Effects of Petroselinum crispum extract versus fat diet and Dexamethasone induced organ injuries in mice

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Abstract. Petroselinum crispum is a herb belonging to the Apiaceae family, it has been usually used for the management of many inflammatory disease due to many pharmacological effects of corticosteroid with a strong anti-inflammatory and immunosuppressant properties (ex. dexamethasone), it is famous to cause side effects. It was clearly observed that dexamethasone could induced liver renal and increase the lipid profile in mice. The Petroselinum crispum methanol extract was prepared by soaking of 200 g of each of the pure dry in 1 liter of 90% methyl alcohol with shaking for 5 days, The selected animal groups (eight animals per each group) were grouped as follows Positive control group 1, negative control group 2, group 3 (10% Petroselinum crispum treated) and group 4 (20%). Petroselinum crispum treated group it was found that Petroselinum crispum has a clear ant atherosclerosis and a potent liver protection effects.

1. Introduction
Petroselinum crispum is a herb related to the Apiaceae family, it is well-known to the middle east area where it is found in the natural form. It is mostly grown outdoors and is seasonally harvest (1). Petroselinum crispum is a leafy vegetable, rich in many medical active compounds, and its name is derived from the Greek for ‘rock celery’; it can be well-known from other plant herbs by its unique aroma. In sunny areas with suitable environmental conditions in a humid soil with a pH of 5.3–7.3 Petroselinum crispum could reach to 60–120 cm in tall (2). It is susceptible to water stress, especially if it is planted in the summer and spring, and to increase the production and to increase its quality, a permanent source of water should be provided. together growth stage and Petroselinum crispum type determine the susceptibility of the plants to water stress (3).

A piece of Petroselinum crispum is a ‘powerhouse’ of nourishment, and is well off in B vitamin, vitamin C, β-carotene and zinc; it is an imperative dietary segment for help bone because of its rich substance of boron and fluorine, and furthermore contains iron and calcium in an absorbable structure (4). The therapeutic advantages/conventional employment of the Petroselinum crispum have been extensive known, Wine overflowed with the Petroselinum crispum was discretionary in medieval occasions as a treatment for joint inflammation and chest pain (5). It was notable as a purgative, diuretic and quinine substitute by the US Pharmacopeia in 1850 (6). Petroselinum crispum has likewise been accounted for as a helpful for postmenopausal women (7).

Petroselinum crispum has been frequently used for the management of allergies and autoimmune and chronic inflammatory disease (7). Tissue injure, neuropathological diseases and autoimmune disease could be from chronic stimulation of the immune system, and myristicin oil from...
Petroselinum crispum can decrease the immune inflammation by inhibiting nitric oxide, cytokine production and the release of inflammatory proteins (9).

Research done on the suppressive effects of Petroselinum crispum essential oil on mouse splenocytes and macrophages, and it was found that it could lead to suppress nitric oxide production and the immune functions of macrophages (10). Petroselinum crispum has an important role in the defense mechanisms against bacteria and fungi (11). Mixing of fresh Petroselinum crispum leaves to ‘Kareish’ cheese decrease the amount of yeast present within 2 h, additionally Petroselinum crispum extracts showed a important inhibitory activity against Staphylococcus aureus and antibacterial activity against other microbial flora in the cheese (12). The diuretic effect of Petroselinum crispum has long been documented in traditional medicine (13). More recently studied found that, Petroselinum crispum extracts increased the urine output/day by inhibiting Na±K±-ATPase, thus leading to an increased K± concentration in the kidney lumen that leads to an osmotic water flow into the lumen and diuresis(14). Petroselinum crispum used for treatment of the kidney stone (15).

