International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Hepatoprotective activity of *Ammi majus* on CCL_4 Induced Albino Mice

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International Journal of Science and Research Archive, 2023, 09(02), 315–324

Publication history: Received on 07 June 2023; revised on 16 July 2023; accepted on 18 July 2023

Article DOI: https://doi.org/10.30574/ijsra.2023.9.2.0449

Abstract

This study was amid to evaluate the possible protective effects of the water and alcoholic extract of *Ammi majus* seeds against liver damage induced in mice by CCL_4. The plant was collected from Baghdad markets, water and alcoholic extract was prepared and used at one concentration for injecting six groups of mouse, the first one is control negative that are mice was not injected, the second was positive control which is the mice was injected with CCL_4, the third group had mice that injected with water extract, the fourth group included mice that injected with CCL_4 and water extract, the fifth group had mice that injected with alcoholic extract , the last group includes mice injected with CCL_4 and water extract. Injected intraperitoneally (0.1 ml) for seven days as single dose\day, Finally mice were sacrificed at day 8 for laboratory assessments. Blood and liver tissue were collecting , blood used for administration of liver enzyme (GOT, GPT, ALP) and liver tissue used for assessment of the hepatoprotective effect of *Ammi majus* extracts.

The results show that for the liver enzymes, the GPT activity was 199±2.1 Unit/ L in control negative (Untreated mice); the GPT value decrease to 76±5.2 Unit/L in positive control suggesting that there was positive damage upon its treatment. Interestingly, it got normal value of GPT which was 142±2.5 Unit/L after its treatment with 100 mg/Kg water extract of *Ammi majus* extract and about same value 145±2.5 Unit/L when injected with water extract and CCL_4, also it got decrease value 154±2.5 when injected the alcoholic extract of *Ammi majus* and highly increased value 203±2.5 Unit/L with injection of alcoholic extract and CCL_4. While GOT activity is checked in order to see the hepatoprotective role of *Ammi majus* plant extract, the GOT value of CCL_4 injected mice had shown high level 67.1±1.2 Unit/L compared with those untreated mice was 33±4.5 Unit/L, while upon the treatment with *Ammi majus* water extract, it got a in GPT about normal value which was 40.3±3 Unit/L 100 mg/Kg, and highly decrease value 16±2.5 Unit/L when injected water extract of *Ammi majus* and CCL_4, also it got increase value 50±2.5 when injected the alcoholic extract of *Ammi majus* and few decrease value 28±2.5 Unit/L with injection of alcoholic extract and CCL_4. Also, ALP activity was the mice that were injected with CCL_4 also produced high levels of ALP 132.3±2.5 Unit/L compared with those untreated mice that was 93.6±4 Unit/L. But, upon the treatment with *Ammi majus* water extract, it got a in ALP about normal value which was 90.3±3 Unit/L with a dose of 100 mg/Kg, and increase value 102±2.5 Unit/L when injected with water extract of *Ammi majus* and CCL_4, also it got decrease value 61±2.5 when injected the alcoholic extract *Ammi majus* and highly decrease value 43±2.5 Unit/L with injection of alcoholic extract and CCL_4.

The Histopathological Evaluation of liver and further assessment of the hepatoprotective effect of *Ammi majus* water and alcoholic extracts, the results show that, the section of normal liver structure, which consists of central vein, surrounded by hepatocyte cells without any changes, while section of liver tissue in mouse treated with CCL_4 as a control positive showing congestion, degenerative and necrosis of paranchymal tissue cells; with mild cells inflammation. In addition, section of a liver tissue in mouse treated with water extract of *Ammi majus*, section of liver showing normal histological structure and section of a liver tissue in mouse treated with water extract of *Ammi majus* and CCL_4, Section showing look like normal histological structure appearance but with mild dilatation of sinusoid and no fatty changes

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seen. On the contrary, section of a liver tissue in mouse treated with alcoholic extract, section of liver showing congestion evident depletion of glycoprotein granules inside the hepatocyte cells and Section of a liver tissue in mouse treated with Section of a liver tissue in mouse treated with alcoholic extract of *Ammi majus* and CCL_4, section showing congestion ,sinusoidal dilation, with few hepatic cells showing fatty changes.

