



Antioxidant and Healing Effect of Aqueous alcoholic Extract of *Rhodomyrtus tomentosa* (Ait.) Hassk on Chronic Gastric Ulcers in rats

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ABSTRACT

Rhodomyrtus tomentosa is a medicinal plant used traditionally in gastric disorders like colic, diarrhoea, dysentery, abscesses, haemorrhage, and as an antiseptic wash for wounds. Based on the ethnomedical information of the plant, the antiulcerogenic activity of an aqueous alcoholic (70%) extract of *Rhodomyrtus tomentosa* was investigated using acetic acid-induced chronic ulcer model in rats. The reduction in ulcer index followed by increase in the levels of superoxide dismutase (SOD) and catalase (CAT) and decrease in lipid peroxidation proved the antiulcer and antioxidant activity of the extract. Histopathological studies further proved the gastroprotective property of the extract. It was concluded that the extract possesses significant anti-ulcer activity, which can be attributed to its antioxidant mechanism of action. The presence of triterpenoids, flavonoids and phenolic compounds is probably related to the potent antiulcerogenic and antioxidant activity.

Keywords: *Rhodomyrtus tomentosa*, Acetic acid, antiulcer, antioxidant activity.

INTRODUCTION

Oxidative stress has been implicated in the pathogenesis of gastrointestinal disorders including gastric ulceration. It has been reported that most injury of the gastric mucosa can be reduced by pre-treatment with scavengers of reactive oxygen species.^[1]

The reactive oxygen species generated by the metabolism of macrophages, platelets, arachidonic acid and smooth muscle cells may contribute to gastric mucosal damage. Therefore, by scavenging the free radicals, the reactive oxygen metabolites might be useful by protecting the gastric mucosa from oxidative damage or by accelerating healing of gastric ulcers^[2]. Mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals^[3]. In the present study, acetic acid has been used to produce mucosal damage as it produces gastric lesions which is similar to human chronic ulcers.

Rhodomyrtus tomentosa (Ceylon hill gooseberry) of family Myrtaceae is traditionally used in colic, diarrhoea, dysentery, abscesses, haemorrhage, and as an antiseptic wash for wounds, and abscesses.^[4] *In vitro* antioxidant activity and gastroprotective activities of the different extracts of *Rhodomyrtus tomentosa* leaves on ethanol induced mucosal damage^[5] has been reported. The present studies was conducted to evaluate the antiulcerogenic effect of the aqueous alcoholic extract of *Rhodomyrtus tomentosa* leaves in terms of its *in vivo* antioxidant status.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Rhodomyrtus tomentosa* were collected in the month of April / June from Ooty district of Tamil Nadu. The plant was identified, confirmed and authenticated by Field Botanist, Dr. S. Rajan. The shade dried leaves was then ground separately into coarse powder by a mechanical grinder. The resulting coarse powder was then used for extraction.

Preparation of Extracts^[6]

The powdered leaves were subjected to successive hot extraction in soxhlet apparatus with various solvents in the increasing order of polarity, such as Petroleum ether, chloroform, ethyl Acetate, aqueous alcohol (70%) and water. The extracts

were filtered, and dried under vacuum in a rotary flash evaporator and the yields of Petroleum Ether, Chloroform, Ethyl Acetate, aqueous alcohol and water extracts were 0.4%, 11%, 15%, 18% and 16% respectively.

Phytochemical screening^[7]

Preliminary phytochemical screening of the powdered leaves extracts was performed to detect the presence of alkaloids, phenolics, flavonoids, saponins, carbohydrates, steroids and terpenoids.

Animals

Albino rats of Wistar strain of either sex weighing between 150-200g were used. They were housed in standard cages at room temp. (25±2°C) and provided with food and water ad libitum. The animals were deprived of food for 24 h before experimentation, but had free access to drinking water. The study was conducted after obtaining institutional ethical committee clearance bearing the number DSCP/PhD/PCol /IAEC/08/08-09.

Drugs and Chemicals

Chemicals of standard grade were purchased from S.D Fine Chemical Pvt. Lt. India, Merck Specialties Pvt. Lt. and Omeprazole, as standard drug was supplied by Dr.Reddy Laboratory, Hyderabad.

Acute Toxicity Studies

Six rats were fasted overnight and were administered a single oral dose (2000 mg/kg, *b.w.*) of the aqueous alcoholic extract of *Rhodomyrtus tomentosa*. After the administration of the extract, food was withheld for further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily, cage side observations including changes in skin and fur, eyes and mucous membrane etc were observed. Mortality, if any, was determined over a period of 2 weeks.

