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SCREENING OF DAMASK ROSE ESSENTIAL OIL IN WISTAR ALBINO RATS FOR ANTIULCER ACTIVITY

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ABSTRACT

Introduction: Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of population in their life time. In recent years; abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. **Objectives**: The present study was carried out to investigate antiulcer activity of *Rosa damascena mill* belonging to family Rosaceae in the albino rats. **Methods**: Pyloric ligation and alcohol induced ulceration methods were used. Ranitidine (20mg/kg) was used as the standard drug. Rose oil was preliminary subjected to the acute oral toxicity study according to OECD guidelines no: 423 based on which, two dose levels i.e. 250 and 500 mg/kg were selected for the further study. In pylorus ligation induced ulcer model, various parameters were studied viz. gastric volume, total acidity, free acidity and ulcer index. Ulcer index and percentage inhibition of ulceration were determined by alcohol induced ulcer model. **Results**: Rose oil at 500mg/kg shown 68.36% inhibition in alcohol induced ulcer model and 56.5 % in pyloric ligation induced ulcer model. **Conclusions** In conclusion, rose oil tested in this investigation deserves further attention due to its importance in prevention and treatment of gastric ulcers. Further molecular level studies are to be done for knowing its exact mechanism of action.

KEYWORDS: *Rosa damascene*, Ulceration, Ligation, Phytochemicals.

INTRODUCTION

Peptic ulcer therapy has undergone many studies over past few years and a number of synthetic drugs are now available for treatment. Reports on clinical evaluation of these drugs show that there are incidences of relapses and several adverse effects and danger of drug interaction during therapy.[1,2] The development of new antiulcer drug from medicinal plants is an attractive proposition because diverse chemical compounds have been isolated from different medicinal plants with antiulcer activity[3] and have been shown to produce promising results in the treatment of gastric ulcers.[4] The bioactive molecules (generally alkaloids, glycosides, essential oils etc.) are isolated/extracted from crude drugs may be used directly as therapeutic agents or as starting materials for the synthesis of useful drugs or serve as a model for pharmacologically active compounds in the period of drugs in synthesis.[5]

An extensive literature survey reveals no pharmacological validation of antiulcer activity of this plant. This made us to screen this plant for antiulcer activity in a scientific manner. Therefore based on the above facts, the present study has been under taken with the main objective of evaluating the extract of plant flower for antiulcer activity using Albino Wistar Strain Rats as experimental animal model.

MATERIALS AND METHODS

DRUGS AND REAGENTS

The chemicals used in the present study were analytical grade Ethanol (90%), Anesthetic ether, Sodium hydroxide, Phenaphthelien indicator, Topers' reagent, Spirit, Ranitidine, Povidone powder from.

EXPERIMENTAL ANIMALS

Albino Wistar strain rats (either sex) weighing 100-150gms were used. The animals were maintained in well-ventilated room temperature with 12/12 natural day-night cycle, in polypropylene cages. They were fed balanced rodent pellet diet obtained from Mahaveera enterprises, Ghatkesar and tap water throughout the experimental period. The animals were housed for one week prior to the experiments to acclimatize to laboratory conditions.

Grouping

The animals were randomly distributed into four different groups with six animals in each group. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

ACUTE TOXICITY STUDIES

The procedure was followed by using OECD guidelines-423 (Acute Toxic Class Method).

The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally Harmonized System (GHS) for the classification of chemical which causes acute toxicity. The extract of *Damask rose oil mill*. starting dose of 2000 mg/kg body weight p.o. was used as most of the crude extracts possess LD50 value more than 2000 mg/kg. Body weights of the rats before and after treatment were noted and any changes in skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behavior pattern were observed. Sign of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted, if any.

EVALUATION OF ANTIULCER ACTIVITY

Dosage fraction

Damask rose oil was administered at different doses (1, 2, 2.5, 5 g/kg p.o/day). $1/10^{th}$ and $1/20^{th}$ dose of lethal dose was selected (i.e.500mg/kg 250mg/kg).

Grouping

Group-1: Control group
Group-2: Ranitidine (20mg/kg)
Group-3: Rose oil (250mg/kg)
Group-4: Rose oil (500mg/kg)

Experimental procedure

Alcohol induced Ulcer procedure[6,7]

Animals were treated with test and standard drugs and rats were fasted for 24 hrs and were orally dosed with 0.04ml & 0.08 ml of 90% ethanol. The animals were sacrificed by cervical dislocation method 4-6hrs after the dose of ulceration. Stomach was removed, incised along with greater curvature and washed under running tap water. Ulcer score was observed.

