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Antiulcer activity of *viscum articulatum* burm f. (viscaceae)

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ABSTRACT

Nature serves humans with medicines which were used to treat and heal many ailments. The anti-ulcer activity of methanolic extract of *Viscum articulatum* Burm.F (Viscaceae) was investigated in Pyrolus ligation ulcer and Ethanol induced ulcer models in Wistar albino rats. Methanolic extract of *Viscum articulatum* (MEVA) at doses of 200 mg/kg p.o, 400 mg/kg p.o produced significant inhibition of the gastric lesions in both the models. The extract (200 mg/kg & 400 mg/kg) showed significant ($P<0.01$) reduction in gastric volume, free acidity and ulcer index when compared to control. Results suggest that methanolic extract was found to possess anti-ulcerogenic as well as ulcer healing properties, which might be due to its anti-secretory activity.

Keywords: *Viscum articulatum*, Pylorus ligation ulcer, Ethanol induced ulcer model, Ulcer index.

INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors. Ulcer is caused due to the continuously exposure of gastric mucosa to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion (Watkinson, 1988), inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants (Yoshikawa, 1993), platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (Susumu Okabe, 1983) (mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide). The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources. *Viscum articulatum Burm.F* (Viscaceae) was one of the traditional medicines used in many folkclaims. This plant is a small shrubs as parasitic, monoecious or dioecious. It grows up to 20-50 cm tall, in regions with high sunlight. Branches opposite, dichotomous, or verticillate; internodes terete or flattened with successive internodes at right angles to each other. Leaves reduced to pairs of ± scarious scales. The plant has been extensively used in Ayurveda as ulcer healing drug (Pradhan, 2007) and also used in treating epilepsy, Epistaxis, Urinary Tract Infection, Rheumatoid arthritis, Leucorrhea, gout, Low back pain, Lumbar muscle strain, as Natriuritent and as anti tumour agent. *Viscum articulatum Burm.F* contains tannin, Oleanolic acid, glycosides, steroids, Poly phenols, Amino acids, Saponins. The pharmacological activities previously reported are Anti Epileptic activity, Anti-carcinogenic, Diuretic and natriuretic activity. However there are no reports on the antiulcer activity of the plant hence the present study was designed to verify the claims of the native.

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MATERIAL AND METHODS

Plant Collection

The whole plant of *Viscum articulatum* Burm. F was collected from Tirumala hills, Chittore district, Andhra Pradesh during the month of November. It was identified and authentificated by Prof. P. Jayaraman Ph.D., Director-Plant Anatomy Research Centre (PARC), Chennai. The voucher specimen number is PARC/2011/898 and it was submitted to Sree Vidyanikethan College of pharmacy, for further reference.

Preparation of extract

The whole plant of *Viscum articulatum* Burm. F was shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was

then subjected to successive extraction by Soxhlet method using petroleum ether, chloroform, and methanol, water solvents in a soxhlet extractor. The different extracts obtained were evaporated at 45°C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of Alcoholic extract was found to be 15.8%w/w and the methanolic extract (MEVA) was used for further studies.

Preliminary phytochemical screening

The phytochemical examination of the MEVA was performed by the standard methods.

Animals used

Twelve-week-old female (body weights around 150–200 gm) and male (body weights around 200–250 gm) albino rats of Wistar strain were used for the present study. They were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. SVCP/ IAEC/930/a/20-0053/CPCSEA).

Ethanol induced ulcer model

Albino wistar rats were divided into six groups of six animals each.

1. Group-I – Control. (60% (v/v) ethanol 1ml in oral administration).
2. Group-II – Standard (Sucralfate suspension (100 mg/kg p.o).
3. Group-V – Ethanolic extract of *Viscum articulatum* (200mg/kg p.o.).
4. Group-VI – Ethanolic extract of *Viscum articulatum* (400mg/kg p.o.).

