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AQUA DISTILLATION ENHANCES THE ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF *ROSA DAMASCENA* MILL.; A PILOT STUDY

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ABSTRACT: *Rosa damascena* Mill. also known as Damask rose, Persian rose or Gul-e-Muhammadi of Rosaceae family is an aromatic small plant cultivated all over the world for its visual beauty, fragrances and therapeutic potential. The plant is rich in tannins, flavonoids, glycosides, carboxylic acids and ascorbic acid. In addition to its aromatic properties, the plant possesses laxative, anti-oxidant, anti-bacterial, wound healing, skin health, cardiotonic, hypnotic and cough suppressant activities. The medicinal uses reported from this plant are of its methanolic, ethanolic or chloroformic extract and of its essential oil. This research is planned to investigate the analgesic and anti-inflammatory potential of aqua distillate of *Rosa damascena* Mill. flower in two different doses *i.e.* 250 mg/kg and 500 mg/kg using Hot Plate, Tail Flick and acetic acid induced paw-edema method. Also as recent studies reported that alcoholic extract of *Rosa damascena* possesses analgesic activity and aqueous extract does not, so this study also aimed to evaluate these activities after its distillation in aqueous medium.

INTRODUCTION: Herbal drugs have played a major role in global health. They have been widely used as medicines since ancient times in the treatment and management of various ailments. In current era, despite of significant advancement in medical sciences, herbal preparations still make a considerable contribution to health care [1]. Herbal medicines have gained an increased researcher's attention and public awareness in last few years [2]. This may be due to the fact that these herbal preparations are believed to be safe with no or minimum adverse effects and are economical. World Health Organization (WHO) states that in comparison to conventional medicine system, utilization of these herbal remedies has increased two to three times.

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The increase awareness and recognition of herbal remedies and preparation throughout the world led to the establishment and foundation of office of alternative medicine by the National Institute of Health USA in 1992. This inspiration and encouragement by the World Health Organization gave boost to the use of herbal remedies all over the world [3]. Plants of Rosaceae family have long been used for food and medicinal purposes [4]. *Rosa damascene* Mill., commonly known as Gul-e-Muhammadi is among the most important species of Rosaceae family flowers [5]. It is cultivated all over the world more commonly in Iran, Turkey, India, and Bulgaria [6].

Rosa damascene Mill. is a globally well-known medicinal herb. In addition to its aromatic properties, in traditional medicine system, several pharmacological and therapeutic uses of this plant have been reported [7]. The flowers of *Rosa damascene* Mill. have a safe and efficacious history of administration in Persian medicine. The plant is famous for its beneficial effects in various diseases including cardiovascular disorders, skin health,

wound healing, gastrointestinal disease, menstrual bleeding, pregnancy-related disorders, as well as some mental disorders specially depression and anxiety. Its flower has been claimed to be beneficial for liver dysfunction and have liver tonic properties [8].

Rosa damascena is widely cultivated throughout the world for ornamental and medicinal purposes. The medicinal uses reported from this plant are of its methanolic, ethanolic or chloroformic extract and of its essential oil. This study is planned to investigate the analgesic and anti-inflammatory potential of *Rosa damascena* flowers after its distillation in aqueous medium in animal model at two different doses.

MATERIALS AND METHODS:

Selection of Animals: The study was conducted on mice and rats. Swiss male albino mice and Wistar male albino rats weighing between 25 to 30 g and 150 to 200 g respectively were purchased from local animal supplier and were kept in animal house of department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, for a conditioning period of 10 days. Animals were maintained at normal room temperature *i.e.* 25 ± 5 ºC with humidity 55-65% under a 12 h light (08:00 a.m. - 08:00 pm.) and dark (08:00 p.m. - 08:00 a.m.) cycle. They were given standard feed and had free access to purified water. Group I, II, III and IV were control, standard, single dose and double dose groups of Hot plate and Tail flick models respectively with 10 mice in each group. Group V, VI, VII and VIII were control, standard, single dose and double dose groups for acetic acid induced paw-edema procedure respectively with 10 rats in each group. Helsinki Resolution 1964 specifications were adopted for animal handling. This research was conducted after approval by Board of Advanced Studies and Research (BASR), University of Karachi [BASR/No./03460/Pharm.Resol.No. 10(P) 04].