In addition, Petroselinum crispum is now planted all over the world due to its handling in the food industry, perfume manufacturing, soaps and creams. Petroselinum crispum is loaded with several antioxidants including volatile oils and flavonoids also carotenoids, ascorbic acid, tocopherol, tannins, sterols, as well as vitamins A, C and K, potassium, calcium and magnesium. so that it is considered as a potent free radical scavenger. these flavonoids also have been shown to be efficient in the management of gastric ulcers. Petroselinum crispum was documented to scavenge the free radicals OH- and this lead to decrease lipid peroxidation. It was also found that Petroselinum crispum extract inhibited in vitro and in vivo platelet aggregation and prolonged bleeding time in rats (16). Warm compresses or poultices of Petroselinum crispum have been used to treat breast engorgement and mastalgia (17). Also an Oral capsules filled with Petroselinum crispum found to decrease milk flow; however, no scientifically valid clinical trials support this use. Galactogogues should never replace evaluation and counseling on modifiable factors that involve milk production (18). On the other hand Dexamethasone is the mainly a strong synthetic glucocorticoid, which distinct from the naturally occurring cortisol and corticosterone, has to nearly pure glucocorticoid activity (19). The effective anti-inflammatory and immunosuppressant properties of dexamethasone cause to be it helpful in many inflammatory and autoimmune diseases the Steroidal anti-inflammatory drugs in general have been found to decrease the production of leukotrienes and prostaglandins release by blocking the secretion of phospholipase A2 and the arachidonic acid release and it's also known to be strong inhibitors of cytokine production and to has a protective effect against the lipopolysaccharide induces death. It was also has been found to have a benefit in the management of human and animal-spinal cord injury or cerebral ischemia (20). On the other hand it was found that dexamethasone has a serious side effect on the renal function and its very important due to their general therapeutic use in patient with renal and other diseases (21). In some research, it was found that 10% increment of plasma creatinine concentration was documented during corticosteroid treatment, with a go back to pretreatment concentration values after taking out corticosteroids linked to effect of corticosteroids on glomerular filtration rate (GFR) (22). for the most of the studies that recounting corticosteroids to the rate of cardiovascular disease have concentrated on myocardial infarction (23). Other research have documented that cerebrovascular events could be associated with corticosteroids treatment (24). Atherosclerosis occurs of the peripheral arteries was three times as frequent in those treated with corticosteroids in compare to those who were not (25). Several researchers found that long term low dose corticosteroids therapy can cause hypertension as well (26). Liver is well known as one of the most sensitive organs to corticosteroids exposure and toxicity (27). Corticosteroids changed the homeostasis of many biological processes in the liver (28). Corticosteroids is well known to have other serious side effects on lipid profile, ranging from deregulation of lipid metabolism and hepatic steatosis (29). The mechanism behind corticosteroids induced hepatic injury are still not completely explained (30). Low doses of corticosteroids are considered safe for the liver, and chronic administration of such drugs could be associated with steatosis or steatohepatitis (31).
2. Materials and Methods

2.1 Plant materials
Petroselinum crispum whole plant leaf, and root were purchased from a local herbal medicine shop in Baghdad, Iraq, and a specimen was deposited in Pharmacognosy Department, Baghdad collage of medical sciences.

2.2. Lipid rich diet
The lipid rich diet consisted of the following: 16% casein, 10% corn oil, 4% N.N cellulose, 4% salt mixture, 1% vitamin mixture, 0.2% choline chloride, 0.2% DL-methionine, and 64.6% corn starch.

2.3. Animals and housing conditions
32 male Swiss albino mice aged from 9 to 10 weeks and weighing from 25 to 30 g included in the experiment and used throughout the study in the (alnahrian biotechnology center) Animals were feed by a similar diet ingredient and had open access to tap water. All mice were reserved under the same experimental circumstances.

2.4 Preparation of methanol extract
The methanol extract was prepared by soaking of 200 g of the pure dry Petroselinum crispum leaves in 1 liter of 90% methyl alcohol and shaking it 5 days and then reserved in a refrigerator. The methanol then was evaporated by using of a rotatory evaporator apparatus linked to vacuum pump. 20 grams of the semisolid methyl extract was diluted in 100 ml distilled water with 2 ml of 80 (as a suspending agent) to set up 20% alcohol.