Selection of the Dose of the Extract

LD₅₀ was done as per OECD guidelines for fixing the dose for biological evaluation. The LD₅₀ of the extracts as per OECD guidelines falls under class four values with no signs of acute toxicity at 2000 mg/kg *b.w.* The antiulcer evaluation of the extract was carried out at 100, 200 and 400mg/kg *b.w.*

Evaluation of Antiulcer Activity using acetic acid induced gastric ulcers

The antiulcer activity of the aqueous alcoholic extract of *Rhodomyrtus tomentosa* was evaluated using acetic acid induced chronic gastric ulcer model.^[8] Glandular portion of the stomach was collected and mucous barrier^[9] was estimated. Estimation of *in vivo* antioxidant enzymes like catalase,^[10] superoxide dismutase^[11] and lipid peroxides^[12] in stomach tissue homogenates were carried out by standard methods

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Short term toxicity of the extract during the study was studied by observing the RBC and WBC counts on the initial and final day and the changes in body weight, food and water intake during the course of experiments.

Statistical analysis

Values were expressed as mean ± SEM. The experimental mean values were compared statistically with that of vehicle control using One Way Analysis of Variance (ANOVA) followed Dunnett's multiple comparison test. The analysis was carried out using Graph Pad Prism software V.4. p values less than 0.5 were considered to be statistically significant.

RESULTS

Effect of *Rhodomyrtus tomentosa* on ulcer protection and healing rate:

Treatment with the aqueous alcoholic extract of *Rhodomyrtus tomentosa* at 100, 200 and 400 mg/kg significantly reduced the ulcer area from 40.22 in 4th day control to 20.40, 10.42 and 8.15 respectively corresponding to the healing rate of 22.72, 60.53 and 69.12 respectively. 400mg/kg was comparable to that of Omeprazole (20mg/kg) treated group (p<0.01). (Table 1)

Effect of *Rhodomyrtus tomentosa* on mucus content

Mucus content of rats treated with *Rhodomyrtus tomentosa* was increased (P<0.001) but the 4th day control animals showed significant decrease (P<0.001) in the mucus content compared to ulcerated control group. The activity of the extract was comparable to that of Omeprazole 20mg/kg treated group (p<0.001). (Table 1)

Table 1: Effect of *Rhodomyrtus tomentosa* on ulcer area and healing rate of chronic acetic acid induced gastric ulcer in rats

Group	Treatment	Dose (mg/kg)	Ulcer area (mm ²)	Healing rate (%)	Mucus content (µg alcian blue/g wet glandular tissue)
I	Non ulcerated control	-	0.00±0.00	-	92.44±0.68
II	Ulcerated control	-	26.40±2.66	-	36.02±0.44
III	4 th day control	-	40.22 ± 1.14	52.34	22.40±0.60
IV	Omeprazole	20	4.40±0.19***	83.33	90.48±0.22***
V	<i>Rhodomyrtus tomentosa</i>	100	20.40 ± 0.9 ^m	22.72	54.44±0.62***
VI	<i>Rhodomyrtus tomentosa</i>	200	10.42±0.68**	60.53	70.26±0.46***
VII	<i>Rhodomyrtus tomentosa</i>	400	8.15±0.43**	69.12	84.32±0.42***

Values are expressed in terms of mean ± S.E.M, ***P<0.001, **p<0.01, *p<0.05 Vs control group.

Histopathological data of acetic acid induced chronic ulcer model.

Histopathology of the stomach of the 4th day control animals showed focal ulceration and few areas of necrosis within the gastric mucosa. The mucosal layer was oedematous and infiltrated by mixed inflammatory cells predominantly comprising of lymphocytes, neutrophils and macrophages. The submucosal layer showed scattered mononuclear inflammatory infiltration along with some congested vascular spaces and areas of haemorrhage. The muscular and serosal layers however appeared within normal limits.

Rats treated with 100mg/kg of extract showed gastric mucosa with intact lining epithelium. The mucosal layer and submucosal layer show dilated and congested vascular spaces but the muscular and serosal layers appeared within normal limits. Rats treated with 200mg/kg showed gastric mucosa with intact lining epithelium. Minimal oedema and infiltration was seen. However rats treated with 400mg/kg of the extract showed complete restoration of gastric epithelium with regular glands, minimal oedema and infiltration. Omeprazole treated groups showed no ulceration, inflammation and infiltration.

