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Studying the Analgesic, Anti-inflammatory and Antipyretic Properties of The Aqueous Extract of *Petroselinum crispum* in Experimental Animal Models

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Abstract : *The aqueous extract of Parsley (Petroselinum crispum) were investigated for anti-inflammatory, analgesic and antipyretic activity at the doses of 2 , 5 , and 10 g/kg, of body weight. The experimental paradigms used were carrageenan, dextran, histamine induced pedal edema and cotton pellet induced granuloma for anti-inflammatory activity, while hot plate and acetic acid induced writhing methods were used to assess analgesic activity. Yeast-induced hyperpyrexia was used to evaluate the antipyretic activity. In acute phase inflammation, a maximum inhibition 50.6% ($P < 0.05$), 51.1% ($P < 0.05$) and 52.3% ($P < 0.05$) were noted at the dose of 10 g/kg after 3 h of treatment with methanol extract of Parsley (Petroselinum crispum) in carrageenan, dextran and histamine induced pedal edema , respectively. In the chronic model (cotton pellet induced granuloma) , the parsley (10 g/kg) and standard drug (Indomethacin 10 mg/kg) showed decreased formation of granuloma tissue by 51.8% ($P < 0.05$) and 56.6% ($P < 0.05$) , respectively. The extract also produced significant ($P < 0.01$) analgesic activity in both paradigms. In addition, the aqueous extract of parsley potentiated the morphine and aspirin induced analgesia. A significant ($P < 0.01$) reduction in hyperpyrexia in rat was also produced by the extract. This study exhibits that methanol extracts of leaves of parsley possess anti-inflammatory, analgesic and antipyretic activities.*

Keywords:*, Parsley (Petroselinum crispum) , Anti-inflammatory, Analgesic, Antipyretic*

I.Introduction

Inflammation or phlogosis is a path physiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be induced, maintain or aggravate many diseases [1] . However, studies have been continuing on inflammatory diseases and the side effects of the currly available anti-inflammatory drugs pose a major problem during their clinical use [2]. . Phenolic phytochemicals are known to exhibit several health beneficial activities such as antioxidant, anti-inflammatory, antihepatotoxic, antitumor, and antimicrobialTherefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary [3].

Parsley *(Petroselinum crispum*) is a medicinal herb used in folk medicine remedy to decrease the blood glucose level in Turkey [4] , and it shows a protective effect against hepatic toxicity caused as a complication of diabetic state [5] .The plant therapy can provide blood glucose homeostasis and cannot regenerate the B-cells of the endogenous pancreas [6] . Also parsley has been claimed in folk medicine to posses laxative properties attributed to the presence of some volatile oil that are more concentrated in seeds than in stems or leaves [6] . Parsley have advocated diuretic effect in folk medicine , and it is mediated through an inhibition of sodium potassium pump that would lead to the reduction in sodium and potassium re absorption leading, thus, to an osmotic water flow in to lumen and diuresis [7] . As a traditional medicine for hyperuricemia and gout, parsley (*Petroselinum crispum*) has been used in folk medicine. Phytochemical screening of parsley (*Petroselinum crispum*) has revealed the presence of several classes of flavonoids . Flavonols (kaempferol and quercetin) and flavones (apigenin and luteolin), which occur as glycosidic form in nature, are major flavonoids found in parsley (*Petroselinum crispum*) and other apiaceous vegetables . Kaempferol and quercetin, which belong to flavonol group, possess a wide ra parsley (*Petroselinum crispum*) has revealed the presence of several classes of flavonoids . Flavonols (kaempferol and quercetin) and flavones (apigenin and luteolin), which occur as glycosidic form in nature, are major flavonoids found in parsley (*Petroselinum crispum*) and other apiaceous vegetables . Kaempferol and quercetin, which belong to flavonol group, possess a wide range of biochemical and pharmacological effects and have been recommended as chemopreventive agents or nutritional supplements . The predominant mechanism of their biological actions is thought to result from antioxidant activity, enzyme inhibition, and the capacity to scavenge free radicals . Therefore, it is speculated that the health promoting effect

of parsley (*Petroselinum crispum*) may be due to its flavonol constituents and the content of flavonoid compounds in parsley is about 100 mg/100 g fresh weight [8] .

