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Study of Antiemetic Potential of *Ruta graveolens* Extracts by Copper Sulphate and *Brassica campestris* Induced Emesis in Chicks

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The antiemetic effect of aqueous and methanolic extracts of *R. graveolens* in young chicks was investigated. In addition, the phytochemical screening of the test plant and its acute toxicity studies were also conducted. Different doses of the extracts were tested for their antiemetic properties and were compared with the positive control antiemetic drug Chlorpromazine (150 mg/kg), Metoclopramide (50 mg/kg) and an untreated control (normal saline) against copper sulphate and *Brassica*-induced emesis. The phytochemical screening of *R. graveolens* showed that it contains certain alkaloids and flavonoids. It was found to be safe up to the dose of 2000 mg/kg body weight. The aqueous extracts in 50 and 150 mg/kg doses produced 41.49% and 66.49% inhibition of emesis, respectively by copper sulphate-induced emesis, while 34.66% and 57.95% inhibition of emesis, respectively by *B. campestris*-induced emesis. The methanolic extracts of *Ruta graveolens*

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in 50 and 150 mg/kg doses produced 46.80% and 70.20% inhibition of emesis, respectively by copper sulphate-induced emesis while 31.95% and 61.94%, respectively in *Brassica campestris*-induced chick emesis model. These results have suggested *R. graveolens* possess significant antiemetic properties that implicate its use as traditional medicine to treat emesis. However, further studies are needed to isolate the active principle(s) i.e. flavonoids contained in the plant drug tested and its real safety and efficacy as antiemetic agent.

Keywords: Ruta graveolens; antiemetic effects; toxicity testing; phytochemical screening.

1. INTRODUCTION

Although helpful, medicines are beyond the means of many people in rural areas where there are high rates of unemployment and rising costs of medicines. People should be empowered through education on the proper utilization of medicinal plants as first-aid remedies in the rural communities where there is limited access to medical facilities [1]. The natural medicinal plants used as traditional medicines are safe, effective, easily available and economical. Due to these benefits, the medicinal plants have been used frequently by the traditional medical practitioners in their daily use [2].

In the U.K. and U.S.A, herbs that have been generally used to treat emesis include such as *Agastache rugosa*, leaves of *Mentha piperita* flowers of *Eriobotrya japonica* Lindley and *Eugenia caryophyllata* fruits of *Cocos nucifera* L., and *Amomum cardamomum* L., roots of *Cyperus rotundus* L., rhizome of ginger [3] (Aslam,1997). Ethnomedical information showed that many antiemetic plants exist in Pakistan flora [4].

Ruta graveolens L.is evergreen shrub with strong odor belonging to the family Rutaceae.It is used as the source of Rue or Rue oil. It has considerable medicinal importance. More than 120 natural compounds including mainly acridone alkaloids, coumarines, essential oils, flavonoids, and furoquinolines have been found in the roots and aerial parts of this plant [5]. *Ruta graveolens* has been counted among the valuable plants of the European pharmacopoeia since ancient times; many other uses were also recognized by some of the greatest Greek and Roman authors including Hippocrates, Dioscorides and Pliny [6].

Kirthar and Basu [7] studied the use of *Ruta graveolens* L. for asthma, bronchitis, cough and as an expectorant. Other reported indications include the relief of pain, eye problems, rheumatism and dermatitis. *R. graveolens* has recently been shown to have antibacterial, analgesic, anti-inflammatory, antidiabetic and insecticidal activities [8].

Ruta graveolens have been used as antiemetic agent among inhabitants of Brazilian urban and rural areas but it was not scientifically validated till date. So an attempt was made in this study to evaluate the antiemetic potential of *Ruta graveolens* aqueous and methanolic extracts in chick models.

2. MATERIALS AND METHODS

2.1 Chemicals Used

Methanol (Merck Chemical Co, Darmastadt Germany), Copper sulfate (Merck Chemical Co, Darmastadt Germany), Chlorpromazine (Unexo labs, Lahore, Pakistan), Metoclopramide (Olive laboratories, Islamabad, Pakistan).

2.2 Plant Material and its Extraction

Ruta graveolens was collected from college of Agriculture, University of Sargodha in March, 2013. Collected plant was identified with morphological and taxonomic keys provided in various texts and by Botanist Dr. Ameen, Department of Botany, University of Sargodha. Voucher specimen was stored thereof. The plant was made free from foreign adulterants and vegetative debris by hand picking. Then it was washed and shade dried at room temperature for a week. Electrical herbal grinder (Electronica, Pakistan) was used to form powder. The powder was stored in airtight container for further use.