Effect of *Rhodomyrtus tomentosa* on Catalase, Superoxide dismutase and Lipid peroxidation activity

The results revealed that at 100, 200 and 400 mg/kg dose levels, the extract significantly increased the catalase and the superoxide dismutase in the glandular tissue. The activity of the 400mg/kg group was comparable to that of Omeprazole at 20mg/kg (p<0.01). At all dose levels (100, 200 and 400 mg/kg) the extract decreased the LPO level significantly in the glandular tissue, in comparison to the 4th day control group. The activity of the 400mg/kg group was comparable to that of Omeprazole at 20mg/kg (p<0.001). (Table 2)

Table 2: Effect of *Rhodomyrtus tomentosa* on in-vivo antioxidant activity in acetic acid induced ulcers in rats

Group	Treatment	Dose (mg/kg)	CAT (unit/min/mg protein)	SOD (unit/min/mg protein)	LPO (unit/min/mg protein)
I	Solvent control (Non ulcerated)	-	36.24±1.24	1.34±0.08	3.24±0.66
II	Solvent control (Ulcerated)	-	28.00±1.09	0.52±0.04	10.62±0.66
III	4 th day control (Ulcerated)	-	24.26±0.48	0.16±0.38	14.22±0.12
IV	Omeprazole	20	44.24±1.08**	1.28±0.08*	3.42±0.08***
V	<i>Rhodomyrtus tomentosa</i>	100	34.02±0.61	1.22±0.24	8.24±0.13***
VI	<i>Rhodomyrtus tomentosa</i>	200	34.24±0.84**	1.24±0.22	5.02±0.42***
VII	<i>Rhodomyrtus tomentosa</i>	400	36.80±0.44**	1.22±0.22*	4.88±0.44***

Values are expressed in terms of mean ± S.E.M, *P<0.05, ** P<0.01, ***P<0.001, p<0.01 Vs Ulcerated control group

Short term toxicity evaluation of *Rhodomyrtus tomentosa*

The results indicated that the extract caused a nonsignificant (p>0.05) change in the body weight, food and water intakes when compared to nonulcerated control groups. Red blood cell count and White blood cell count data did not show any statistically significant change in all the extract treated groups when compared with non-ulcerated control group. No statistically significant behavioural changes were noted in all the extract treated groups of compared with non-ulcerated control groups. (Table 3)

Table 3: Effect of *Rhodomyrtus tomentosa* on the RBC and WBC count in chronic acetic acid induced gastric ulcer in rats

Group	Treatment	Dose (mg/kg)	Red blood cells (× 10 ⁶)		White blood cells (× 10 ³)	
			Initial	Final	Initial	Final
I	Non ulcerated control	-	7.66±2.53	7.28±3.73	6.80±2.44	6.78±1.53
II	Ulcerated control	-	7.56±2.79	7.29±4.67	6.78±2.42	6.64±1.27
III	Omeprazole	20	7.66±4.58	7.38±4.05	6.70±2.64	6.74±1.48
IV	<i>Rhodomyrtus tomentosa</i>	100	7.80±4.37	7.41±2.28	6.72±3.74	6.86±2.85*
V	<i>Rhodomyrtus tomentosa</i>	200	7.88±3.46	7.42±1.05	6.44±2.78	6.80±2.31*
VI	<i>Rhodomyrtus tomentosa</i>	400	7.00±3.59	7.49±2.09	6.24±3.40	6.88±2.69*

Values are expressed in terms of mean ± S.E.M, *P<0.05, Vs Ulcerated control group

Fig. 1. Section of ulcerated stomach obtained from rats of control group treated in acetic acid induced ulcer model

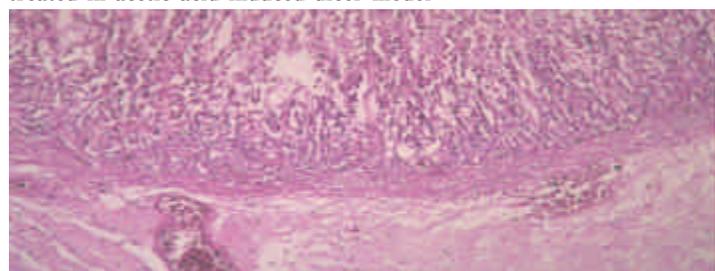


Fig. 2. Section of ulcerated stomach obtained from rats of the group treated with *Rhodomyrtus tomentosa* (100mg/kg) in acetic acid induced ulcer model

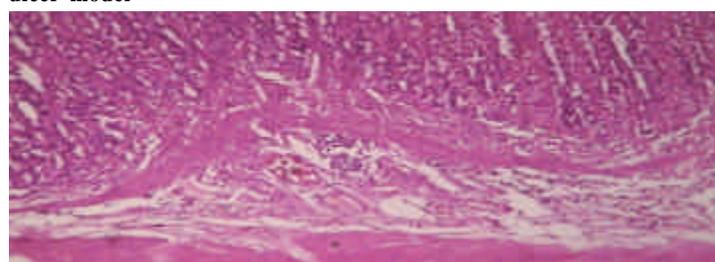


Fig. 3. Section of ulcerated stomach obtained from rats of the group treated with *Rhodomyrtus tomentosa* (200mg/kg) in acetic acid induced ulcer model