From the result above it was concluded that the water extract give a good protection activity for liver enzyme and histology while the alcoholic extract was toxic, harmful, toxic and not produce productive activity for liver enzyme and histology.

Keywords: *Ammi majus*; Medicinal Plant; Hepatoprotective; Antioxidant; Enzyme.

1. Introduction

Coumarins and flavonoids are both secondary metabolic heterocyclic organic phytochemicals, which are very important phytochemicals for the formulation of drugs due to their medicinal values. Recently, the literature showed that both the phytochemicals have been extensively used as synthetic antioxidants to foodstuffs. There is some extensive curiosity in protective medicine and in food manufacturing in the expansion of natural antioxidants found from plant sources, especially herbal plants (Djilas *et al*., 2003).

The liver is a key organ in regulating the homeostasis of the body by carrying various essential functions.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 (Recknagel *et al.,* 1989, Weber *et al*., 2003).

Ammi majus is considered as a wild vegetable *plant* which is used traditionally to treat different ailments. The roots, leaves, stems, fruits and their paste of the selected plant are equally used traditionally to treat particular diseases (Satrani *et al*., 2004 , Abdul-Jalil *et al*., 2010).

A. majus is considered as a wild vegetable plant which is used traditionally to treat different ailments. The roots, leaves, stems, fruits and their paste of the selected plant are equally used traditionally to treat particular diseases (Satrani *et al*., 2004 , Abdul-Jalil *et al*., 2010).

Since ancient times, scientists are working continuously on the various parts of the selected plant species related to phytochemicals and pharmacological activities for medicinal uses (Rose, 1992) [Based on pharmacological activities, the researchers are working continuously on different parts as well as the different polarities of extracts of the selected plant and isolate and characterized some pharmacologically active phytochemicals which can be used as remedy to treat diseases (Satrani *et al* ., 2004).

Our group is trying to work on that selected plant species to isolate the bioactive phytochemicals that can be used to treat different diseases.

2. Methods

2.1. Plant Collection

The seeds of Plant were collected from the local market in Baghdad in December , 2020. The plant was previously identified by Iraqi center of herbs then it homogenized to fine powder .

2.2. Plant extraction(water and Alcoholic extract)

For water extract the powder seeds of plant (10 g) were added to 100 ml of distilled water in cleaned flask, put in shaker incubator for 24 h at 37C. then the mixture was filtered by gauze in to glass tube , centrifuged at 500 rpm for 10 min, the upper phase was filtered by filter paper then distribute it in sterile petry dishes and put in oven at 60 C for about 5 day utile obtained dry scaled of the extract (chanda and Parekh, 2007).Then put the extract in sterile sealed tube and keeping in freezing at -20°C until used. For alcoholic extract the seeds of plant (20 g) were extracted with 250 ml of methanol (70%) by soxhlet extraction apparatus for 6 hours at 55°C (10). After that the extract filtered and evaporated by using rotary evaporator to dry extract and stored at 4°Cuntil used (Harborn *el al*., 1975).

2.3. Plant Tested Doses

The selection of these doses which used in this research were (10%) of LD50 (20 mg /kg) which determined in according to EFSA journal (2008).

2.4. Experimental animals

Twenty four adult males' albino mice were used. Their weights at the beginning of experiment were (22- 28g). These mice which were obtained from General Company of veterinary medicine, maintained in well-conditioned room, on special pellets regime and water. The animals were separated into six groups as below :

- Group 1: mice were administrated with D.W (negative control negative group 4 animals).
- Group 2: mice were administrated with water extract 20 mg/kg of plant extract (4 animals).
- Group3: mice were administrated water extract 20 mg/Kg and CCL_4 2o mg/kg (4 animals).
- Group4: mice were administrated with alcoholic extract 20 mg/kg of extract (4 animals).
- Group5 : mice were administrated with 0.1 ml of alcoholic extract 20 mg/kg and CCL_4 20 mg/kg (4 animals).
- Group 6: mice were administrated with CCL_4 20 mg/ml as control positive (4 animals).

In order to repair these doses, the water plant extract dissolved in distilled water directly, while alcoholic plant extract dissolved in a few drops of DMSO and then completed by distilled water to reach the required doses. In addition the CCL_4 dissolved in virgin olive oil. Injected intraperitoneally (0.1 ml) for seven days as single dose\day, Finally mice were sacrificed at day 8 for laboratory assessments.