Preparation of Aqua Distillate of *Rosa damascena* Mill.: Fresh flowers of *Rosa damascena* Mill. were collected from Botanical garden of University of Karachi, Karachi and were authenticated and identified by Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi [Voucher no: RDF-01-16/17]. Petals were separated and allowed to air-dry at room temperature. Aqua distillate of *Rosa Damascena* was prepared by distillation process. Distillation was performed in a distillation unit with stainless steel tank, cohobation column, condenser and receiver. Air-dried *Rosa damascena* petals (3500 g) were placed in the distillation unit along with 10 liters of water. Air vent was closed after complete removal of air and the unit is then operated as a closed system to distill the rose flower under high temperature and pressure. The vapors produced were cohobated in cohobated column and condensed in a receiver with cold water circulation. The rate of distillation, temperature and pressure were maintained and controlled throughout the distillation process. Distillation was completed after collection of 2.5 liters of distillate [9].

Dosing Protocol: Control groups were given distilled water only. Aspirin 300 mg per 70 kg and Ibuprofen 200 mg per kg were given as standard drug in hot plate & tail flick and acetic acid induced paw-edema model respectively [10, 11]. Single and double dose groups were given aqua distillate of *Rosa damascena* Mill. flower in the doses of 250 mg per kg and 500 mg per kg respectively [12].

Evaluation of Antinociceptive Potential:

Hot Plate Method: To evaluate anti-nociceptive activity of *Rosa damascena* Mill. hot plate method was used. After 30 min of oral administration of distilled water, standard drug and test drug to Group I, II, III and IV respectively, each animal was placed in hot plate made up of plexiglas cylinder with a maintained temperature of 51 ºC ± 1 ºC. Time for jumping or paw licking was observed individually at 30, 60, 90, 120 and 180 min. Cut-off time was 30 sec to avoid any tissue damage [13].

Tail Flick Method: Tail flick method was also used in this study to evaluate anti-nociceptive activity of *Rosa damascena* Mill. Half an hour after oral administration of distilled water, standard drug and test drug to Group I, II, III and IV respectively, each animal's tail was immersed individually in water maintained at a temperature of 55 ºC ± 1 ºC. Time taken by animal to withdraw its tail from hot

water was observed at 30, 60, 90, 120 and 180 min. To prevent any damage to the tissues, cut-off time was set at 10 sec [14, 15].

Acetic Acid Induced Paw Edema Method: Acetic acid in conc. of 1% was used to induce inflammation in paw. 0.1 ml of 1% acetic acid was injected to rat's hind paw in the sub-plantar tissue after 30 min of oral administration of distilled water, standard drug and test drug to Group V, VI, VII and VIII respectively. Plethysmometer was used to observe the signs of inflammation or edema in the rat's hind paw. Hind paw of each rat was dipped in the plethysmometer measuring tube. When the hind paw is immersed in the tube, water is displaced which is sensed by platinum electrode. Plethysmometer determines the change in conductance and an output result is transmitted on digital display that shows measured volume displacement (resolution - 0.01 ml). Observations were taken just before and immediately after administration of acetic acid and at 1, 2, 3 and 4th hour post acetic acid administration[16]. Formula used to calculate percent inhibition in edema was [17]:

$$\frac{\text{Paw edema of control} - \text{Paw edema of treated}}{\text{Paw edema of control}} \times 100$$

Statistical Analysis: Data was analyzed by SPSS version 20. All values in the table were presented as mean ± SD (n=10). One-way ANOVA followed by multiple comparison post-hoc analysis was used for statistical calculations where $P<0.001^{***, ###}$was highly significant, $P<0.01^{**, ##}$ was very significant and $P<0.05^{*, #}$ was significant in comparison to control and standard respectively.

RESULTS:

Hot Plate Method: Table 1 shows the anti-nociceptive effect of *Rosa damascena* Mill. using hot plate method. Analysis by one-way ANOVA followed by post-hoc Tukey's test showed significant analgesic effect of *Rosa damascena* Mill.

In comparison to control, standard and our test drug (*Rosa damascena* Mill.) at both doses *i.e.* 250 mg/kg and 500 mg/kg highly significantly ($p<0.001$) increased the latency time at 30, 60, 90, 120 and 180 min. Maximum analgesic effect was observed at 120 min against both doses.

In comparison to standard, our test drug (*Rosa damascena* Mill.) at both doses *i.e.* 250 mg/kg and 500 mg/kg does not affect the latency time and showed similar analgesic response as that of standard drug except at 180 min, where the test drug in dose of 500 mg/kg significantly ($p<0.05$) increased the latency time.