However, no work has been reported on the anti-inflammatory effects on acute and chronic phases of inflammation, analgesic and antipyretic activity of *Petroselinum crispum* . Keeping this in view, the present study has been undertaken to investigate these of the aqueous extract of *Petroselinum crispum* in experimental animal models.

II.Materials and Methods

Plant material and extraction

Dried parsley was obtained from commercial source and a voucher specimen of the plant was identified at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib . Parsley was shed dried at 25 C. Then a ground material was obtained by grinding dried parsley flakes with a coffee grinder and passing the powdered material through a standard sieve number 20, and stored at room temperature , then , the aqueous extract of parsley was prepared by decoction process of powdered material, in brief a suspension of 25 g of the powder in 100 ml of distilled water was stirred magnetically overnight (16 hours) at room temperature , and this was repeated three consecutive times . The residue was removed by filtration and the extract was evaporated to dryness at a low temperature (40 C) under reduced pressure in a rotator evaporator rotary vacuum (model Zirbus 302R) for about 1 h , and kept in deep freeze . The residual extract were dissolved in normal saline whenever used in the experiments [10] .

Animals

Swiss albino mice of both sex weighing between (18-22 g) and Albino Wister rats of the either sex (180-200 g) were used for the study. They were maintained under standard environmental conditions and were fed with standard pellet diet, and water *ad libitum*.

Chemicals and Drugs

Carrageenan (S. D. Fine Chemicals Limited, Bom-bay), histamine (Sigma, USA), dextran (Sigma, USA) were used in the study and indomethacin (Re-con, Bangalore), aspirin (USV, Bombay) , paracetamol (IPCA, Bombay), and morphine (M.M. Pharma, New Delhi) were used as the standard drugs.

I-Anti-inflammatory models

a-Carrageenan-induced paw edema in rats.

The rats were divided into 5 groups (n = 6) for eah group . The different groups were treated with parsley (2, 5 and 10 g/kg PO) , indomethacin (10 mg/kg PO) and control vehicle per oral and the paw volume was measured at 0 h and 3 h after carrageenan injection using a plethysmometer [11,12] . Animals were pretreated with the extract 1 h before the administration of carrageenan. Acute inflammation was produced by the sub planter administration of 0.1 ml of (1%, w/v) carrageenan in normal saline in the right paw of the rats. The ratio of the anti-inflammatory effect of the aqueous extract of parsley was calculated by the following equation:

anti-inflammatory activity (%) = (1 - D / C) x 100

where D represents the percentage difference in paw volume after parsley was administered to the rats, and C represents the percentage difference of volume in the control groups [12] .

b-Dextran-induced paw edema in rats.

Animals were treated in a manner similar to that of carrageenan induced paw edema model; dextran (0.1 ml, 1% w/v in normal saline) was used in place of carrageenan [13]

c-Histamine-induced paw edema in rats.

The anti-inflammatory activity of parsley was measured with histamine (phlogistic agents) which acts as mediator of inflammation. The paw edema was induced in rats by sub planter injection of 0.1 ml (1% w/v) of freshly prepared histamine solution, and the paw edema was measured [14] .

d-Cotton pellets-induced granuloma.

The rats were divided into five groups (n = 6) for each group. After shaving the fur, the rats were anaesthetized with ether , and 10 mg of sterile cotton pellets were inserted, one in each axils. The aqueous extract of parsley (2 , 5, and 10 g/kg, PO), indomethacin (10 mg/kg, PO) and control vehicle were administered orally for 7 consecutive days from the day of cotton pellet implantation. Animals were then anaesthetized on the eighth day and cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation [15] .

II-Analgesic activity

The aqueous extract of parsley at the dose of 2 , 5 , and 10 g/kg and combination of above doses of extract with the standard drug aspirin 100 mg/kg (Acetic acid induced writhing response in mice) and morphine 5 mg/kg (Hot plate reaction time in mice) were administered to eight groups (six mice in each paradigm) [16] .

a-Acetic acid-induced writhing test.

Acetic acid solution (15 mg/ml) at the dose of 300 mg/kg body weight was injected (i.p.) and the number of writhes during the following 30 min period was observed [17] . A significant reduction in the number of writhes by drug treatment as compared to vehicle treated animals was considered as a positive analgesic response. The percentage inhibition of writhing was then calculated. Aspirin (100 mg/kg, i.p.) was used as standard.

b-Hot plate reaction time in mice.