2.3 Aqueous Extract Preparation

Aqueous extract of *Ruta graveolens L.* (whole plant) was prepared following the method of Halvaei et al. [9]. The 200 g herb was immersed in 400mL of distilled water, put on stirrer for 48 hours in room temperature and a dark place, and filtered through a paper filter (11µm pore size). Afterwards, the container

was placed into a water bath for evaporation. The brown jelly extract was kept at -4 °C for further experiments.

2.4 Methanol Extract Preparation

The powdered material (2 kg) of plant was subjected separately to maceration in 70% methanol in amber colored glass bottle at room temperature for 8 days with occasional shaking [10]. The soaked material was passed through muslin cloth to remove the Vegetative material and the fluid obtained was filtered through Whattman-1 Filter paper(11µm pore size). The filtrate was evaporated on a rotary evaporator (Yamato, Rotary evaporator, model – RE 801) under reduced pressure to obtain dried crude extracts.

2.5 Animals

Young chicks of either sex; 10days old weighing 332-345 g were obtained from the Government poultry farm, Sargodha. They were divided into group of 6 and kept in different cages with marks of identification. All chicks were kept under laboratory conditions of room temperature with 12 hour light and dark cycle and were allowed to free access to food and water.

2.6 Acute Toxicity Studies

As illustrated by Hailu and Engidawork, [11], toxicity of *Ruta graveolens* methanolic extract was observed at doses of 500, 1000, 1500 and 2000 mg/kg/5mL p.o to albino mice. The animals were observed for overt toxicities like diarrhea, weight loss, tremor, lethargy and paralysis periodically for the first four hours during the 24hr period and then followed for 2 weeks for mortality.

2.7 Preliminary Phytochemical Screening

The extracts were subjected to different qualitative tests for identification of various active constituents using the methods described by Palanisamy et al., [12].

2.7.1 Identification of flavonoids

Sodium hydroxide test: 2 mL of the extract was dissolved in 10% aqueous sodium hydroxide solution and filtered to give yellow colour,a change in colour from yellow to colourless on addition of dilute HCl indicate the presence of flavonoids.

2.7.2 Identification of carbohydrates

A small quantity of methanolic extract was dissolved in 4mL of distilled water and filtered. The filtrate was subjected to following test to detect the presence of carbohydrates.

2.7.3 Fehling's test

The filtrate was treated with each 1ml of Fehling's solution A and B and heated on a water bath for the identification of carbohydrates.

2.7.4 Detection for fixed oils and fats

A small quantity of the extract was pressed separately between filter paper. Appearance of oil stains indicated the presence of fixed oils.

2.7.5 Detection of proteins and free amino acids

To the extract, equal volume of 5% sodium hydroxide and 1% copper sulfate solution were added. The violet colour produced showed the presence of proteins and free amino acids.

2.7.6 Detection of tannins and phenolic compounds

A small quantity of the extract was taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the reagents i.e. 5% ferric chloride solution and10% lead acetate solution.

2.7.7 Detection of alkaloids

0.5 g of the extract was stirred with 5 mL of 1% aqueous hydrochloric acid on a water bath and filtered. 3 mL of the filtrate was divided into three. To the first 1 mL few drops of freshly prepared Dragendroff reagent was added and observed for formation of orange to brownish precipitate. To the second,1 drop of Mayer reagent was added and observed for formation of white to yellowish or cream colour precipitate. To the third 1 ml, 1 drop of Wagner reagent was added to give a brown or reddish or reddish-brown precipitate.

2.7.8 Detection of gums and muscilages

A small quantity of the extract was added separately to 25mLof absolute alcohol with constant stirring and filtered. The precipitate was air dried and examined for its swelling properties that is indicative of the presence of gums and mucilage.

2.8 Determination of Antiemetic Activity by Copper Sulfate-induced Chick Emesis Model

The anti-emetic activity was determined following the protocols Akita, (1996). Each chick was set aside for 10 minutes to stabilize in a large beaker. The methanolic extract of *Ruta graveolens* was dissolved in 0.9 % normal saline containing 5 % DMSO and 1% tween 80 and administered in doses of 50 mg/kg b.w and 150 mg/kg b.w orally in a volume of 10 mg/kg to the chicks. The control group received only 0.9% normal saline. After 10 minutes, copper sulfate pentahydrate (as an emetic agent) was administered orally at 50 mg/kg, and then the number of retching was observed during next 20 minutes. Chlorpromazine and metoclopramide were used as standard antiemetic drug (100 mg/kg body weight).