Tail Flick Method: Table 2 shows the anti-nociceptive effect of *Rosa damascena* Mill. using Tail Flick method. Analysis by one-way ANOVA followed by post-hoc Tukey's test showed significant analgesic effect of *Rosa damascena* Mill.

In comparison to control, standard and our test drug (*Rosa damascena* Mill.) at both doses *i.e.* 250 mg/kg and 500 mg/kg highly significantly ($p<0.001$) increased the latency time at 30, 60, 90, 120 and 180 min. Maximum analgesic effect was observed at 120 min against both doses.

In comparison to standard, our test drug (*Rosa damascena* Mill.) at both doses *i.e.* 250 mg/kg and 500 mg/kg does not affect the latency time and showed similar analgesic response as that of standard drug except at 60 and 120 min, where the test drug in dose of 250 mg/kg significantly ($p<0.05$) and highly significantly ($p<0.001$) decreased the latency time respectively.

Acetic Acid Induced Paw-Edema Method: Table 3 shows the anti-inflammatory effect of *Rosa damascena* Mill. using acetic acid induced paw edema method. Analysis by one-way ANOVA followed by post-hoc Tukey's test showed significant anti-inflammatory effect of *Rosa damascena* Mill.

In comparison to control, standard and our test drug (*Rosa damascena* Mill.) at both doses *i.e.* 250 mg/kg and 500 mg/kg showed highly significantly ($p<0.001$) decrease in rat paw edema at 1, 2, 3 and 4 h post acetic acid administration.

In comparison to standard, our test drug (*Rosa damascena* Mill.) at both doses *i.e.* 250 mg/kg and 500 mg/kg showed highly significantly ($p<0.001$) increase in rat paw edema at 1, 2 and 3 h post acetic acid administration. At 4th hour post acetic acid administration, there was no difference between standard and test drug.

TABLE 1: ANALGESIC EFFECT OF *ROSA DAMASCENA* MILL. BY HOT PLATE METHOD

Groups	Latency time in seconds					
	0 min	30 min	60 min	90 min	120 min	180 min
Control (I)	7.18±0.68	7.30±.75	7.60±0.26	7.44±0.63	7.09±0.63	7.13±0.37
Standard (II)	7.27±0.75	***12.54±1.16	***14.10±1.03	***15.09±0.76	***16.01±0.42	***12.88±0.73
Test (III)	7.16±0.74	***12.26±1.61	***14.43±0.49	***15.0±0.87	***15.99±0.19	***13.15±0.51
Test (IV)	7.20±0.65	***12.35±1.7	***14.89±0.73	***15.56±0.93	***16.23±0.41	***#13.59±0.68

TABLE 2: ANALGESIC EFFECT OF *ROSA DAMASCENA* MILL. BY TAIL FLICK METHOD

Groups	Latency time in seconds					
	0 min	30 min	60 min	90 min	120 min	180 min
Control (I)	0.89±0.66	0.9±0.77	0.87±0.58	0.86±0.65	0.85±0.45	0.84±0.54
Standard (II)	0.87±0.38	***2.51±0.33	***2.98±0.4	***4.26±0.62	***4.9±0.1	***2.85±0.56
Test (III)	0.85±0.84	***2.46±0.29	***#2.54±0.32	***4.31±0.32	***###4.54±0.31	***2.79±0.65
Test (IV)	0.91±0.88	***2.39±0.2	***2.65±0.24	***4.5±0.2	***4.75±0.66	***2.91±0.57

TABLE 3: ANTI-INFLAMMATORY EFFECT OF *ROSA DAMASCENA* MILL. BY ACETIC ACID INDUCED PAW EDEMA METHOD

Groups	Before Drug treatment (displacement in ml) Mean ± S.D	Immediately after acetic acid administration (displacement in ml) Mean ± S.D	Results shown as Displacement in "ml" and percent inhibition after acetic acid administration	1h	2h	3h	4h
Control (V)	2.24±0.12	2.26±0.18		3.65±0.1	3.87±0.1	4.28±0.09	3.86±0.14
Standard (VI)	2.20±0.19	2.23±0.21		***2.75±0.09 (24.65%)	***2.82±0.09 (27.13%)	***2.97±0.07 (30.60%)	***2.96±0.06 (23.31%)
Test (VII)	2.34±0.34	2.30±0.12		***###2.99±0.08 (18.08%)	***###3.24±0.11 (16.27%)	***###3.26±0.1 (23.83%)	***3.14±0.29 (18.65%)
Test (VIII)	2.39±0.11	2.41±0.28		***#2.89±0.07 (20.82%)	***###3.21±0.08 (17.05%)	***###3.25±0.09 (24.06%)	***3.07±0.07 (20.46%)

DISCUSSION: This study was designed to explore analgesic and anti-inflammatory potential of *Rosa damascena* Mill. flower at two different doses after its aqueous distillation by using hot plate, tail flick and acetic acid induced paw edema method and to compare its effects with standard drug.

