly 3A) to form the trichloromethyl radical $CCl_3{}^{*}$[3], which can either binds to cellular molecule (nucleic acid, protein, and lipid) impairing crucial cellular processes such as lipid metabolism by hypo-methylation of the ribosomal RNA at 2-ortho ribose which results in decreasing protein synthesis, with potential outcome of

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fatty degeneration (steatosis) or reacts with oxygen to form the trichloromethylperoxy radical CCl_3OO^{*}[3,4], a highly reactive species that initiates chain reaction of lipid peroxidation[5]. Natural products research is one of the most promising sources of medicine for the future.[6,7]

Ammi majus, F. Umbelliferea has different names, the Arabic name Khillah, Khillah shytani, English name Bishops weed, Latin and German name Ammi, French name Ammi commun.[8] Its seeds contain different active ingredients namely, xanthotoxin, bergapten, imperatorin, isoimpinellin and marmesinin[9] that may have possible hepatoprotective activity. Thus, this study was designed to evaluate the possible protective effects the alcoholic extract of *Ammi majus* seeds against liver damage induced in rats by CCl_4.

MATERIALS AND METHODS

Plant material

Dried seeds of Iraqi *Ammi majus L.* obtained from Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad after taxonomic identification performed by Iraqi National Herbarium.

Experimental model

Eighty albino rats of both sexes weighing 200-250gm were utilized in this study. They were obtained from and maintained in the Animal House of the College of Pharmacy, University of Baghdad under the conditions of controlled temperature. Animals were fed commercial pellet and tap water in free access *ad libitum.*

Methodology

The alcoholic extract of *Ammi majus L.* seeds was prepared according to the method of B Meier.[10]

To study the possible protective effect of different doses of *Ammi majus* extract against CCl_4-induced liver damage, rats were allocated as follows: **Group I**– 10 rats treated with single oral dose of 2ml/kg/day D.W for 14 days. The animals were sacrificed by anesthetic ether on the day 15. The group served as control. **Group II**- 10 rats treated with single oral dose of *Ammi majus* seeds extract 16mg/rat/day alone for 14 days. The animals were sacrificed by anesthetic ether on the day 15.

Group III – 10 rats received single oral dose of 2ml/kg/day D.W. for 14 days. At the day 15, the animals received single dose of CCl_4 (99%) (2ml of a mixture of 1:1 V/V in corn oil /kg/day) orally to induce liver damage.[11] The animals were sacrificed by anesthetic ether 24 hr after CCl_4 administration. The group served as positive control. **Group IV-** 50 rats were used to study the possible protective effects of different doses of *Ammi majus* seeds extract, and subdivided as follows: **Group IV**A- 10 rats treated with single oral dose of *Ammi majus* seeds extract 1mg/rat/day started 14 days prior to treatment with CCl_4. **Group IVB-** 10 rats treated with single oral dose of *Ammi majus* seeds extract 2mg /rat/day started 14 days prior to treatment with CCl_4. **Group IVC-** 10 rats treated with single oral daily dose of *Ammi majus* seeds extract 4mg/rat/day started 14 days prior to treatment with CCl_4. **Group IVD-** 10 rats treated with single oral daily dose of *Ammi majus* seeds extract 8mg/rat/day started 14 days prior to treatment with CCl_4. **Group IVE-** 10 rats treated with single oral daily dose of *Ammi majus* seeds extract 16mg/rat/day started 14 days prior to treatment with CCL_4. The animals of groups IVA, IVB, IVC, IVD and IVE were sacrificed by anesthetic ether on the day 16.

After the animals have been sacrificed by anesthetic ether, liver were quickly excised, homogenized and utilized for the estimation of MDA contents[12] and GSH levels[13].

Blood was collected by intra-cardiac puncture and then centrifuged at 3000 rpm for 15 minute to obtain serum, which was used for the estimation of ALT, AST[14] and ALP[15] as parameters of liver function tests and total serum bilirubin as excretory function test[16].

The hepatoprotective activity can be calculated according to the formula of Singh co workers[17]:

$$Hepatoprotective\ activity\ \% = \frac{1 - (A\ majus + CCl_4 - C)}{CCl_4 - C} \times 100$$

Where, *A. majus*+CCl_4, CCl_4 and C are measurable variable biochemical parameters estimated in rats treated with *A. majus* plus CCl_4, CCl_4 alone and control groups(C), respectively.

Statistical analysis

The significance of differences between the mean values was calculated using unpaired Student's-test. Multiple group comparisons were made using analysis of variance (ANOVA). *P-values* less than 0.05 were considered significant for all data showed in our results.