Adult Albino Wistar rats, 6 per group, were tested according to the method of (Biswaas, 2003). Thirty minutes after drugs administration, each rat was given orally solution of in 60% (v/v) ethanol. The animals were sacrificed one hour later. The stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcer score was determined. MEVA and was suspended in distilled water and administered at the doses of 200 and 400 mg/kg, respectively. Control groups received saline water. Sucralfate suspension (100 mg/kg) was given orally as reference drugs. The tissues were subjected to Histo-pathological studies. Ulcer index was calculated as per the formula given below (Gerhard Vogel, 2002).

Pylorus ligation ulcer model

Animals are divided into four groups, each consisting of six rats (Bandyopadhyay, 2002). Control group were received distilled water orally. Omeprazole, in the dose of 20 mg/kg was being administered orally for Group two as a reference drug for ulcer protective studies. Third & Fourth Groups received methanolic extract of *Viscum articulatum* in a dose of 200 and 400 mg/kg.. After 45 min of MEVA and Omeprazole treatment, pyloric ligation was be done by ligating the pyloric end of stomach of rats of respective groups under ether anaesthesia at a dose of 35 mg/kg of body weight. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed.

Scoring of ulcer

0 = Normal stomach; 0.5 = Red coloration; 1 = Spot ulcers; 1.5 = Haemorrhagic streaks; 2 = Ulcer > 3 mm but > 5 mm; 3 = ulcers > 5 mm.

Ulcer index=UA+US+UP/10

Where, UA=Average number of ulcers per animal, US=Ulcer severity score, UP=Percentage of animals with ulcers. UP=Total ulcers in a group/total number of animals x 100.

Percentage ulcer inhibition was calculated by the formula,

Percentage inhibition=UIC-UIT/UIC X 100

Where UIC=Ulcer index of control group, UIT= Ulcer index of test group.

Histopathology

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity of *Viscum articulatum* Burm.F The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5 μm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for Pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes.

Statistical analysis

The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of one way ANOVA, followed by Dunnet's test where $P \geq 0.01$.

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the Methanolic extract of *Viscum articulatum*, MEVA revealed that presence of flavonoids, carbohydrates, glycosides, tannins, steroids, Phenols and absence of fixed oils and terpenoids.

Ethanol-induced gastric ulcer

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black & dark red lesions. MEVA has shown significant protection index of 41.07% and 60.83% with the dose of 200 and 400 mg/kg respectively in comparison to control, Sucralfate suspension (100 mg/kg) as reference standard drug was

reduction of ulcer 45.5%. Results are tabulated in Table 1.

Table: 1. Ulcer index and Percentage inhibition in Ethanol induced gastric ulceration model

S.No.	*Group*	*Ulcer index*	*% inhibition*
1.	Control	26.17±0.91	-
2.	Standard	14.25±0.82*	45.5%
3.	MEVA 200mg/kg	15.42±0.53*	41.07%
4.	MEVA 400mg/kg	10.25±0.70*	60.83%

MEVA– Methanolic extract of *Viscum articulatum*. *p<0.01 compared to control

Table: 2. Ulcer index and Percentage inhibition in Pylorus ligation ulcer model

S.No.	*Group*	*Vol of gastric juice(mL)*	*Free acidity (MEQ/ L)*	*Total acidity (MEQ/ L)*	*Ulcer index*	*% inhibition*
1.	Control	8.70±0.24	90.4±2.52	125.3±2.85	10.5±0.50	-
2.	Standard	2.89±0.25	36.10±2.68	41.35±2.26	2.58±0.32*	75.42%
3.	MEVA 200mg/kg	6.08±0.43	78.3±3.21	109.7±2.90	5.86±0.92*	44.12%
4.	MEVA 400mg/kg	3.02±0.42	37.67±1.73	48.84±1.42	2.93±0.31*	72.19%

MEVA– Methanolic extract of *Viscum articulatum*. *p<0.01 compared to control

Fig: 1. Anti-ulcer activity of *V. articulatum* in Ethanol induced gastric ulceration model