The results of hot plate and tail flick model confirmed that distillation of *Rosa damascena* Mill. flower in aqueous medium showed good analgesic activity comparable to standard reference drug Aspirin at both doses of 250 mg/kg and 500 mg/kg with maximum analgesic activity observed at 120 min.

The main components of *Rosa damascena*flower are flavonoids, terpenes, tannins, ascorbic acid etc. [18]. Analgesic potential of *Rosa damascena* can be due to the presence of flavonoids, as flavonoids are involved in delaying the arachidonic acid metabolism of cyclogeneses pathway [19]. Flavonoids are also involved in inhibition of phospholipase A_2, cyclooxygenases and lipoxygenases and in turn can inhibits the prostaglandins and leukotrienes production [20]. Thus flavonoids rich composition of *Rosa damascena* can be one of the main reason of its analgesic potential.

A study conducted in 2008 reported that ethanolic extract of *Rosa damascene* possesses good analgesic activity while chloroformic and aqueous extracts have no analgesic response [21]. On the other hand, our results showed significant analgesic response of *Rosa damascena* aqua distillate. As discussed earlier the analgesic activity of this plant can be due to the presence of flavonoids and phenolic compounds. But difference in analgesic activity with respect to solvent extracts can be due to the fact that maximum efficacy of biological substances depends upon the extraction process and selection of solvents [22]. Yield of flavonoids and phenolic compounds highly depends on solvent's polarity. With decrease in polarity of solvent from methanol to chloroform, the yield of phenolic compounds also decreases [23]. This can be the reason that ethanolic extract of *Rosa damascena* showed analgesic and aqueous and chloroformic extract showed no analgesic activity in 2008 study.

Also in our study aqua distillation of *Rosa damascena* showed significant anti-nociceptive activity which can be due to the fact that distillation of *Rosa damascena* petals yields good source of phenolic compounds and flavonoids which in turn is responsible for its significant analgesic activity[24]. Thus in view of these observations we can say that distillation improves the analgesic activity of *Rosa damascena* flower.

The results of acetic acid induced paw edema method confirmed the anti-inflammatory potential of aqua distillate of *Rosa damascena* Mill. flower by both doses *i.e.* 250 mg/kg and 500 mg/kg. The anti-inflammatory effect can be due to presence of phenolic compounds and flavonoids which are responsible for inhibition of prostaglandins as discussed above. Also, its anti-oxidant nature can be a cause of its anti-inflammatory activity [25]. It is commonly observed that if any plant possesses anti-oxidant property than it will show significant anti-inflammatory response [26]. Moreover, the plant also possesses anti-histaminic activity and has an anti-allergic effect which further contributes to its anti-inflammatory response [27].

The anti-inflammatory response of *Rosa damascena* was slightly lower than that of standard drug ibuprofen. Ibuprofen is a potent inhibitor of prostaglandin-E_2 which is the most common eicosanoid found in inflammatory conditions ranging from acute edema to chronic arthritis [28]. Anti-inflammatory effects of NSAIDs are due to inhibition of cyclooxygenases along with several inflammatory mediators such as inflammatory cytokines, interleukins *etc.* which makes them potent anti-inflammatory agents [29]. *Rosa damascena* Mill. also inhibits cyclooxygenase pathway as discussed above but their ability to inhibit inflammatory markers is still questionable, which can be one of the possible reason that ibuprofen showed significant and our test drug showed mild anti-inflammatory activity.

Thus, the findings of this study confirmed that distillation of *Rosa damascena* Mill. in aqueous medium enhances its analgesic and anti-inflammatory potential.

CONCLUSION: In the light of our results, it is concluded that aqua distillate of *Rosa damascena* Mill. flower possesses significant analgesic and anti-inflammatory activity at doses of 250 mg/kg and 500 mg/kg. It can be used in traditional system of medicine as an anti-inflammatory agent and analgesic in managing and treating various inflammatory conditions. Also as majority of conventional pain killers are associated with unavoidable adverse effects, therefore, it can be a good choice for pain management. Further research could be done to investigate its use in humans in different conditions and to determine its exact mechanism of action.

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