Mice were screened by placing them on a hot plate maintained at 55 ± 1°C and the reaction time in seconds for hind paw licking or jumping were recorded [18] . Only mice which reacted within 15 sec and which did not show large variation when tested on four separated occasions, each 15 min apart, were used in this study. Morphine (5 mg/kg, i.p.) was used as standard. The time for hind paw licking or jumping on the heated plate of analgesiometer was taken as the reaction time.

II-Induction of Yeast-induced pyrexia

Rats were divided into five groups (six rats in each) , and were trained to remain quiet in a restraint cage. A thermister probe was inserted 3-4 cm deep into the rectum and fastened to the tail by an adhesive tape and the temperature was measured on a thermometer. The normal body temperature of each rat was measured rectally at predetermined intervals and recorded. Fever was induced by a subcutaneous injection of 20 ml/kg body wt. of 20% w/v yeast suspended in methyl cellulose solution . Rats were then returned to their housing cage. After 24 h of yeast injection, animals were again restrained in individual cages for recording of their rectal temperatures as described previously. Then parsley was administered orally at doses of 2 , 5 , and 10 g/kg body wt. to three groups of animals , respectively. 10% Propylene glycol (5ml/kg, body wt.) was administered orally to the control group of animals. The fifth group of animals received the standard drug paracetamol (150 mg/kg, body wt.) orally. Rats were restrained for recording of their rectal temperatures at intervals of one hour, after the drug administration [15].

Statistical analysis

The experimental results were expressed as the mean ± S.E.M. Data were assessed by the method of analysis of ANOVA followed by student's t-test. *P* value of < 0.05 was considered as statistically significant.

III. Results

I-Anti-inflammatory effects

The anti-inflammatory potential of parsley 2 , 5 , and 10 g/kg against various experimental animal models exhibited significant ($P < 0.05$) anti-inflammatory activity. The effects of parsley and indomethacin on the inflammation induced by carrageenan, dextran, histamine and cotton pellet induced granuloma are summarized in Fig 1 and Fig 2.

The percentage of inhibition was calculated according to the following equation :-

$$\% \text{ Inhibition} = (C-T)/C \times 100$$

As shown in Fig 1, parsley showed maximum inhibition of 50.6% at the dose of 10g/kg after 3 h of treatment in carrageenan induced paw oedema, whereas the standard drug (Indomethacin 10 mg/kg) showed 72.4% of inhibition ($P < 0.05$). In case of dextran induced paw oedema, the parsley showed significant inhibition (33.0%, 43.2% and 51.1%) in a dose dependent manner as compared with control. The parsley showed 52.3% of inhibition at the dose of 10 g/kg whereas indomethacin showed 72.1% of inhibition in histamine induced paw oedema. In the chronic model (cotton pellet induced granuloma), the parsley (2 , 5 , and 10 g/kg) and indomethacin showed decreased formation of granuloma tissue at 29.6%, 41.3%, 51.8% and 56.6% respectively (Fig 2). Regarding the effect of parsley on acute phase of inflammation (carrageenan, dextran and histamine) and chronic (cotton pellet induced granuloma), a maximum inhibition (69.9%, 74.9%, 72.5 and 91.5% respectively) was noted at the dose of 10 g/kg when compared with standard drug.

II-Analgesic effects

The results presented in Table 1, shows that parsley at the doses of 2 , 5 , and 10 g /kg and aspirin at 100 mg / kg exhibited highly significant ($P < 0.01$) inhibition of the control writhes . The percentage of inhibition was calculated according to the following equation

$$\% \text{ Inhibition} = (C-T)/C \times 100$$

The inhibition was at rate of 16.5%, 27.8%, 48.8% and 66.0%, respectively , when compared to that of control. In addition, parsley at the above mentioned doses, potentiated the analgesic activity of aspirin shown by further decreasing the writhing response when given in combination.
As shown in Table 2, the parsley produce a highly significant ($P < 0.01$) analgesic activity when compared to the that of control. Additionally, parsley at different doses potentiated the analgesic activity of the standard drug (Morphine 5 mg/kg).