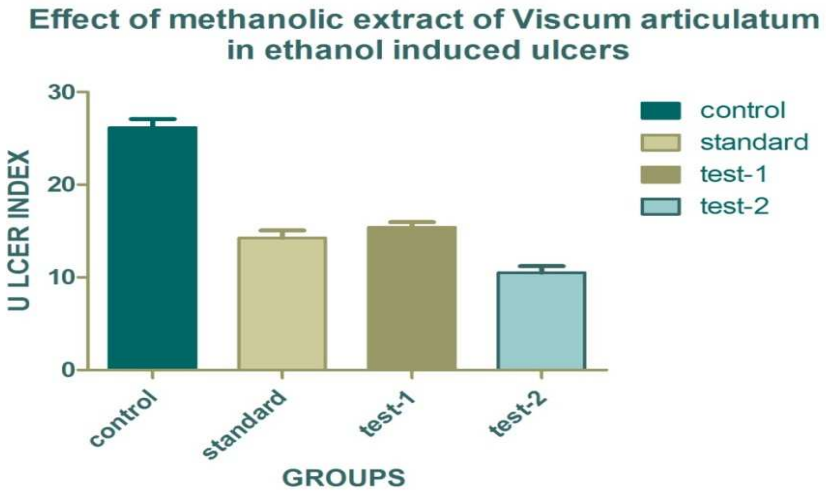


Fig: 2. Anti-ulcer activity of *V. articulatum* in pylorus ligation induced gastric ulceration model

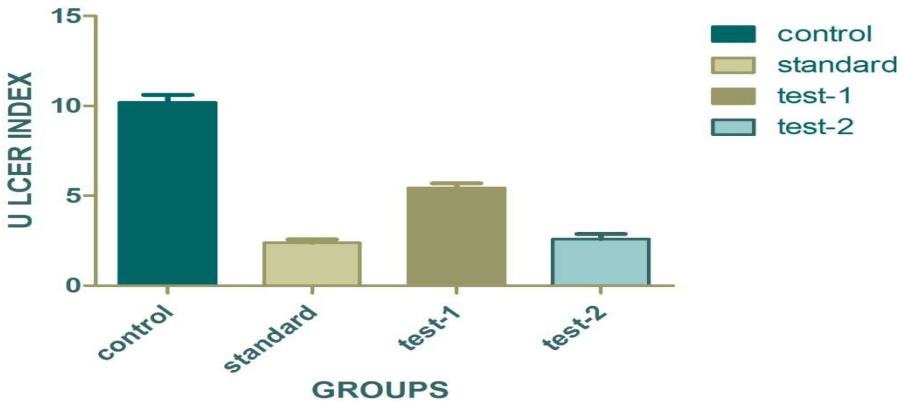
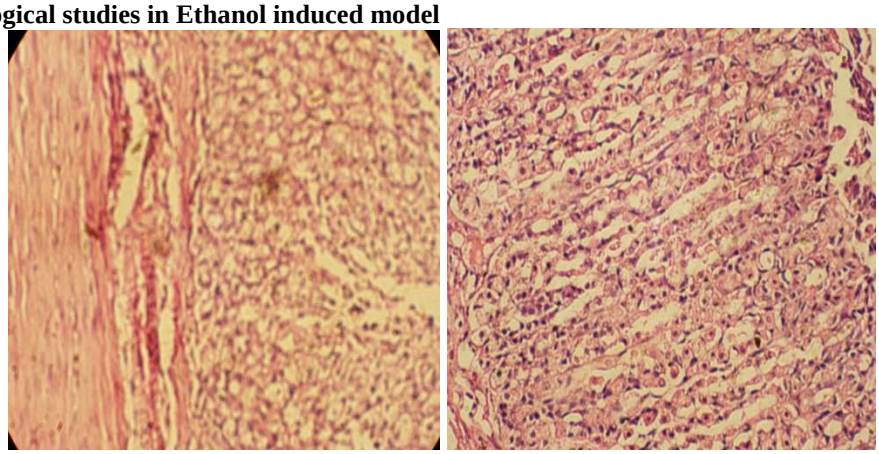
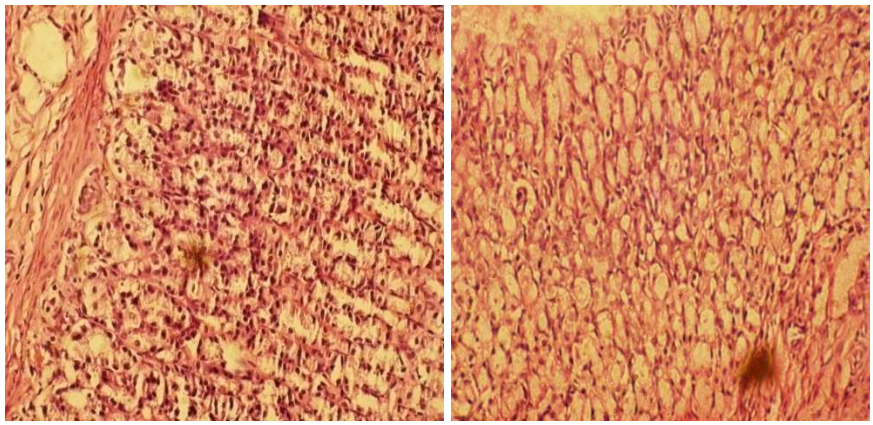