2.9 Determination of Antiemetic Activity by *Brassica campestris*-induced Chick Emesis Model

Procedure was adopted for fresh aqueous extract of *Brassica campestris*-induced emesis as described by Musaddique, et al. 2015). 100 gm of powdered *B.campestris* (seeds and leaves) was taken and mixed with 500mL of distilled water. It was magnetically stirred overnight in a container at room temperature. The residue was removed by filteration and the aqueous extracts were kept on water bath for evaporation. Emesis was induced by *B.campestris* in aqueous solution.

Same each chick was placed in a large separate beaker and left to settle for 10 minutes. Aqueous and methanolic extracts of *Ruta graveolens* were prepared in a dose of 50 and 150 mg/kg body weight in a volume of 10ml/kg in 0.9% saline containing 5% DMSO and 1% Tween80. The dose was administered orally. The control group received 0.9% normal saline. After 10 minutes, fresh leaves extract of *Brassica campestris* was administered orally to irritate gastric mucosa. Then number of retches was observed during next 20 minutes. Chlorpromazine and metoclopramide were used as standard antiemetic drugs (150 and 50 mg/kg body weight respectively).

The percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = (A-B/A) \times 100$$

where: A= Frequency of retching in control group, B= Frequency of retching in test group

2.10 Statistical Analysis

Data for antiemetic activity was expressed as S.E.M. The software used to analyze the data was SPSS for Windows. The statistical significance of the difference was determined by analysis of variance ANOVA test followed by Student t test to evaluate their effect and comparison in both models respectively. Similar letters showed insignificance at 5% level of significance.

3. RESULTS

3.1 Phytochemical Screening of *Ruta graveolens*

Ruta graveolens powder was tested for the presence of tannins, phenolics, saponins, glycosides, flavonoids and alkaloids. Its leaves showed positive test for saponins, tannins, alkaloids, glycosides and flavanoids as summarized in Table 1.

3.2 Acute Toxicity Studies

R. graveolens was found safe upto 2000 mg/kg body weight of albino mice. Both extracts of the plant did not produce any visible signs of toxicity up to the dose of 2000 mg/kg. This was confirmed when there was no loss of weight, tremor, stress, paralysis, lethargy or adverse behaviors.

3.3 Antiemetic Activity Evaluation of Aqueous and Methanolic Extracts of *Ruta graveolens* L

Results of the antiemetic activity of aqueous and methanolic extracts of *Ruta graveolens* at various concentrations are given in Table 2 and 3. Both the extracts inhibited emesis to an extent greater than cyclizine at a dose of 150 mg/kg. At test dose of 50mg/kg *Ruta graveolens* showed same antiemetic activity as reference drug cyclizine but less than chlorpromazine. At test dose of 150 mg/kg *Ruta graveolens* showed more antiemetic activity as compared to cyclizine (reference drug) used. Highest antiemetic activity showed by *Ruta graveolens* was 79.22% inhibition and the lowest antiemetic activity showed was 58.94 % inhibition in copper sulphate emesis model. From Table 2 and 3, it is

clear that both extracts of *Ruta graveolens* possess antiemetic potential which is comparable to reference drugs. Chlorpromazine and metoclopramide showed 29.25% inhibition and 66.48% inhibition respectively and were somewhat similar in both models.

3.4 *Brassica campestris*-induced Chick Emesis Model

Table 3 shows the antiemetic activity of the aqueous and methanolic extracts compared with standards after inducing emesis with *Brassica campestris* at the dose of 50mg/kg body weight. Group I was taken as control group, Group II and III were administered standard antiemetic drugs Chlorpromazine and metochlopramide at the dose of 150 and 50 mg/kg body weight. Aqueous and methanolic extracts were each administered at the doses 50mg/kg body weight (Group III and Group V respectively) and 150 mg/kg body weight (Group IV and VI respectively). Values were presented as Mean ± S.E.M (n=????).

3.5 Comparison of Antiemetic Activity of Both Extracts of *Ruta graveolens*

Fig. 1 shows the comparison of antiemetic activity of *Ruta graveolens* aqueous extractand methanolic extract in copper sulphate and *Brassica campestris* induced chicks models.