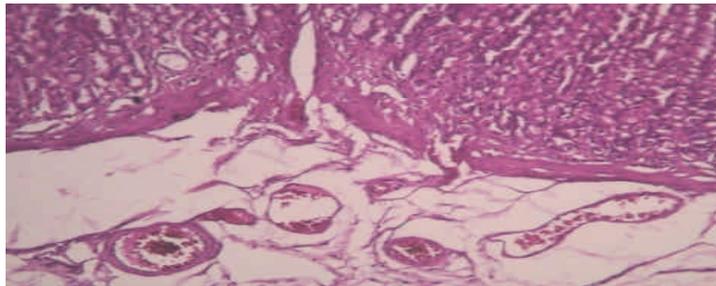


Fig. 4. Section of ulcerated stomach obtained from rats of the group treated with *Rhodomyrtus tomentosa* (400mg/kg) in acetic acid induced ulcer model

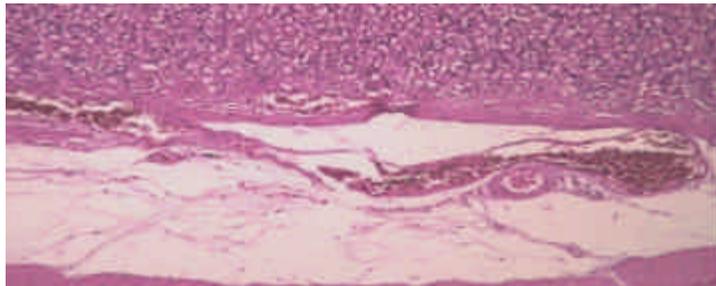
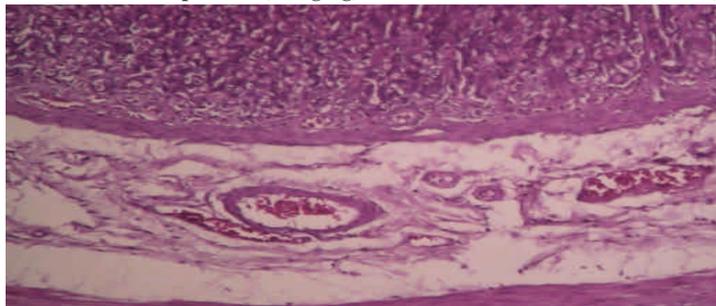


Fig. 5. Section of ulcerated stomach obtained from rats of the group treated with Omeprazole (20mg/kg) in acetic acid induced ulcer model)



DISCUSSIONS

One of the major underlying factor for gastric ulcers is the generation of free radicals. Free radical scavengers play an important role in the healing of gastric ulcers. Antioxidative therapy is known to stimulate the healing of therapy resistant ulcers.^[13] It has been widely accepted that antioxidants like alfa tocopherol and carotenoids play a significant role in protecting membranes from oxidative damage.

In the present study oral administration of 100, 200 and 400mg/kg of the aqueous alcoholic extract of *Rhodomyrtus tomentosa* for 20 days caused a significant reduction in the occurrence of gastric mucosal lesions in a dose dependant manner.

Earlier studies revealed that the different extracts of the plant was effective in the scavenging of DPPH, ABTS and Nitric oxide radicals. The extract of the plant

was also found effective in the prevention of ulcers induced by ulcerogens like ethanol. Our present study also revealed the healing effect of the extract in chronic ulcers induced by acetic acid which was confirmed by histopathological studies. The mucous production was also found to be increased during the experimental period. Preliminary phytochemical analysis and GCMS standardisation of the extract revealed the presence of important phytoconstituents like triterpenes. Triterpenoids are reported to possess antiulcer and antioxidant activity^[14]. Mucous is capable of acting as an antioxidant and can reduce mucosal damage mediated by oxygen free radicals. Hence the mucous production and the ulcer healing may be attributed to the presence of triterpenes in the extract. Oxygen handling cells have antioxidant enzymes such as CAT and SOD which are the first line of cellular defence against oxidative injury^[15] and are known to decrease the gastric mucosal damage induced by ulcerogens. Many researchers have also proved that antioxidants play an important role in the protection of gastric mucosal injury and inhibits the progression of gastric ulcers.^[16] In our present study, the SOD and catalase activity was significantly increased in the glandular tissue whereas the lipid peroxidation was decreased in a dose dependant manner.

Since the study involved 20 days of treatment with the extract it was required to look for toxicity signs during the treatment period. However no observable signs of toxicity was noted.

In conclusion it can be said that the aqueous alcoholic (70%) extract of *Rhodomyrtus tomentosa* exhibit a protective and healing effect on gastric ulcers through a free radical scavenging effect and reduces oxidative damage caused by acetic acid. A further detailed study on various other parameters is required to elucidate the exact mechanism of action for its use in the treatment of gastric ulcers.

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