RESULTS AND DISCUSSION

In group of rats treated with CCl_4, the results of table **1** showed, a significant increase ($p<0.05$) in the hepatic contents of MDA compared with control rats. Moreover, treatment of rats with either 1mg or 2mg/rat/day of *Ammi majus* extract for 14 days prior to orally-administered CCl_4, showed non-significant ($p>0.05$) decline in the hepatic contents of MDA compared to CCl_4-treated animals and a significant increase ($P<0.05$) compared to control group.

Table **1** also showed significant decline in hepatic MDA contents in rats treated with either (4 or 8 or 16mg/rat/day) of *Ammi majus* extract for 14 days prior to CCl_4 compared to CCl_4 treated rats but still significant ($P<0.0\%$) high levels concerning hepatic MDA contents compared to control.

Rats treated with 16 mg/rat/day of *Ammi majus* extract alone showed a non-significant difference ($P>0.05$) in the MDA contents of liver tissue homogenate compared to control group (Table **1**).

Table 1. Effects of treatment with different doses of *Ammi majus* extract prior to CCl_4 on the hepatic contents of MDA and GSH levels compared to CCl_4-treated and control groups.

Animal group	N	MDA(nmole/g)	GSH (µg/g)
Group I	10	108.91±10.19[a]	11.87±2.95[a]
Group II	10	117.1±15.7[a]	10.87±2.95[a]
Group III	10	413.68±7.31[b]	2.09±0. 63[b]
Group IV-A	10	409.2±5.20[b]	2.1±0.23[b]
Group IV-B	10	407.62±5.64[b]	2.4±0.89[b]
Group IV-C	10	295.95±10.04[c]	4±0.36[c]
Group IV-D	10	204.21±16.12[d]	6.06±0.38[d]
Group IV-E	10	140.11±9.98[e]	9.04±0.32[e]

Each value represents mean ± SD; Values with non-identical superscripts (a, b, c, d & e) considered significantly different ($p< 0.05$); N= number of animals; Group I= Control, Group II=animals treated with 16mg/rat/day, Group III=CCl4-treated group, Group IV-A=animals pretreated 1mg/rat/day,_Group IV-B= animals pretreated 2mg/rat/day, Group IV-C=animals pretreated 4mg/rat/day, Group IV-D=animals pretreated 8mg/rat/day, Group IV-E=animals pretreated with 16mg/rat/day.

Concerning the effect of different doses of *Ammi majus* extract on hepatic GSH level, the result of table **1** showed that there was a significant ($P<0.05$) decrease in the level of hepatic GSH in CCl_4 treated rats compared to control group.

Pre-treatment of rats with either 1mg or 2mg/rat/day of *Ammi majus* extract for 14 days showed nonsignificant increase (P>0.05) in the levels of hepatic GSH compared to CCl_4 treated rats; while there was a significant decrease (P<0.05) in such level compared to control. Moreover, Table **1** showed significant increase (P<0.05) in the levels of hepatic GSH in groups of animals treated with either (4 or 8 or 16mg) /rat/day of *Ammi majus* extract for 14 days prior to CCl_4 compared to CCl_4 treated rats and there were still significant (P<0.05) lower levels of hepatic GSH obtained from this study compared to control group. Additionally, rats treated with 16 mg/rat of *Ammi majus* extract for 14 days alone showed a non-significant differences (P>0.05) in the level of hepatic GSH compared to control group.

In group of rats treated with CCl_4, the results of table **2** showed significant (p<0.05) increase in the serum activity of AST compared to control rats. Rats treated with either 1mg or 2mg/rat/day of *Ammi majus* for 14 days prior to CCl_4, showed non-significant decrease (P>0.05) compared to CCl_4-traeted rats; while there was a significant increase (P<0.05) in the serum activity of AST compared to control group. Table **2** also showed a significant decline (P<0,05) in the serum activity of AST in rats treated with either (4mg or 8mg or 16mg) of *Ammi majus* extract /rat/day for 14 days prior to CCl_4 administration compared to CCl_4-treated rats, but still significant (P<0.05) higher activity of serum AST were observed in table **2** compared to controls. Rats treated with 16 mg/rat of *Ammi majus* extract for 14 days alone showed a non-significant differences (P>0.05) in the serum activity of AST compared to control group.

Table 2. Effects of treatment of rats with different doses of *Ammi majus* extract on the serum activities of AST and ALT prior to CCl_4 compared to CCl_4-treated and control groups.