Pyloric ligation procedure[7,8]

Albino Wistar strain rats of either sex weighing between (100-150gms) were divided into four groups of six animals in each. In this method albino rats were fasted in individual cages for 24 hrs. Care is taken to avoid caprophagy. Control vehicle, Standard drug Ranitidine 20mg/kg p.o, Rose oil of *Rosa damacena mill* 250 mg/kg, 500mg/kg p.o is administered to group I, II, III, IV respectively 30 minutes prior to pyloric ligation. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4hrs of ligation, the animals were sacrificed by cervical decapitation or deep anesthesia and the abdomen is opened and ligature is placed around esophagus, close to diaphragm. The stomach was dissected out, gastric juice was collected in graduated tubes and then centrifuged at 1000 rpm for 10 minutes and the volume is noted. The pH of the gastric juice was recorded by pH meter. Then the contents were subjected for analysis of free and total acidity. The stomachs were washed with running water to see for ulcers in glandular portion of the stomach. The number of ulcers per stomach was noted and severity of the ulcers scored microscopically with the help of hand lens (10 x) and scoring is done.[9,10]

0 = Normal stomach
0.5 = Red coloration
1 = Spot ulcers
1.5 = Hemorrhagic streaks
2 = ulcers > 3 mm but < 5mm,
3 = ulcers > 5mm

Percentage protection = $[100\text{-}U_t\backslash U_c] \times 100$

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection is calculated using the above formula where,

U_t = ulcer index of treated group and
U_c = ulcer index of control group

DETERMINATION OF FREE ACIDITY AND TOTAL ACIDITY

1ml of gastric juice is pipette out in 100ml conical flask, 2-3 drop of topfer's reagent is then added and titrated with 0.01 N sodium hydroxide until all traces of pink colour disappears and the colour of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Titration is continued until pink color of solution reappears. Again the total volume of alkali added is noted, this volume corresponds to total acidity.

Acidity (MEq/l/100g) can be calculated by using the formula.

Acidity = volume of NaoH x Normality of NaOH x100 MEq/l/100gm\01.

STATISTICAL ANALYSIS

The data was expressed as mean ± SEM. Results were analyzed statistically by One-way ANOVA followed by DUNNETT's TEST using standard statistical software package of social science (SPSS). The difference was considered significant if $p<0.05$.

RESULTS

The Rose oil was subjected to preliminary chemical screening for the presence or absence of active phytochemical constituents by following methods and resulted positively for the presence of alkaloids, carbohydrates, terpenes and anthocyanin's as shown in **Table-1** The extract did not produce any toxic symptoms of mortality up to the dose level of 2000 mg/kg body weight in rats and hence the drugs were considered safe for further pharmacological screening. According to the OECD-423 guidelines for acute oral toxicity, the LD50 dose of 2000 mg/kg and above is categorized as unclassified. Effect of Standard drug Ranitidine and test Rose oil on gastric volume, free acid, total acid, pH and ulcer index in Alcohol induced and pylorus ligated rats were studied. Table 2 and figure 1 represents the effect of standard and test drug in alcohol induced ulcers. Table 3 and figure 2 represents the effect of standard and test drugs in pylorus ligated rats.

Table: 1 Preliminary phytochemical test of Rose oil

Tests	Rose oil
Test for carbohydrates	
1. Molisch's test	+
2. Barfoed's test	-
3. Benedict's test	+
Test for Glycosides	
1.Borntranger's test	-
2.Legal's test	+
Test for Flavonoids	
1. Schinoda test	+
2. Alkaline reagent test	+
Test for anthocyanins:	+
Test For Terpenoids	
1.Noller's test	+

Table: 2 Effect of Rose oil on Alcohol induced ulcer in rat

Drug dose(mg/kg)	Ulcer area $(mm)^2$ (mean ± SD)	% Ulcer inhibition
Disease control	2.75±0.5	-
Ranitidine(20mg/kg)	0.5±0.00	81.2
Rose oil(250mg/kg)	1.87±0.25	31.8
Rose oil(500mg/kg)	0.87±0.48	68.36