2.5. Determinations of Hepatoprotecive effects

For Hepatoprotective determinations, the parameters of assessment were ALT, AST and ALP enzymes in serum, The activity of enzyme of ALT was determined in mouse serum according to (Reitman and Frankel, 1957), as in GOT determination using a commercial kit (Randox Company). The enzyme activity of Aspartate Amino-Transferees (GPT) was calculated in mouse serum according to evaluation method of (Gometi *et al*., 2014). For this purpose, a commercial kit (Randox Company) was used.

The ALP enzyme was measured in mouse serum using a specific kit manufactured by Bio Merieux Company and the greatest traditional way used is that of (Camargo and Martinez, 2007)

2.6. Histopathological Study

Samples were obtained & cut into small pieces (2×2×2 mm.) then pre-fixed in 2.5% gluteraldehyde diluted in phosphate buffer PH (7.4). After that specimen were rinsed in the same buffer for several times and left in PBS for 12 hrs, and the procedure of (Dogan and Celik,2012) was followed to prepare histopathological sections.

3. Results and discussion

3.1. Effect of water extract and alcoholic extract on liver enzymes (GOT, GPT, Alkaline Phosphatase)

Here, we would like to refer that we injected the mice with CCL_4 before their treatment with our plant extract in order to produce certain damage in liver function.

3.1.1. GPT activity

The results had shown that the GPT activity was 199±2.1 Unit/ L in control negative (Untreated mice) while, upon its injection with CCL_4 as a positive control; the GPT value decrease to 76±5.2 Unit/L, suggesting that there was positive damage upon its treatment. Interestingly, it got normal value of GPT which was 142±2.5 Unit/L after its treatment with 100 mg/Kg of water extract of *Ammi majus* extract and about same value 145±2.5 Unit/L when injected with water extract and CCL_4, also it got decrease value 154±2.5 when injected the alcoholic extract *Ammi majus* and highly increased value 203±2.5 Unit/L with injection of alcoholic extract and CCL_4. As shown in Table (1).

Table 1 The values of GPT level of liver in control negative (untreated), control positive (**CCL_4**), water extract *Ammi majus* and water extract with CCL2, and alcoholic extract of *Ammi majus* and alcoholic extract with **CCL_4**

Liver enzyme	**Negative control Unit/L**	**Positive control CCL_4 Unit/L**	**Water extract of *Ammi majus* Unit/L**	**Water extract of *Ammi majus* and CCL_4 Unit/L**	**Alcoholic extract of *Ammi majus* Unit/L**	**Alcoholic extract of *Ammi majusa*nd CCL_4 Unit/L**
GPT	199±2.1	76±5.2	142±2.5	145±2.5	154±2.5	203±2.5

It was found that from the result that the water extract, water extract with CCL_4 and alcoholic extract are given a good protection effect for GPT enzyme level while the alcoholic extract with CCL_4 is not give protection effect for enzyme level.

3.1.2. GOT activity

GOT activity is another parameter checked in order to see the hepato-protective role of *Ammi majus* plant extract. In table (3-2), the GOT value of CCL_4 injected mice had shown high level 67.1±1.2 Unit/L compared with those untreated mice was 33±4.5 Unit/L, while upon the treatment with *Ammi majus* water extract, it got a in GPT about normal value which was 40.3±3 Unit/L 100 mg/Kg, and highly decrease value 16±2.5 Unit/L when injected with water extract of *Ammi majus* and CCL_4, also it got increase value 50±2.5 when injected the alcoholic extract *Ammi majus* and few decrease value 28±2.5 Unit/L with injection of alcoholic extract and CCL_4 (table 2).

Table 2 The values of GOT level of liver in control negative(untreated), control positive (**CCL_4**), water extract *Ammi majus* and water extract with CCL2, and alcoholic extract of *Ammi majus* and alcoholic extract with **CCL_4**

Liver enzyme	**Negative control Unit/L**	**Positive control CCL_4 Unit/L**	**Water extract of *Ammi majus* Unit/L**	**Water extract of *Ammi majus* and CCL_4 Unit/L**	**Alcoholic extract of *Ammi majus* Unit/L**	**Alcoholic extract of *Ammi majusand* CCL_4 Unit/L**
GOT	33±4.5	67.1±1.2	40.3±3	16±2.5	50±2.5	28±2.5

From the result above it was found that no good effect for protection level of GOT enzyme with water extract while alcoholic extract have good effect that's mean the alcoholic extract helped in reregulation of liver activity upon the reduction of GOT in CCL_4 mice.