Animal group	N	AST U/L	ALT U/L
Group I	10	15.6±2.91[a]	11.36±4.27[a]
Group II	10	15.58±2.85[a]	15.1±2.65[a]
Group III	10	65.6±7.79[b]	67±8.7[b]
Group IV-A	10	62.6±3.57[b]	63.56±1.66[b]
Group IV-B	10	59.2±1.64[b]	61.18±1.92[b]
Group IV-C	10	45.8±3.11[c]	46.6±2.4[c]
Group IV-D	10	34.6±2.3[d]	35.8±3.03[d]
Group IV-E	10	27.2±1.48[e]	25±2[e]

Each value represents mean ± SD; Values with non-identical superscripted (a, b, c, d& e) considered significantly different (p<0.05); N= number of animals; Group I= Control; Group II=animals treated with 16mg/rat/day; Group III=CCl4-treated group; Group IV-A=animals pretreated 1mg/rat/day; Group IV-B= animals pretreated 2mg/rat/day; Group IV-C=animals pretreated 4mg/rat/day; Group IV-D=animals pretreated 8mg/rat/day; Group IV-E=animals pretreated with 16mg/rat/day.

In group of rats treated with CCl_4, the results of table **2** showed a significant (p<0.05) increase in the serum activity of ALT compared to control rats. Treatment of rats with either 1mg or 2mg/rat/day of *Ammi majus* extract for 14 days prior to CCl_4 showed non-significant decrease (P>0.05) compared to CCl_4-treated rats but a significant (P<0.05) increase in the respected serum enzyme activity were seen compared to control group as shown in table **2**. Additionally, table **2** showed significant (P<0.05) decline in the serum activity of ALT in groups of rats treated with either (4 or 8 or 16mg) of *Ammi majus* extract /rat for 14 days prior to CCl_4 compared to CCl_4-treated rats, but still significant (P<0.05) higher serum activity of ALT was observed compared to control group. Rats treated with 16mg/rat/day of *Ammi majus* extract for 14 days alone showed a non-significant (P>0.05) difference in the respected serum enzyme activity compared to control group.

In group of rats treated with CCl_4, table **3** showed a significant increase (p<0.05) in the serum activity of ALP compared to control rats. Treatment of rats with either 1mg or 2mg/rat/day of *Ammi majus* extract for 14 days prior to CCl_4, showed non-significant (P<0.05) decrease in the serum ALP activity compared to CCl_4-treated rats with a significant (P<0.05) increase was seen in the respected enzyme activity compared to control group. Moreover, Table **3** showed significant (P<0.05) decline in the serum activity of ALP in groups of animals treated with either (4 or 8 or 16 mg) of *Ammi majus* extract /rat for 14 days prior to CCl_4 compared to CCl_4 treated rats, but still significant (P<0.05) higher serum activity of the respected enzyme were observed compared to control group. Rats treated with 16 mg/rat/day of *Ammi majus* extract for 14 days alone result in a non-significant (P>0.05) difference in the serum activity of ALP compared to control group.

Total serum bilirubin level (TSB) was shown to be significantly (P<0.05) increased in CCl_4 treated rats compared to control rats. Treatment of rats with either 1mg or 2mg/rat/day of *Ammi majus* extract for 14 days prior to CCl_4, showed non-significant (P>0.05) decrease in the serum levels of TSB compared to CCl_4-treated rats and still significant (P<0.05) increase in the respected serum enzyme activity compared to control group. Table **3** showed significant (P<0.05) decline in the level of TSB in groups of rats treated with either (4 or 8 or 16mg) of *Ammi majus* extract /rat/day for 14 days prior to CCl_4 compared to CCl_4-treated rats and still significant (P<0.05) higher levels of TSB were seen compared to control group. Rats treated with 16mg/rat/day of *Ammi majus* extract for 14 days alone showed a non-significant (P>0.05) difference in the level of TSB compared to control group.

Table 3. Effects of treatment with different doses of *Ammi majus* extract prior to CCl_4 on serum activity of ALP and TSB levels compared to CCl_4-treated and control groups.

Animal group	N	ALP(Ku/dL)	TSB (mmole/L)
Group I	10	11.62±2.14[a]	0.45±0.22[a]
Group II	10	13.65±2.1[a]	0.51.±0.39[a]
Group III	10	71.8±7.19[b]	4.76±0.21[b]
Group IV-A	10	66±4.52[b]	4.18±0.72[b]
Group IV-B	10	65.6±0.89[b]	3.96±0.6[b]
Group IV-C	10	45.2±2.86[c]	3.2±0.89[c]
Group IV-D	10	38.5±1.87[d]	2±0.95[d]
Group IV-E	10	24.6±1.3[e]	0.82±0.39[e]

Each value represents mean ± SD; values with non-identical superscripted (a, b, c, d & e) considered significantly different (p<0.05); N= number of animals; Group I= Control; Group II=animals treated with 16mg/rat/day, Group III=CCl4-treated group, Group IV-A=animals pretreated 1mg/rat/day,_Group IV-B= animals pretreated 2mg/rat/day, Group IV-C=animals pretreated 4mg/rat/day, Group IV-D=animals pretreated 8mg/rat/day, Group IV-E=animals pretreated with 16mg/rat/day.