2.5 Experimental design.
All experimental animals were feed with the high lipid diet and then putted under observation in about 2 weeks before the start of the experiment to keep out any undercurrent infection. The mice were then divided randomly into four groups each of 6 mice as follows: the first group (G1) was the normal untreated negative control group fed with normal diet without any exposure to dexamethasone, the second group (G2) was fed with 2% cholesterol in the diet to induce hypercholesterolemia, the third group (G3) was fed with 2% cholesterol and cotreated with 20% Petroselinum crispus seeds methanol extract using stomach tube, and the fourth group (G4) was fed with 2% cholesterol and cotreated with 20% carob legumes methanol extract using stomach tube. The experiment was conducted for 8 weeks as an adequate period to induce hypercholesterolemic(22). After 8 weeks acclimatization period, the selected animals of nearly a similar weight were divided into four experimental groups so as to keep more or less the same mean body weight within the individual groups. The selected animal groups (eight animals per each group) were treated as follows: Positive control group 1 (dexamethasone at the dose of 4mg/kg body weight per day) fed with 2% cholesterol in the diet to induce hypercholesterolemia, negative control group 2 (fed with normal diet and no dexamethasone injection), group 3 Petroselinum crispus treated group with 10% parsley methanol extract using stomach tube plus dexamethasone at the dose of 4mg/kg body weight per day, and group 4 Petroselinum crispus treated group with 20% parsley methanol extract using stomach tube plus dexamethasone at the dose of 4mg/kg body weight per day. At the ending of the experiment, animals were fasted 14–16 hours after their last feeding and blood was taken from the heart of dimethyl-ether preanaesthetized mice in plain tubes for biochemical analysis. Blood serum was taken by centrifugation at 1000 rpm for 10 min at room temperature and then stored at −20°C until analysis was performed. Animals were sacrificed by cervical dislocation.

2.6 Experimental design.
Serum total cholesterol (TC) and triglycerides (TG) were obtained by colorimetric methods as described by Young using Spinreact Kit (Spain) according to the instruction of the supplier. Serum
high density lipoprotein (HDL) was taken according to the colorimetric method of Naito using Spinreact Kit (Spain) according to the instruction of the supplier. Serum LDL and VLDL were calculated according to the equation of Srivastava et al (26) as follows: 
\[ \text{LDL} = \text{TC} - (\text{HDL} \pm \text{TG}/5) \]
\[ \text{VLDL} = \text{TC} - (\text{LDL} \pm \text{HDL}) \]

### 2.7 Liver enzymes

Serum alanine transaminase (ALT) and alkaline phosphatase (ALP) were taken spectrophotometrically according to the method of Thelfeld et al. and Schlebusch et al., respectively, using Human Kit (Germany) according to the instruction of the supplier. Serum aspartate aminotransferase (AST) was taken spectrophotometrically depending on the method of Thelfeld et al. using Swemed Diagnostics kit (India).

### 2.8 Analysis of renal function

Serum concentrations of creatinine and blood urea nitrogen (BUN) were measured automatically in a Dimension RxL autoanalyzer (Dade-Behring, Siemens, Eschborn, Germany). This study is conducted after approval from institutional animal ethics committee.

### 2.9 Statistical analysis

Data are presented as mean ± standard error mean and analyzed by using one-way analysis of variance followed by Bonferroni’s multiple comparison test (post-test); \( P \leq 0.05 \) was considered as statistically significant in all analyse.

### 3. Results and Discussion

On high light the results that taken from the experiment it was found that cholesterol concentrations in negative control group found to be 85±3 mg/dl (with a significant difference in compare to positive control) and it increase after exposure to dexamethasone drug plus feeding with a high fat diet and it found to be found to be 121±11.5 mg/dl, on the other hand looking in Petroselinum crispum 10% treated group the cholesterol concentration has been found to be 81.6±1.5 mg/dl, which is un-significant differences in comparing to negative group and a significant changes in comparing to positive group. In Petroselinum crispum 20% treated group the cholesterol concentration was found to be 76±1.5mg/dl, which has highly significant differences in compare to positive control group (Figure 1). Also after analysis the result that has been taken in this experiment it was found that triglycerides concentration in taken from the negative group was 95±18mg/dl and after it exposure to dexamethasone plus high fat diet (the positive control group) it was found to be 119±16mg/dl with a significant difference in compare to it. In Petroselinum crispum 10% treated group the triglyceride concentration was 75±2 mg/dl, and in comparing these results to the positive control group it found that it has a significant differences and un-significant to negative control group that did not expose to dexamethasone plus high fat diet. In Petroselinum crispum 20% treated group the triglyceride concentration was found to be 68±1 mg/dl, which is un-significant to negative group and highly significant differences to positive group (Figure 2).