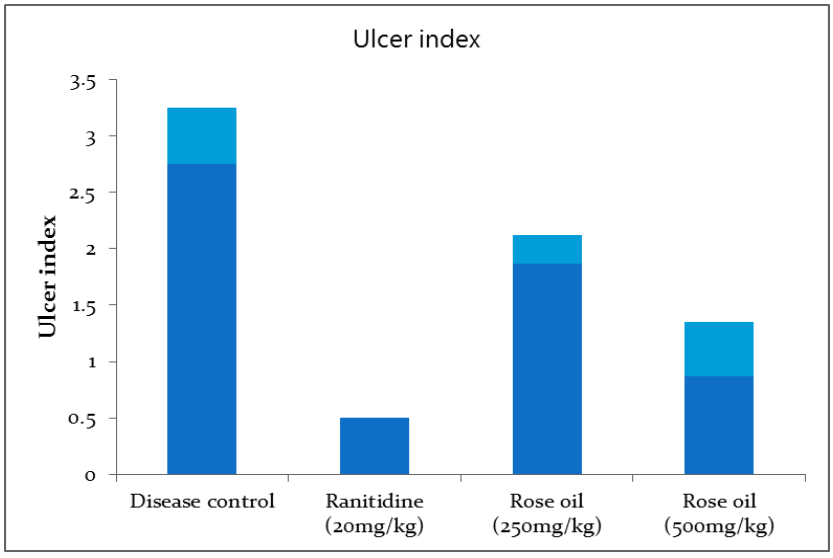

Fig: 2 Effect of Rose oil on Alcohol induced Ulcer in rats.

Table: 3 Effect of Rose oil on Pylorus ligated rats.

Drug	Drug dose(mg/kg)	Volume of gastric acid(ml)	Free acidμEq/l	Total acid(μEq/l)
Disease control	-	2.29±0.05	31.47±2.18	46.23±1.59
Ranitidine	20	0.95±0.05	10.92±1.41	17.15±1.10
Rose oil	250	1.75±0.06	25.84±1.82	36.97±2.83
Rose oil	500	1.26±0.04	15.24±3.14	23.12±2.24

