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ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF ROSA DAMASCINA MILL - AN INVITRO ANALYSIS

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ABSTRACT

Introduction: Rosa damascena Mill (Rose) is a shrub 1–2 mts in height from the Rosaceae family. Flowers of this plant are large, showy and colorful. Rosa damascena today are highly cultivated all over the world, because it is a popular garden plant and for fragnance. In addition to its perfuming effect, flowers, petals and hips (seed-pot) of Rosa damascena are used for medical purposes. **Aims and Objectives:** To check these medicinal values of this plant specifically the anti-inflammatory, analgesic and wound healing property this study was undertaken. The aim of this study is to check anti-inflammatory, analgesic and wound healing properties of Rosa Damascena Mill. **Materials and Methods:** For the application convenience the rose oil converted into two forms one is GEL and other is MOUTH WASH. This was done in JSS College of Pharmacy, Ooty. To check the effects of prepared rose gel and mouthwash it has been tried on 24 Wister rats and 24 Albino mice with the approval of animal ethical committee of JSS College of Pharmacy, Ooty. **Results:** Animal study confirmed the analgesic, anti-inflammatory and wound healing properties of Rosa damascena mill. To check analgesic property of R.damacena mill two tests were carried out; A.Acetic acid induced Writhing in Mice B. Hot plate method. Both of them proved presence of analgesic activity. Carrageenins induces paw edema test showed the anti-inflammatory property R.Damascena Mill. **Conclusion:** These two invitro studies proved that the medicinal properties of R.Damascena mill.

KEYWORDS: Rosa damascena mill, anti-inflammatory, analgesic, wound healing.

INTRODUCTION

Rose is a common name given to the thorny shrubs and climbing vines of the genus Rosa in the Rosaceae family. More than 100 Rosa species have been recorded throughout the world. One of these is **Rosa damascena Mill.**[1,2]

Rosa damascena Mill is an erect shrub 1–2 m in height from the Rosaceae family. Flowers of this plant are large, showy and colorful.[3] Rosa damascena today are highly cultivated all over the world, because it is a popular garden plant and for fragnance.[4] In addition to its perfuming effect, flowers, petals and hips (seed-pot) of Rosa damascena are used for medical purposes.[5,6]

To check the medicinal values of this plant specifically the anti-inflammatory, analgesic and wound healing property this study was undertaken.For this study commercially available Rose oil is used. According to the manufacturer rose oil was derived from dried petals by steam distillation method.[7] This study was conducted at JSS College of Pharmacy, Ooty.

AIM AND OBJECTIVES

The aim of this study is to check anti-inflammatory, analgesic and wound healing properties of Rosa Damascena Mill.

MATERIALS AND METHODS

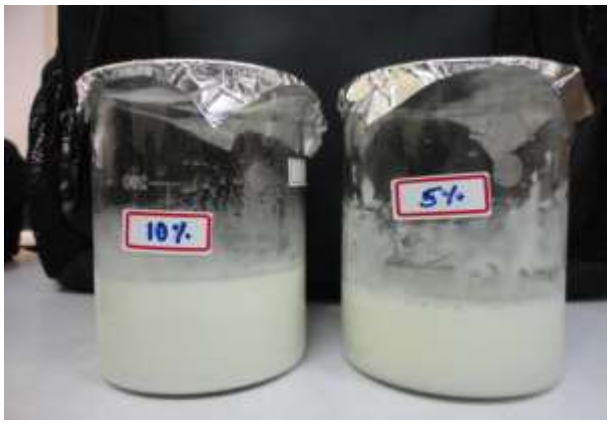
In order to apply with convenience the rose oil is converted into two forms, one is GEL and other is MOUTH WASH. This preparation was done in JSS College of Pharmacy, Ooty. The procedure followed to prepare GEL and MOUTH WASH is explained below:

Formulation of Mouth Gel and Mouthwash

Preparation of Mouth Gel

All the required ingredients of the formulation were weighed accurately. Hydroxy propyl methyl cellulose (HPMC K14) was dispersed in 50 ml of distilled water maintained at 70°C. The dispersion was stirred at 70°C for 20 min using a magnetic stirrer. Then poly ethylene glycol-400, sucrose, citric acid, and preservatives (methylparaben / propylparaben) were added with

stirring, required amount of sodium citrate was dissolved in 10 ml of distilled water and added to the mixture. Finally, rose oil was added slowly under stirring and speed was maintained appropriately. The mixture was allowed to cool to room temperature to form gel (**image:1**).