On the other hand results obtained from this experiment found that high density lipoprotein concentration in negative group was 37.3±2.5 mg/dl and after exposure to dexamethasone plus high fat diet it was found to be 46±3.4 mg/dl (with a significant difference in compare to positive control) In Petroselinum crispum 10% treated group the high density lipoprotein concentration was found to be 62±0.5 mg/dl, which has significant differences to positive group. In Petroselinum crispum 20% treated group the high density lipoprotein concentration was found to be 52±4.1mg/dl, which has significant differences to negative group and unsignificant to positive group (Figure 3).

Analysis the result which we had in this experiment it was found that low density lipoprotein concentration in negative group was 38±6.5 mg/dl and after administration to dexamethasone drug plus high high fat diet it was found to be 71±5.13 mg/dl which is high significant differences between both positive and negative group In Petroselinum crispum 10% treated group the high density
lipoprotein concentration was found to be 58±1.5 mg/dl, which has high significant to negative group. After analysis the results of Petroselinum crispum 20% treated group the high density lipoprotein concentration was found to be 53±2.3mg/dl, which has significant to both negative and positive group (Figure 4). Looking at the result which we had in this experiment it was found that very high density lipoprotein concentration in negative group was 20±2 mg/dl and after exposure to dexamethasone plus high fat diet it was found to be 31.6±1.15 mg/dl which has significant differences between negative and positive control group. In Petroselinum crispum10% treated group the very high density lipoprotein concentration was found to be 32±0.5 mg/dl, which has high significant differences to both positive and negative group. Results which we had from the data of renal function test was shown that Petroselinum crispum treated group (10 and 20%) both has un significant differences in compare to positive control group. Also high light the result which we had in this experiment it was found that SGPT concentration in negative group was 62±10 mg/dl and after exposure to dexamethasone plus high fat diet it was found to be 78±1.7 mg/dl with a significant differences between both groups. In Petroselinum crispum10% treated group the SGPT concentration was found to be 56.6±7.6 mg/dl, with high significant differences in compare to negative group and with high significant differences was also found from the results taken from Petroselinum crispum treated group 2(20% Petroselinum crispum extract) in SGOT and alkaline phosphates results (Figures 5 and 6). Maryam Rezzad et al found in her research about the protective effect of Petroselinum crispum extract in abortion against prostadin-induced renal dysfunction in female rats. The Petroselinum crispum reduced the dysfunction in rat's kidney caused by prostadin-induced abortion and could have beneficial effect in reducing the progression of prostaglandin-induced edema (32). Nabila Y. Mahmoud et al found in her research about effect of Petroselinum crispum against gentamicin induced nephrotoxicity in rate, showed significant decrease in uric acid, creatinine and urea (33). From the above results with this study it was found that Petroselinum crispumin both concentration ( 10 and 20%) failed to protect the kidney from dexamethasone induced damage and this could be due to differences in dose and duration of administered of Petroselinum crispum. A research of Adnan M. Jassim about Protective Effect of Petroselinum crispum(Petroselinum crispum)extract on histopathological changes in liver induced by Sodium Valproate- In male Rats reveal mild degree of steatosis reflected by micro vacuoles of the cytoplasm, thus Loss of normal arraying of hepatocytes indicates a mild degree of liver atrophy , central vein. Focal liver necrosis (34). Mohamed M. Abdel_Daim also found in her research about effect of Petroselinum crispum oil against cisplatin induce hepatotoxicity that the Administration of Petroselinum crispum oil either as a preventative medicine or as treatment significantly enhanced all the observed deleterious effects induced by Cisplatin in rat liver thus Petroselinum crispum oil,with its antioxidant, anti-inflammatory, and anti-apoptotic activities, can potentially be used in the treatment of cisplatin-induced hepatic injuries (35). The above results found to be lined with results obtained from the experiment that found a high protection effects of Petroselinum crispum against dexamethasone induced liver damage .SGOT has been decreases from 57± 2 mg/dl to 48.6 ±8 mg/dl and this is also found in the results taken from ALK ,phosphatase which has a decrease from positive control group 134 ± 3.2 mg/dl to Petroselinum crispum 20% treated group 113.3 ± 11.5 mg/dl. Mohamed abd el-Aleim et al found in her research about effect of Petroselinum crispum extract on lipid profile in adult male albino rates with hypercholesterolemic Treated with Petroselinum crispum extract, showed significant lower concentrations of serum total cholesterol (TG), triglycerides (TGs), low density lipoprotein cholesterol (LDL-C), and very low density lipoprotein cholesterol (VLDL-C) , and significant increase in high density lipoprotein cholesterol (HDL-C) and significant decrease in AST ,ALT and GGT(36). Ayman F.khalil etal found in her research about effect of Petroselinum crispum leaves oil against carbon tetrachloride in hepatotoxicity which resulted in significant elevation of serum triglycerides, total cholesterol, low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C) and decreasing in serum high density lipoprotein (HDL-C). Moreover, kidney function tests for serum urea nitrogen, creatinine, and uric acid were found to be increased. Petroselinum crispum and their mixture oils have strong radical scavenging activity and antioxidant activity, and all parameters returned near to the
normal value (37). These results have found to be compatible to our results. Group 2 Petroselinum crispum treated (20 % Petroselinum crispum extract ) was found to have highly significant decrease in cholesterol, TG , LDL and VLDL in comparing to positive control group.