III-Anti-pyretic effects
The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration . Treatment with the parsley at the dose of 2 . 5 , and 10g / kg., body weight decreased the rectal temperature of the rat in a dose dependent manner. The antipyretic effect started from the first hour and was maintained for 4 h, after administration of the extract. The result obtained from both the standard and parsley treated rats were compared with the control group and a high significant reduction($P < 0.01$) in the yeast induced elevated rectal temperature was observed (Table 3).

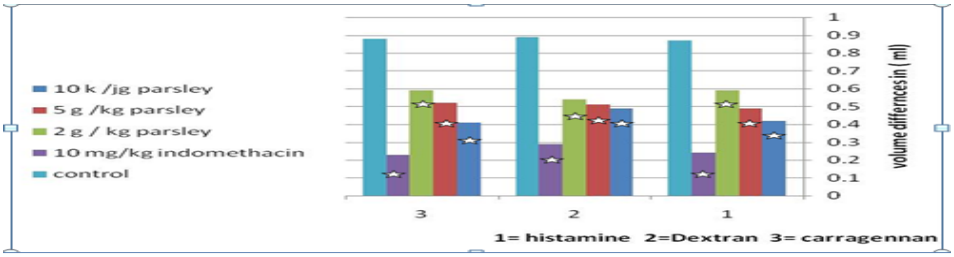

Figure 1: Effect of parsley and indomethacin on carragennan , dextran , histamine induced rat paw oedema . Differences of mean oedema volume (ml) between control and treatment values at different doses SEM ± Variation compared to the control animals . ANOVA followed by Students t test . * p<0.05 .

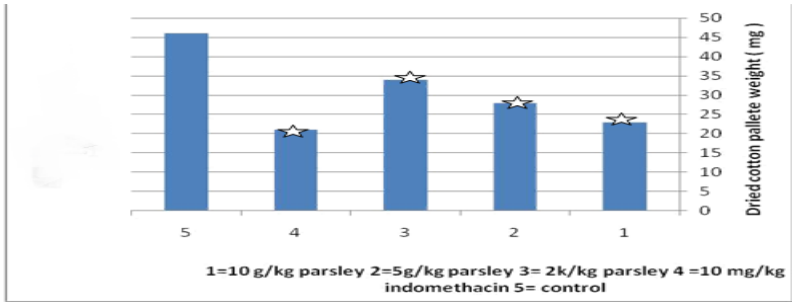

Figure 2 : Effect of Parsley and Indomethacin on the proliferative phase of inflammation in rats Difference of means of dried cotton pellet weight (mg) between control and treatment values at different doses S.E.M±Variation compared to the control animals. ANOVA followed by Student's t- test * $P < 0.05$.

Table 1: Effects of parsley and aspirin on writhing induced by acetic acid in mice.

Treatment	Dose (g/kg)	Number of writhes (per 30 min)	Inhibition (%)
Control	-	30.33±2.68	-
parsley	2	25.00±2.23 *	16.5
parsley	5	21.33±1.18 *	27.8
parsley	10	14.54±1.49 *	48.8
Aspirin	0.1	9.11 ±1.40 *	66.0
parsley +Aspirin	2+0.1	9.82±1.43 *	67.5
parsley +Aspirin	5+0.1	6.9 4±0.62*	71.7
parsley +Aspirin	10+0.1	5.96±0.46*	79.4

*P< 0.01 means highly significant difference compared with control

Table 2: Effects of parsley and morphine on hot plate reaction time in mice.

Treatment	Dose (g/kg)	Mean latent time	
		Initial	After 30 min
Control	-	9.94±0.62	8.14±0.56
parsley	2	10.43±0.53*	13.34±0.93**
parsley	5	10.77±0.91*	14.25±1.22**
parsley	10	10.32±0.59*	15.74±1.26**
Morphine	0.005	10.94±0.79*	19.17±1.33**
parsley +Morphine	2+0.005	10.77±0.48*	21.37±1.57***
parsley +Morphine	5+0.005	9.93±0.29*	23.68±1.32***
parsley +Morphine	10+0.005	10.43±0.59*	27.53±1.21***

*P< 0.01 ,**P<0.001, P<0.005 , means highly significant difference compared with control

Table 3: The effect of parsley and paracetamol on yeast-induced pyrexia in rats.