3.1.3. ALP activity

The mice that were injected with CCL_4 also produced high levels of ALP 132.3±2.5 Unit/L compared with those untreated mice that was 93.6±4 Unit/L. But, upon the treatment with *Ammi majus* water extract, it got a in ALP about normal value which was 90.3±3 Unit/L with a dose of 100 mg/Kg, and increase value 102±2.5 Unit/L when injected with water extract of *Ammi majus* and CCL_4, also it got decrease value 61±2.5 when injected the alcoholic extract *Ammi majus* and highly decrease value 43±2.5 Unit/L with injection of alcoholic extract and CCL_4 (table 3).

Table 3 The values of ALP level of liver in control negative(untreated), control positive (**CCL_4**), water extract *Ammi majus* and water extract with CCL2, and alcoholic extract of *Ammi majus* and alcoholic extract with **CCL_4**.

Liver enzyme	**Negative control Unit/L**	**Positive control CCL_4 Unit/L**	**Water extract of *Ammi majus* Unit/L**	**Water extract of *Ammi majus* and CCL_4 Unit/L**	**Alcoholic extract of *Ammi majus* Unit/L**	**Alcoholic extract of *Ammi majus and* CCL_4 Unit/L**
ALP	93.6±4	132.3±2.5	90.3±3	102±2.5	61±2.5	43±2.5

From the result it was shown that water extract and water extract with CCL_4 provide a protection effect for ALP level while there is decrease in this enzyme when treated with alcoholic extract and alcoholic extract and CCL_4.

From the results mentioned above which explain the important role of *Ammi majus* plant extract (water and alcohol)in controlling the regulation of liver function that were represented by GPT, Got and ALP enzymes, we should look for the mechanism or any explanation behind that positive effect. To answer this question, we treated the mice with both of our plant extract and CCL_4 together in order to check if this reduction in enzymatic activity that came from plant treatment is due to its ability to interact with the CCL_4 or not. Regarding GPT activity, the dual treatment with CCL_4 and water *Ammi majus* plant extract had shown positive increase in its values which were 142±2.5 and 203±2.5 Unit/L compared with the group which was treated with CCL_4 alone. while this result was confirmed by GOT values that produce decrease in its activity upon dual treatment which were 16±2.5 and 28±2.5Unit/L compared with CCL_4 treatment alone. Again, ALP activity was down regulated significantly 102±2.5and 43±2.5Unit/ L if we compare it with single treatment of CCL_4. So, we can conclude that the dual treatment of mice with CCL_4 and *Ammi majus* produced a fundamental increase in GPT and reduction in Got & ALP activity.

Liver is the major site of detoxification and the primary target of drug exposure in the body. High levels of drugs cause various hepatic disorders by producing pro-oxidants/reactive oxygen species (ROS), which are able to induce cellular damage in a variety of ways by affecting the cellular biomolecules, such as lipids, DNA and proteins (Ziech *et al*., 2010). The hepatotoxicity induced by CCL_4 is mainly due to its metabolite CCl_3, which is a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids. In the presence of oxygen, lipid peroxides are produced, leading to liver damage, which is characterized by fatty liver, cirrhosis and necrosis (Zeashan *et al*., 2008).

In addition, in CCL_4 induced hepatotoxicity, the extent of hepatic damage is assessed by the increased level of cytoplasmatic enzymes (ALT, AST and ALP), which leads to leakage of large quantities of the enzymes into the blood circulation and could be regarded as an index of the liver parenchymal cells damage (Shankar *et al*., 2008). Hepatocellular necrosis and liver injury leads to elevation of these serum marker enzymes, which are released from the liver into blood (Rezende *et al*., 2014). The present study revealed a significant increase in the activities of GPT, and decrease in GOT and ALP upon exposure to CCL_4, indicating considerable hepatocellular injury. Clinically, the general strategy for prevention and treatment of the CCL_4-induced hepatotoxicity includes reducing the production of reactive metabolites (Wong *et al*., 2012), increasing evidence indicates that oxidative stress causes organ injury and carcinogenesis (Kucharská *et al*., 2004, Cheng *et al*., 2013).