The percent of hepatoprotective activity of different doses of *Ammi majus* extract treated 14 days prior to CCl_4 was illustrated in table **4**.

Table 4. The (%) of hepatoprotective activity of treatment with different doses of *Ammi majus* extract prior to CCl_4 for all biochemical parameters.

Group	MDA %	GSH %	ALT %	AST %	ALP %	TSB %
Group IVA	1.4	0.056	7.5	6	9.6	13.48
Group IVB	2.38	2.86	11.7	12.8	10.3	18.59
Group IVC	38.6	17.86	37.5	39.6	44	36.2
Group IVD	68.7	37	56.6	62	55	64.2
Group IVE	89	65	75.8	76.8	78.4	91.3

Group IVA=animals pretreated 1mg/kg/day; Group IVB=animals pretreated 2mg/kg/day; Group IVC= animals pretreated 4mg/kg/day; Group IVD=animals pretreated 8mg/kg/day; Group IVE= animals pretreated 16mg/kg/day.

The results obtained from this work clearly demonstrated the state of oxidative stress induced in hepatic tissues by CCl_4, manifested by the elevation of MDA content in the tissue homogenate and is associated with severe depletion of GSH content in hepatic tissue homogenate (Table **1**); these results are consistent with those observed by others.[18]

The reductions in glutathione contents, the important water-soluble anti-oxidant, which can directly scavenge reactive species produced during metabolism of CCl_4 in the hepatocytes, worsen the state of oxidative stress and as much as more GSH were consumed for conjugation of metabolites, the redox potential of the tissue was impaired.[19] As a result of increased lipid peroxidation and subsequent degradation of biomembranes, the permeability of the plasma membranes was severely affected, and may lead to leakage of AST and ALT and increasing in their activities in the serum. This picture was observed in the CCl_4 treated rats compared to controls (Table **2**) and seems to be consistent with those obtained by others.[20] The high values of serum activities of both cytosolic enzymes (AST and ALT) may be attributed to the alteration in the structure and function of the hepatocellular membrane as a result of binding of toxic metabolites of CCl_4 to the lipid and protein components of the membrane.[21] Similarly, the serum activity of alkaline phosphatase (ALP) that is present in the lining membrane of the hepatocytes was also increased in the CCl_4 treated rats compared to control animals, and the results of our study (Table **3**) are consistent with other investigators.[20] Total serum bilirubin (TSB) was increased significantly in CCl_4 treated rats (Table **3**) due to hepatic cellular damage which leads to disability of liver cells to metabolize and excrete bilirubin.[22]

A great number of medicinal plants contain flavonoids which have been reported by many authors as having antibacterial[23], anti-inflammatory and antineoplastic actions[24] in addition to antioxidant activity through scavenging lipid peroxyl radicals[25].

The antioxidant properties of *Ammi majus* extract may be attributed to the presence of quercetine.[26] It has been shown that chronic administration of an oral dose of quercetine (10mg/kg/day) for 5 weeks reduce blood pressure, increase glutathione activity and reduce both plasma and hepatic malondialdehyde (MDA) levels.[27]

It was shown that the stimulation or inhibition of cytochrome p-450 (CYP2E1) lead to induction or inhibition of CCl_4-induced hepatotoxicty through increase or decrease, respectively the formation of CCl_3^*.[28]

The GSH level was preserved by *Ammi majus* extract treatment against CCl_4 toxicity and it could be one of the important factors which prevent lipid peroxidation seen in rats treated with CCl_4. (Table **2**)

Our results are in agreement with the study performed by Said AM[29], using fenugreek seeds (FGS) extract as a hepatoprotective agent against CCl_4 toxicity in a dose of 347.282mg/kg/day for 30 days. The extract lowers the level of MDA and elevates GSH due to the antioxidant action of its active constituent, quercetine that counteracts and scavenges the intermediate free radicals generating by CCl_4 during the peroxidation.

The level of serum bilirubin was increased abnormally during CCl_4 toxicity due to the defect in their metabolism and excretion making them retained in hepatic tissue as observed in table 3 and the results were in agreement with that observed by others.[30]

Regarding cholestasis, a form of liver injury results from either decrease in the volume of bile formed or an impaired secretion of specific solutes into bile is characterized biochemically by a sharp elevation in serum activities of enzymes localized to bile ducts, particularly alkaline phosphatase.[31]

CONCLUSION

The results of this study clearly demonstrated that treatment of rats with different doses of *Ammi majus* seeds extract prior to CCl_4 produce hepatoprotective effects against CCl_4-induced liver damage and their effects were in dose-dependent manner.

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