The composition of gel is shown in table 1.

S. No.	Ingredients	F1 (%)	F2 (%)
1	Rose oil	5	10
2	HPMCK14	3	3
3	PEG 400	10	10
4	Sucrose	2	2
5	Sodium citrate	0.05	0.05
6	Citric acid	0.2	0.2
7	Methyl paraben	0.2	0.2
8	Water	Q.S.	Q.S.

PREPARATION OF MOUTH WASH

The composition of mouthwash is shown in table 2.

S. No.	Ingredients	F1 (%)	F2 (%)
1	Rose oil	5	10
2	Tergitol-N9	2	2
3	Water	Q.S.	Q.S.

Rose oil and tergitol-N9 was taken and added to the water under stirring at appropriate speed to make the mouth wash (**Image: 2**)

Triton X-**100**(TX-100) (Hydrophillic nonionic Surfactant)
Tween-40 (T-40) (water soluble Nonionic surfactant)
Span—80 (s-80) (Oil soluble Nonionic surfactant)
Tergitol-N9 (N-9) (water soluble Nonionic surfactant)
All are based on **USFDA** Approved standards.

To check the effects of prepared rose gel and mouthwash, it has been tried on 24 Wister rats and 24 Albino mice with the approval of animal ethical committee of JSS COLLEGE OF PHARMACY, Ooty. Animal study confirmed the analgesic, anti-inflammatory and wound healing properties of Rosa damascena mill.(report attached).

ANALGESIC ACTIVITY

A. Acetic acid induced Writhing in Mice

Procedure

Acetic acid induced writhing method was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing is considered as a positive response.

Drugs

- Acetic acid 1% v/v (1ml/100 g) inject 1ml/100 g body weight of mice (i.p)
- Aspirin - (Dose 25 mg/kg i.p Stock solution containing 2.5 mg/ml of the drug) and
- Test drugs – A,B,C,D (1ml/100 g) inject 1ml/100 g body weight of mice (i.p)

Swiss albino mice weighing between 25-30g were used for evaluation of analgesic activity.

1. The animals were divided in to six groups of six animals each.
2. Group-I Acetic treated group, Group-II Aspirin and acetic acid treated group and Group-III –VI ABCD and acetic acid treated groups.
3. Approximate volume of acetic acid solution was administered to first group (control) and was placed individually for observation.
4. The onset and severity of writhing response was noted. The number of abdominal contractions, trunk twist response and extension of hind limbs as well as the number of animals showing such response during a period of 10 min were recorded.
5. Aspirin and test drugs ABCD were injected to II to VI groups respectively. Fifteen minutes later, acetic acid solution was administered to these animals. The onset and severity of writhing response was noted.
6. The mean writhing scores and percentage inhibition of control, aspirin and ABCD treated group were calculated.

Table 3: Analgesic Activity by Acetic Acid Induced Writhing in Mice.

Treatment	Dose (mg/kg)	No. of Wriths in 10 mins	Inhibition (%)
Control	-	25.17 ± 0.70	-
Aspirin	25	11.67 ± 0.42*	56
A	5	17.24 ± 0.36*	41
B	10	15.36 ± 0.25*	40
C	5	14.83 ± 0.31*	44
D	10	13.50 ± 0.42*	48

B. Hot plate method

Procedure

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging to the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch.

Drugs

- Acetic acid 1% v/v (1ml/100 g) inject 1ml/100 g body weight of mice (i.p).
- Aspirin - (Dose 25 mg/kg i.p Stock solution containing 2.5 mg/ml of the drug). and
- Test drugs – A,B,C,D (1ml/100 g) inject 1ml/100 g body weight of mice (i.p).