Treatment of rats with different doses of *A. majus* seeds' extract could cause hepatoprotective effects against CC14-induced liver damage, in a dose-dependent fashion(Mutlag *et al*., 2011).

3.2. Histopathological Evaluation of Liver

For a further assessment of the hepatoprotective effect of *Ammi majus* water and alcoholic extracts, the liver of the six groups in the present experiment was examined blindly by the histpathologist Professor Dr. Salim R. Hamoudi (Department of Pathology, College of Medicine, University of Baghdad). The results of examination are presented for each treated group of mice under represented pictures (Figures 1,2,3,4,5,6,).

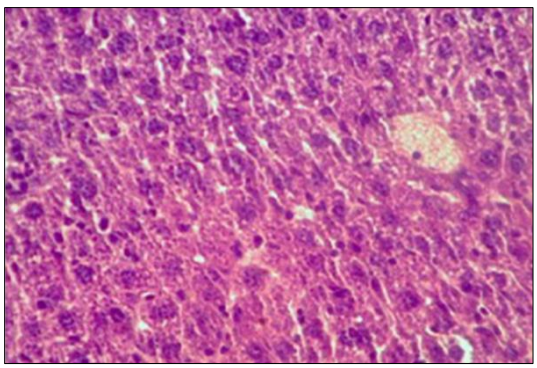

Figure 1 Section of a liver tissue in mouse as a negative control, section of normal liver structure, which consists of central vein, surrounded by hepatocyte cells (400X; H and E)

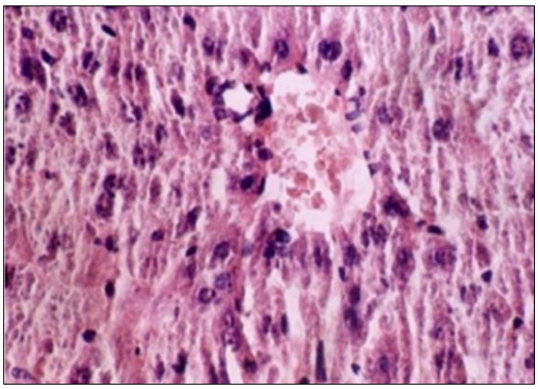

Figure 2 section of liver tissue in mouse treated with **CCL_4** showing congestion, degenerative and necrosis of paranchymal tissue cells; with mild cells inflammation as a control positive (H&E, x400)

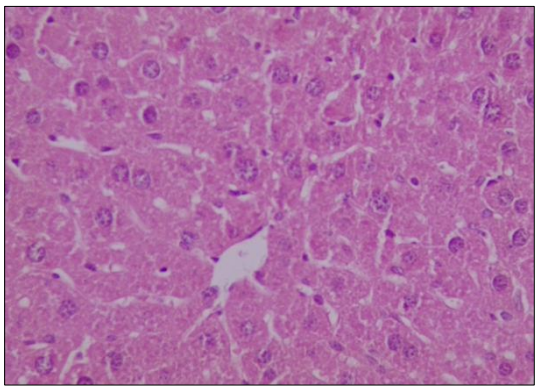

Figure 3 Section of a liver tissue in mouse treated with water extract of *Ammi majus*, Section of liver showing normal histological structure (200X; H and E)

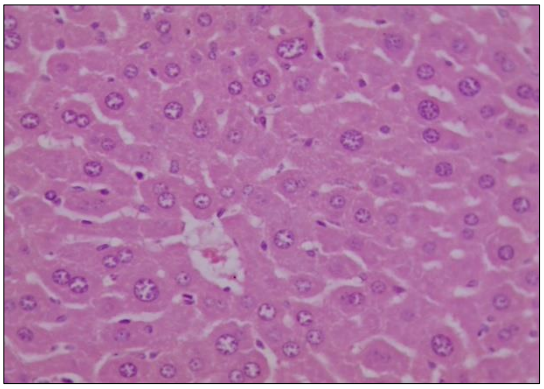

Figure 4 Section of a liver tissue in mouse treated with water extract of *Ammi majus* and **CCL_4**, Section showing look like normal histological structure appearance but with mild dilatation of sinusoid and no fatty changes seen (200X; H and E)