**Table 1:** The mean of lipid profile, liver function test and renal function test of the various treatment at the end of the experiment. Group 1 positive treatment, Group 2 negative treatment, Group 3 10% Petroselinum crispum and Group 4 20% Petroselinum crispum.

| Group | Cholesterol mg/dL | TG mg/dL | HDL mg/dL | LDL mg/dL | VLDL mg/dL | UREA mg/dL | Creatinine mg/dL | SGPT IU/L | SGOT IU/L | ALK.PH IU/L |
|-------|-------------------|----------|-----------|-----------|------------|------------|-----------------|-----------|-----------|-------------|
| Group 1 85±3 * | 95±18 * | 37.3±2.5 * | 38±6.5 ▪▪ | 20±2 * | 39±4 * | 0.5±0.1 | 62±10 * | 58±2.8 * | 125±5 * |
| Group 2 121±11.5 | 119±16 | 46±3.4 | 71±5.13 | 31.6±1.15 | 48±5.2 | 0.6±0.7 | 78±1.7 | 75±2 | 143±3.2 |
| Group 3 81.6±1.5* | 75±2* | 62±0.5* | 58±1.5*** | 32±0.5*** | 44±2.5 | 0.55±0.5 | 56.6±7.6 | 58.6±3.2** | 121±10** |
| Group 4 76±1.5** | 68±1** | 52±4.1* | 53±2.3*** | 33±1.7*** | 37.6±1.2 | 0.46±0.1 | 50±5** | 48.6±8** | 113.3±11.5** |

* represent Significant differences in compare to positive control group  
** represent Highly Significant differences in compare to positive control group  
* represent Significant differences in compare between positive and negative control group  
** represent highly Significant differences in compare between positive and negative control group  
▪ represent Significant differences in compare to negative control group  
▪▪ represent Highly Significant differences in compare to negative control group.

All values are mean ± SD data were analyzed by using spss 22 statistical test.

**Figure 1.** Represent the difference of cholesterol mean concentrations at the end of the experiment between positive, negative, Petroselinum crispum treated group 1, Petroselinum crispum treated group 2.
Figure 2. Represent the difference of triglyceride mean concentrations at the end of the experiment between positive, negative, Petroselinum crispum treated group 1, Petroselinum crispum treated group 2.

Figure 3. Represent the difference of HDL mean concentrations at the end of the experiment between positive, negative, Petroselinum crispum treated group 1, Petroselinum crispum treated group 2.
Figure 4. represent the difference of LDL mean concentrations at the end of the experiment between positive, negative, Petroselinum crispum treated group 1, Petroselinum crispum treated group 2.

Figure 5. represent the difference of alkaline phosphatase mean concentrations at the end of the experiment between positive, negative, Petroselinum crispum treated group 1, Petroselinum crispum treated group 2.
Figure 6. Represent the mean difference of SGOT concentrations between positive (positive treated group, negative, Petroselinum crispum treated group 1, Petroselinum crispum treat 2

4. Conclusion
Petroselinum crispum has extract shows a significant hepatic protection effects against dexamethasone with a significant decrease the lipid profile against diet induced atherosclerosis to an extent that highlight a strong anti-atherosclerotic hepatoprotective effects.

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