Fig: 3. Histopathological studies in Ethanol induced model



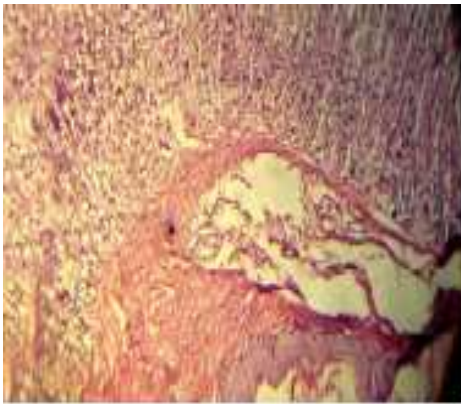
Control Standard



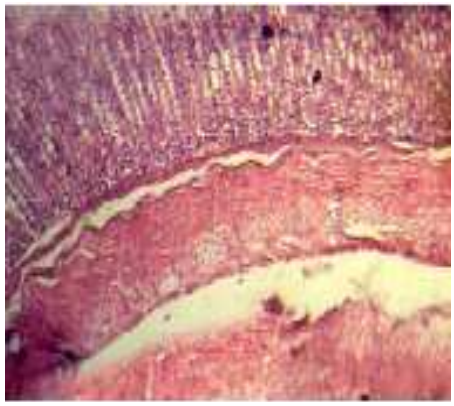
MEVA 200mg/kg MEVA 400mg/kg

Fig: 4. Histopathological studies in pylorus ligation induced model

Control Standard



MEVA 200mg/kg



MEVA 400mg/kg

Pylorus ligation-induced gastric ulcer

In control animal, ligation produced characteristic lesions in the glandular portion of rat stomach and the lesions appeared to as red bands yielding in blood. MEVA has shown significant protection index of 44.12% and 72.19% with the dose of 200 and 400 mg/kg respectively in comparison to control, Omeprazole (20 mg/kg) as reference standard drug was reduction of ulcer 75.42%. Results are tabulated in Table 2.

Macroscopical and Histopathological Evaluation

Histopathological changes on pylorus ligation chronic gastric ulcer and ethanol induced ulcer showed the degeneration, hemorrhage, oedematous appearance of the gastric tissue, where as MEVA (400 mg/kg) and, Sucralfate suspension, Omeprazole treated groups showed regeneration and prevents the formation of hemorrhage and edema and it was shown in figure 3 and figure 4.

ACKNOWLEDGEMENTS

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