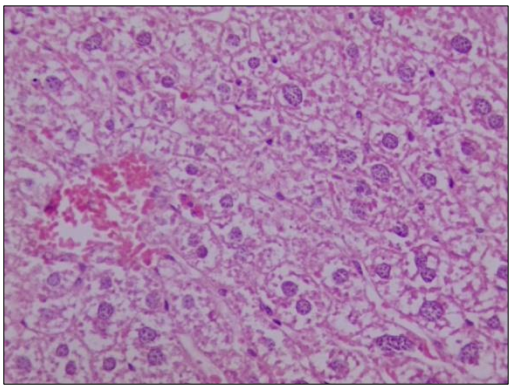

Figure 5 Section of a liver tissue in mouse treated with alcoholic extract, section of liver showing congestion evident depletion of glycoprotein granules inside the hepatocyte cells (200X; H and E).

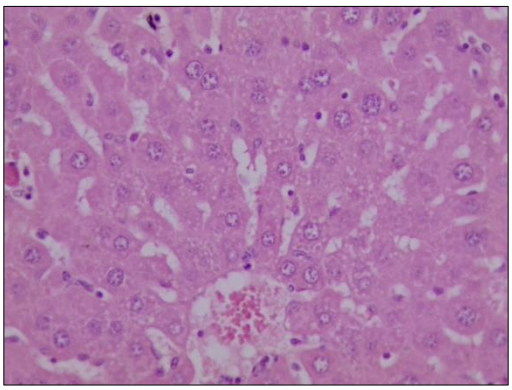

Figure 6 Section of a liver tissue in mouse treated with Section of a liver tissue in mouse treated with alcoholic extract of *Ammi majus* and **CCL_4**, Section showing congestion ,sinusoidal dilation, with few hepatic cells showing fatty changes (200X; H and E)

Histopathological examination of liver confirmed the results of tissue homogenate and serum markers, and also indicated that CCL_4 induced liver injury was less severe in the water extract of *Ammi majus* treated groups. The present results show that water extract of *Ammi majus* reduces the extent of hepatic injury caused by CCL_4 in mice. This suggests the beneficial effect of *water extract of Ammi majus* in modulating the hepatotoxic effects of CCL_4 in treated mice; an observation that is favored by Abdel-Salam *et al*. (2012).

The histopathological examinations of liver sections in CCL_4-treated mice revealed a regeneration of hepatic cells after treatments with the water plant extract especially at a dose of 100 mg/kg; therefore it is possible to suggest that the plant extract being able to condition the hepatic cells to a state of accelerated regeneration (Aydin *et al*., 2014).

Ammi majus contain Linear furancoumarins (xanthotoxin , bergabten , imperatorin and isioimpiellin) which inhibt human liver CYP450 simple coumarins induce number of enzymes like aldehyde reductase , glutathione Stransferase(GST), and NAD(P)H quinone oxidoredctase in the liver that are possible for detoxification of alfatoxin B1 and also contain flavoniods (quercetin and keampferol) which has antioxidant and anti-tumor activity (lin *et al*., 2006 and Donnini *et al*., 2006).

The antioxidant properties of *Ammi majus* extract may be attributed to the presence of quercetine (Muragundla and Kanwaljit, 2004). 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 (Duarte *et al*., 2001).

4. Conclusion

- CCL_4 had a toxic and harmful effect at liver enzyme and histology.
- Water extract have benefit effect and productive activity for liver enzyme and histology.

- Alcoholic extract have toxic effect and not give productive activity.

Recommendation

- Investigated on active compound that are found in *Ammi majus* water extract and study.
- Study effect of water extract of *Ammi majus* with different concentration and know toxic dose for it.
- Study effect of water extract of *Ammi majus* on another organ.
- Investigated on active compound found in alcoholic extract and why it was toxic.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to disclosed.

Statement of ethical approval

This work done after take a permission from the head of animal laboratory house\Biotechnology Research Center\Al-Nahrain University \Baghdad\Iraq.

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