Swiss albino mice weighing between 25-30g were used for evaluation of analgesic activity.

1. The animals were divided in to six groups of six animals each.
2. Group-I Control (Normal saline), Group-II Aspirin and Group-III –VI ABCD.
3. The basal reaction time was taken by observing hind paw licking or jump response (which ever appear first) in animals, when placed on the hot plate maintained at constant temperature (55^0C).
4. Normally animals show such response in 6-8 sec. A cut off period of 15 sec is observed to avoid damage to the paws.
5. Aspirin and test drugs ABCD were injected to their respective group animals and the reaction times of animals on hot plate were noted at 30,60, 90 and 120 min.
6. When the reaction time increases, 15 sec was taken as maximum analgesia and the animals were removed from the hot plate to avoid injury to paws. The results were tabulated in **Table 4.**

Table 4: Analgesic Activity by Hot Plate Method in Mice.

Treatment	Dose (mg/kg)	Reaction time in seconds at time Minutes				
		0	**30**	**60**	**90**	**120**
Control	-	7.33 ± 0.33	7 ± 0.36	**6.83** ± 0.30	**7.12**± 0.36	**7.16** ± 0.37
Aspirin	25	7.16 ± 0.3	**5.83** ± 0.37*	**5** ± 0.25*	**3.14** ±0.41*	**3.33** ± 0.211*
A	5	7.16 ± 0.16	**5.33** ± 0.21*	**4.33** ± 0.21*	**3.64** ± 0.31*	**3.83** ± 0.30*
B	10	7.83 ± 0.16	**5.56** ± 0.22*	**4.16** ± 0.16*	**3.66** ± 0.21*	**3.83** ± 0.16*
C	5	7.43 ± 0.18	**5.50** ± 0.26*	**4.5**± 0.34*	**3.16** ± 0.30*	**3.66** ± **0.21***
D	10	7.15 ± 0.12	**5.54** ± 0.36*	**3.66** ± 0.21*	3± 0.25*	3.5 ± **0.22***

ANTI-INFLAMMATORY ACTIVITY

Carragennin induces paw edema

1. The Wistar albino rats were divided into six groups of six animals each as follows.
2. **Group I** - Solvent control 1ml/kg 0.3% CMC orally, **Group II** - Indomethacin 10 mg/kg in 0.3% CMC orally and **Group III- VI** - A,B,C,D (1ml/100 g) inject 1ml/100 g body weight of mice (p.o).
3. Acute paw edema was produced by injecting carragennin 1% w/w (0.1ml) into the sub plantar region of the left hind paw in the rats.
4. The test drugs A, B, C and D and Indomethacin 10 mg/kg administered orally one hour before testing. The control group received vehicle 0.1 ml/100gm.
5. The paw volume was measured by using digital plethysmometer (UGO Basile. Italy) at 0, 1, 2, 3, 4, and 6 hrs after carrageenin challenge. The percent increase in the edema (paw volume) was calculated by comparing it with zero minute reading.
6. The percentage inhibition of edema was calculated at 4^{th} hour assuming 100% inflammation in vehicle group.

RESULTS

Analgesic Activity

a) Acetic acid induced writhing in mice

Results were shown in Table 3. Data were analyzed by using One-way ANOVA followed by Tukeys test. All the test and standard drugs significantly ($p<0.05$) reduced the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid comparing to the control. The test drugs ABCD showed 41, 40,44 and 48 % of inhibtion against control and however standard drug showed 56%.

b) Hot Plate Method in Mice

Results were shown in Table 4. Data were analyzed by using One-way ANOVA followed by Tukey's test. All the test and standard drugs showed significant change ($p<0.05$) in reaction time compared to control. Treatment groups showed decrease in reaction time from 30 to 90 minutes and thereafter showed increase in reaction time in 120 minutes and the same effect was also observed with standard drug aspirin.

ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of test drugs on carrageenin (0.1ml) induced paw oedema indicated that test drugs had significant ($p<0.05$) anti-inflammatory activity from 3^{rd} hour onwards and it was maintained up to 6^{th} hour, when compared to control group. (**Table. 5).**

Table 5: Inhibitory effects of test drugson carrageenan-induced edema of the hind paw in rats.