Table 1. Phytochemical constituents and identification tests for *Ruta graveolens*

TEST	OBSERVATIONS	INFERENCES
Frothing test for saponins	Thick persistent froth for 10 minutes	Saponins present
Dragendroff reagent test	Presence of colored ppt.	Alkaloids present in excess
Ferric chloride test for tannins	Appearance of green color	Tannins present (hydrolysable tannins present)
Fehling test for carbohydrates	Brown ring appears at junction of two solutions.	Glycosides present
Test for flavonoids HCl test	Yellow colour solution does not turns colourless with HCl	Flavonoids present in excess.
Gums and mucilage	Swollen precipitates	Present
Resins	Turbidity formation	Present

Table 2. Antiemetic activity using Copper sulphate-induced chick emesis model

Sr.No.	Groups (N=6)	Mean number of retches ±S.E.M	Inhibition (%) of emesis	Significant digit
1	Control (Normal saline0.9%)10ml/kg	62.66 ± 3.28295	_____	D
2	Chlorpromazine 150mg/kg b.w.	44.33 ± 2.90593	29.25%	C
3	Metochlopramide 50mg/kg b.w.	21.00 ± 2.08167	66.48%	A
4	RGAE 50mg/kg b.w.	36.66 ± 2.02759	41.49%	BC
5	RGAE 150mg/kg b.w.	21.00 ± 57735	66.49%	B
6	RGME 50mg/kg b.w.	33.33 ± 1.45297	46.80%	B
7	RGME 150mg/kg b.w.	18.66 ± 2.18581	70.20%	A

Similar letter shows insignificant results at 5% level of significance; RGAE: Ruta graveolens aqueous extract and RGME: Ruta graveolens methanolic extract; RGAE: Ruta graveolens aqueous extract and RGME: Ruta graveolens methanolic extract

Table 3. Antiemetic activity using *Brassica* -induced chick emesis model

Sr.No.	Groups (N=6)	Mean number of retches ±S.E.M	Inhibition (%)ofemesis	Significant digits
I.	Control (Normal saline 0.9%)10ml/kg	58.67 ± 3.48010	--------	C
II.	Chlorpromazine 150mg/kg b.w.	44.33 ± 2.90593	24.44%	B
III.	Metoclopramide 50mg/kg b.w.	21.00 ± 2.08167	64.20%	A
IV.	RGAE 50mg/kg b.w.	38.33 ± 3.75648	34.66%	B
V.	RGAE 150mg/kg b.w.	24.67 ± 1.20185	57.95%	A
VI.	RGME 50mg/kg b.w.	40.33 ± 5.23874	31.26%	B
VII.	RGME 150mg/kg b.w.	22.33 ± 3.52767	61.94%	A

Similar letter shows insignificant results at 5% level of significance

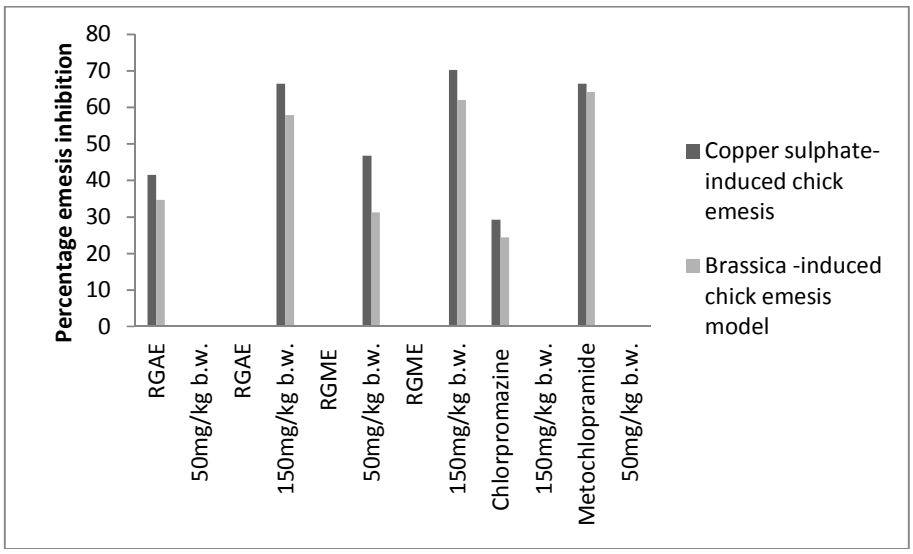

Fig. 1. Comparison of antiemetic activity of *Ruta graveolens* extracts

4. DISCUSSION

In the present study, *Ruta graveolens* L. known by the common name of Rue, Sudaab, sodab [13] was scientifically evaluated for its antiemetic potential. This is perhaps the pioneer study showing the antiemetic effect of *Ruta graveolens* and thus provides scientific basis for its use in folkloric medicine for the management of emesis in human patients.