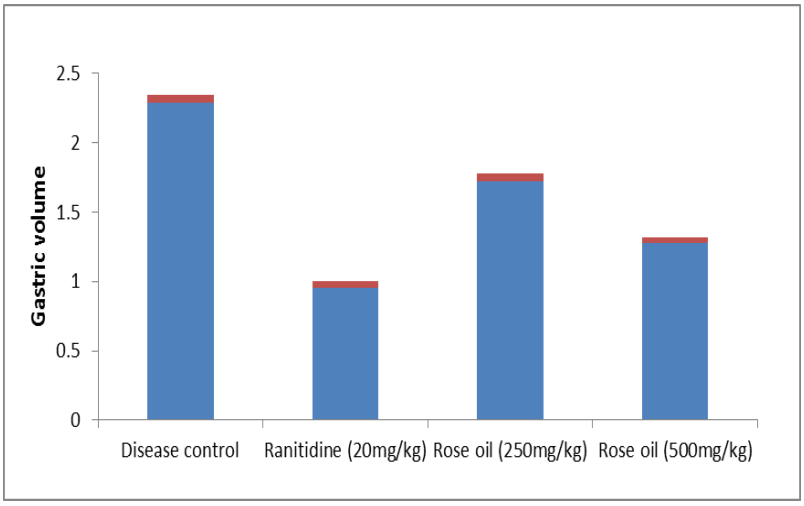

Fig: 3 Effect of Rose oil on Gastric volume

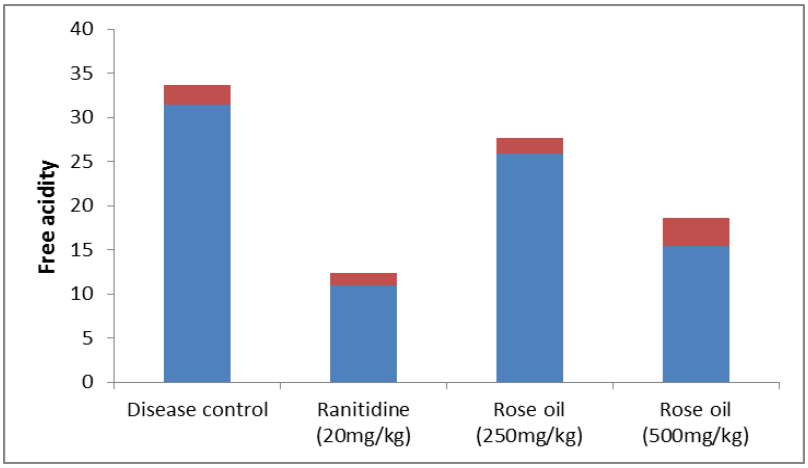

Fig: 4 The effect of rose oil on free acidity

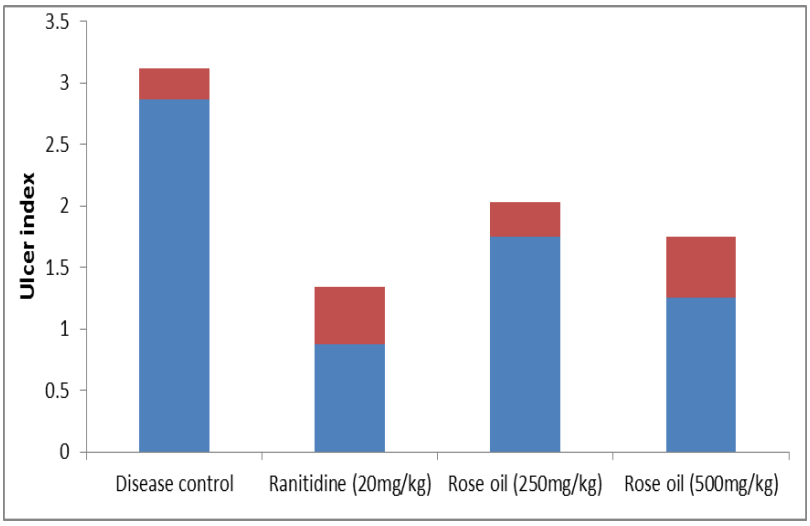

Fig: 5 The effect of rose oil on Ulcer index

Table: 4 Effect of rose oil on pylorus ligated ulcer in rats

Drug dose (mg/kg)	Ulcer area (mm)² Mean± SD	% Ulcer inhibition
Disease control	2.85±0.25	-
Ranitidine 20	0.87±0.47	69.5
Rose oil 250	1.75±0.28	39.1
Rose oil 500	1.25±0.5	56.5

DISCUSSION

Peptic ulcer disease (PUD) encompassing gastric and duodenal ulcers is the most prevalent GIT disorder that affects a considerable number of people in the world[11] and some authors consider gastric ulcer as the new "plague" of 21st century.[12] The defense potential of gastric mucosa depends upon a delicate balance between the processes affecting the synthesis and secretion of its mucin constituents. To regain the balance, different therapeutic agents including plant extracts are used. They inhibit the gastric acid secretion or encourage the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells, or interfere with the prostaglandin synthesis. Thus the primary therapeutic approach of an antiulcer agent involves maintenance of a delicate balance of gastric acid secretion and defense mechanism factors.[13] In the present study the anti-ulcer activity of *Rosa damascene* was explored with the help of pharmacological experiments and statistical analysis. The oil produced a dose dependent protection against alcohol induced peptic ulcer though not better than rantidine. Ethanol provoked gastric mucosal lesions by reducing the mucous production, decreasing the gastric mucosal blood flow and bicarbonate secretion. It also lowers endogenous glutathione and prostaglandin levels. It increases the release of histamine, influx of calcium ions, free radicals and leukotrienes which leads to the cause of peptic ulcers. As per the present investigation, in alcohol induced ulcer method, Ranitidine (20mg/kg), the standard drug reduced the ulcer index by 81.2% whereas rose oil in doses 250mg/Kg and 500mg/Kg reduced it by 31.8% and 68.36% respectively. In pylorus ligation method gastric volume, free acid, total acid and ulcer index were compared. Rantidine 20mg/Kg reduced the volume of gastric juice from 2.29 ± 0.05 to 0.95±0.05 (58.6%) whereas it was reduced to 1.72±0.06 (24.9%) and 1.28±0.04 (44.2%) by 250mg/Kg and 500 mg/Kg doses of rose oil.

The free acid levels were reduced in standard group from 31.47 ± to 10.92±1.41 (34.6%) and to 25.84±1.82 (17.9%), 15.24±3.14 (51.6%) in III and IV groups respectively. The total acid levels were also decreased from 46.23±1.59 to 17.15 ± 1.10 (37.09%) in standard group and to 36.97± 2.83(20.0%), 23.12±2.24 (50.0%) in animals treated with rose oil 250mg/Kg and 500mg/Kg respectively. Ulcer index was also reduced by 69.5% in standard group and by 39.1%, 56.5% in III and IV group respectively. The antiulcer effect of rose oil is may be due to presence of aliphatic alcohols.[20] The active constituents of the rose oil revealed by the phytochemical screening especially flavanoids, terpenoids and anthocyanins may play a contributory role in its anti ulcer activity.

CONCLUSION

In conclusion, rose oil tested in this investigation deserves further attention due to its importance in prevention and treatment of gastric ulcers. Further molecular level studies are to be done for knowing its exact mechanism of action.

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