Group	Dose (mg/kg)	Swelling volume (mL)				
		1hr	2hr	3hr	4hr	6hr
Control	-	1.53±0.07	2.33±0.09	3.31±0.05	3.65±0.06	3.38±0.07
Indomethacin	10	1.54±0.05	1.86±0.10	2.13±0.08*	2.11±0.12*	1.89±0.06*
A	5	1.56±0.21	2.29±0.21	2.98±0.11*	2.59±0.43*	2.46±0.04
B	10	1.52±0.19	2.14±0.06	2.58±0.44*	2.49±0.07*	2.43±0.25*
C	5	1.57±0.68	1.98±0.23	2.16±0.51*	2.02± 0.14*	1.93±0.08*
D	10	1.57±0.58	1.96±0.43	2.15±0.51*	2.01± 0.14*	1.93±0.08*

DISCUSSION

Most of the modern medicines are based on the synthetic derivatives of plants. Since these derivatives are having their own adverse effects, the major Pharmaceutical companies started to do research in herbs as an alternative. R.Damascena is such a plant which is being used since longtime for its medicinal properties such as analgesic, anti inflammatory and wound healing.[8]

The analgesic effect

The analgesic effect of R. damascena is also reported. In a study, the effect of aqueous, ethanolic and chlorphormic extracts in mice on hot plate and tail flick was evaluated and only ethanolic extract showed analgesic effect.[9] However, studies have shown that hydroalcoholic extract has a potent analgesic effect in acetic and formalin tests and no effect on tail flick test.[10] In our study we used acetic acid induced writhing in mice and hot plate method to evaluate the analgesic effect of R. Damascena Mill. Test results showed promising effect ($p< 0.05$) in comparison with other drugs (**Table: 4**). Based on the study's results, it can be derived that ingredients of the plant that not soluble in water may be responsible for the analgesic effect. The non water soluble components of rose oil quercetin and kaempferol may be responsible for the analgesic effect.[11,12]

The anti-inflammatory effect

Studies have shown that R.damacena mill is having anti-inflammatory effect.[13,14] The effect of anti inflammatory property was demonstrated by carragennin induced paw edema test. In this study it has been proved that, the R.Damascena mill oil gel also showed the same results as the previous studies (TABLE.5). Essential oil had no anti-inflammatory effect while the extract could significantly reduce edema which may be acted by inhibiting the mediators of acute inflammation.[15] In addition, R. damascena contains vitamin C which has antioxidant and anti-inflammatory effects.[16]

Antioxidant effects

The phenolic compounds present in the plants are the causative agents for antioxidant properties.[17] The presence of phenolic compounds in R.damascena mill was proven by Kumar et al in 2009 and determined the antioxidant effects by 1,1-picrylhydrazyl (DPPH) free radical analytical method[18] & they also proved antioxidant property of hydro alcoholic of petals and essential oil of R.damascena mill. In addition to these flavonoid glycosides of R.damascena mill quercetin-3-0-glucoside, kaempferol-3-0-rhamnoside and kaempferol-3-0-arabinoside have antioxidant activities. Invivo studies showed that R.damascena mill also having inhibitory effect on lipid oxidation. These antioxidative inhibitory effects are comparable with α tocopherol and can be utilized in the prevention of free radicals.[19] This anti oxidant property helps indirectly in the wound healing tendency of R.Damacena Mill.[20]

CONCLUSION

The main advantages of using herbs are easy availability, cost effectiveness and less toxicity. Herbs are widely used in dentistry for the purpose of anti inflammation, analgesic, antibacterial and acceleration of wound healing. They also aid in healing and are effective in controlling microbial plaque in gingivitis and periodontitis and thereby improving immunity. Though the in vitro results showing promising hope, more clinical trials are needed to evaluate the biocompatibility and safe factor before they can be recommended for clinical use conclusively.

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Conflicts of Interest: NIL.

Criteria for inclusion in the authors: They have contributed in this study design, analyzing data and writing article.

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