On the basis of the results obtained in the present investigation, conceivably all the tested extracts possess anti-emetic potential comparable to reference standards i.e. chlorpromazine and metoclopramide. Chlorpromazine which has already been reported to produce antiemetic effect through acceleration of gastrointestinal tract movement [14] was seen to be even less effective than the aqueous and methanolic extracts though closer to the antiemetic effect produced by the 50mg/kg dose of the extracts. Considering the increase in antiemetic activity with the increasing doses (from 50 to 150 mg/kg), the antiemetic activity of aqueous and methanolic extracts against *Brassica campestris* and copper sulfate was dose dependent as shown in Fig. 1.

For antiemetic activity, flavonoids are known to play a key role as described by Kinoshita et al. [15]. Hariparasad [16] has observed that *Rumex vesicaeius L.* showed antiemetic activity in chick emesis model and flavonoids and terpenes were considered the major active constituents responsible for this activity. Phytochemical screening of *Ruta graveolens* showed the presence of flavanoids and alkaloids along with resins (Table 1). Thus, antiemetic effect of the test extracts may be caused by the presence of these flavonoids. In another study, Hassan et al. [17] have reported that the antiemetic activity may also be the result of alkaloidal content(s) present in the extract. Similarly, it was proposed on the basis of the presence of excessive amounts of alkaloids present in *Ruta graveolens* L. that this plant's active principle could be its alkaloid. However, these results need to be confirmed in separate experiments. Also the activity oriented fractionation studies are still required to further specify the constituent (s) responsible for the anti-emetic activity.

Copper sulfate-induced retching in chicks is a useful experimental model in screening for anti-emetic effects of plant extracts and other natural compounds. It was therefore selected for present anti-emetic evaluation study. This model is considered comparable with acute emesis seen in man and hence, serves as a useful model for evaluating the involvement of the brain in the observed anti-emetic effects of the *R. graveolens* extracts (Salman et al.,2012).Furthermore, Nosiri (2009) has demonstrated that oral copper sulfate induces emesis by peripheral action through stimulation of visceral afferent nerve fibres of the gastrointestinal tract and fact that peripheral 5-HT_4 plays a vital role in copper sulphate-induced emesis. Interestingly, the test extracts were able to effectively prevent this induced antiemetic effect too. Therefore, it could be implied that the test extracts also possess a peripheral antiemetic potential. The peripheral antiemetic activity of plant was may be due to the chelation of copper sulfate by chelating compounds present in the extract (e.g. tannins, alkaloids and other negatively charged compounds). The action of extract may be as a physical barrier might have decreased the irritant effect of copper sulphate on gastric mucosa.

In the present investigation, there was significant retardation of *B. campestris*-induced emesis may be inferred that aqueous and methanolic extracts of *Ruta graveolens* might also be acting centrally (on CTZ) as well as peripherally (on gastric mucosa) to exert the antiemetic effects. Moreover, aqueous and methanolic extracts have also effectively prevented emesis induced by oral copper sulphate indicating the peripheral antiemetic activity of these extracts as the mechanism of emesis induced by copper sulphate is mainly peripheral [18]. It has also been established that the peripheral 5- HT_4 receptors play an important role in copper sulphate-induced emesis [19].

Moallem et al. [20] have observed direct relationship between polarity of extracts and their antiemetic activity. It is therefore suggested that the active principle responsible for antiemetic effect may be non-polar in nature because in the aqueous extract of *R. graveolens*, non-polar constituents perhaps are not present or exist in very low concentrations; showing no effect. However, non-polar nature of methanol has possibly helped to picked up the higher concentrations of the non-polar constituents. Thus, as shown in Table 2, a very promising antiemetic activity has been observed to the extent that it could even precede the positive control at 150mg/kg dose in copper sulphate-induced emesis model. Methanolic extract of *Ruta graveolens* at dose 150mg/kg body weight showed highest 70.20% and 61.94% inhibition of retches in copper sulphate and *Brassica* models, respectively where as aqueous extract of *Ruta graveolens*at dose of 150mg/kg body weight showed lower i.e. 66.49% and 57.95% inhibition of retches in copper sulphate and *Brassica* models respectively (Table 2 and 3).

5. CONCLUSION

From the present investigation it was clear that the *Ruta graveolens* L. aqueous and methanolic extracts possess antiemetic activity and methanolic extract shows more antiemetic potential than the aqueous extract. The safety profile of the extracts is an added benefit that provides for executing further research to ascertain the findings reported in this study. Further investigations can be done to identify and isolate the active constituents such as flavonoids, alkaloids responsible for antiemetic potential and further toxicological and hematological studies to authenticate it as a potent antiemetic agent with fewer side effects.

CONSENT

It is not applicable.

ETHICAL APPPROVAL

The animals were treated complying with the international standards duly approved by the Ethical Committee for Animals, University of Sargodha

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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