Treatment	Rectal temperature					
	After yeast injection		After drug administration			
	0 h	24 h	1 h	2 h	3 h	4 h
Control Vehicle 5ml/kg	39.9±0.02	41.9±0.03	42.1 ±0.02	41.9±0.05	41.9±0.05	41.9±0.04
Paracetamol 150mg/kg	39.9±0.02	42±0.038*	40.9±0.02*	40.3±0.02*	39.8±0.04*	39.7±0.03*
Parsley 2 g/kg	39.7±0.04	41.8±0.03*	41.2 ±0.02*	40.7±0.02*	40.4±0.04*	40.1±0.06*
parsley 5g/kg	38.9±0.02	41.7±0.04*	41.8±0.05*	40.5±0.05*	40.1±0.03*	39.8±0.06*
Parsley 10mg/kg	39.7±0.07	42.1±0.06*	41.8±0.02*	40.2±0.03*	39.7±0.05*	39.6±0.04*

*P< 0.01, means highly significant difference compared with control

IV. Discussion

Parsley was evaluated for its anti inflammatory activity in acute and chronic models. A significant (p < 0.05) anti-inflammatory activity was observed for parsley in *c*arrageenan, dextran, histamine induced edema and cotton pellet-induced granuloma models. Carrageenan-induced rat paw edema has been used as an inflammation model in order to investigate the anti-inflammatory effect of drug [18] . There are two phases of carrageenan-induced inflammatory reaction: early or (first phase) and later or (second phase). It has been proposed that early phase results from histamine, serotonin and bradykinin liberation , while late phase is associated with the release of prostaglandin [19] . In carrageenan induced paw edema , the parsley showed maximum inhibition of 50.6% at the dose of 10 g/kg after 3 h of drug treatment. Dextran induced paw edema is known to be mediated both by histamine and serotonin. Dextran induces fluid accumulation, which contains little protein few neutrophils, whereas carrageenan induces protein rich exudation containing large number of neutrophil [18] . The parsley also exhibited significant anti-inflammatory activity in dextran induced paw edema. Histamine is one of the important inflammation mediators and it is a potent vasodilator substance and increases the vascular permeability [20.21] . This study showed that all the doses of parsley effectively suppressed the edema produced by the histamine, which indicates that the extract exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators via. histamine, serotonin and prostaglandin might be involved in the inflammation .

From these results , it is suggested that the anti- oedematogenic effect of parsley on carrageennan , dextran and histamine induced edema may be related to the inhibition of inflammatory mediator formation .

Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate pro inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation [22.23] . Chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or granuloma form . Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation [24.25]. The parsley showed significant ($P < 0.05$) anti-inflammatory activity in cotton pellet induced granuloma and , thus , found to be a effective in chronic inflammatory condition.

In order to distinguish between the central and peripheral analgesic action of parsley , acetic acid induced writhing responses in mice were used to examine the effect. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test, the animals react with characteristic stretching behavior, which is called writhing. It was found that parsley significantly ($P < 0.01$) inhibited the acetic acid induced writhing response and potentiated the analgesic activity of aspirin as well. The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is , therefore , possible that parsley produced analgesic effect by inhibition of synthesis or action of prostaglandins [25].

The hot plate method was originally described by Woolfe and Mac Donald [26] .This test has been found to be suitable for the evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance [27] .The present findings of the study indicate that the parsley may be centrally acting.

Fever may be a result of infection or one of the sequel of tissue damage, inflammation, graft rejection, or other disease states. Antipyretics are drugs, which reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained . In fever , this set point is elevated and a drug like paracetamol do not influence body temperature when it is elevated by factors such as exercise or increases in ambient temperature [28] . The present result show that the parsley possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats and its effect is comparable to that of paracetamol .

Based on the results of the present study , it can be concluded that parsley has potential activity against both acute and chronic phases at a dosage range of 2-10 g/kg. PO . Of the three doses , the dose of 10 g/kg is found to be more potent and efficacious towards the anti-inflammatory, analgesic and antipyretic activity, when compared with control , and the activity is in a dose-dependent manner. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism(s) of action.

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