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Phytochemical and anti-ulcer investigations of the whole plant extract of *Neregamia alata* Wight & Arn. in albino rat model.

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ABSTRACT

The purpose of the present study is to investigate the chemical constituents and anti-ulcer profile of the methanolic extract of *Neregamia alata* Wight & Arn. (MENA) whole plant in albino rats. The phytochemical examination of methanolic extract of whole plant of *Neregamia alata* was performed by the standard methods. MENA at the doses of 125, 250 and 500 mg/kg body weight orally was administered to evaluate anti-ulcer activity by using pyloric ligation (PL) and hypothermic-restraint stress (HRS) induced gastric ulcer models in Albino rats. MENA, at doses of 125, 250 and 500 mg/kg were found to be protective (36.47, 45.86 and 75.02% respectively), in PL induced ulcer models and significantly reduced free and total acidity by *P Neregamia alata* as a potent anti-ulcer agent.

KEYWORDS: *Neregamia alata, MENA, anti-ulcerogenic, pyloric ligation, hypothermic-restraint stress.*

INTRODUCTION

Neregamia alata W&A. (Meliaceae) is one of the ancient plants in the world which is used in traditional system of medicine, Ayurveda and Siddha. It is a small branching under shrub and distributed in Konkan, N.Kanala and Western Ghats of Madras state in all districts up to 3000ft and Africa also. The root is sweet and cooling; alexiteric, vulnerary; cures, asthma, bronchitis, biliousness, ulcer (Ayurveda). In the Konkan, the leaves and stem are given in decoction with bitters and aromatics as remedy for biliousness. The root is a good emetic and cholagogue. It has been found useful in acute dysentery and as an expectorant. In Southern India the plant is used in rheumatism and itching. Root and bark contains alkaloid like neregamin. Stem and root contains heneicosone, β-sitosterol and palmitic acid (1, 2).

From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate the constituents and anti-ulcer profile of the methanolic extract of *Neregamia alata* Wight & Arn. whole plant is being reported here.

MATERIALS AND METHODS

Plant material

The whole plant of *Neregamia alata* was collected from Kolli Hills, Salem District, Tamilnadu, India. It was identified and authenticated by Prof.R.Arivukarsu, Pharmacognosist, Sangaralingam College of Pharmacy, Sivakasi, Tamilnadu. A voucher specimen (NA-P-06-S3) has been kept in our laboratory for future reference.

Preparation of plant extract

The collected plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. The powdered materials were extracted with 95% methanol using maceration for 7 days. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in 5% acacia suspension and used for the experiment. The percentage yield of prepared extract was around 2.1%w/w.

Phytochemical Screening

The phytochemical examination of methanol extract of whole plant of *Neregamia alata* was performed by the standard methods (3–6).

Animals Used

Albino rats of either sex weighing about (100–125 g) provided by the Animal House in polyethylene-walled cages in groups of five, with food and water. The animals were kept on a 12 hrs light 12 hrs dark regime (lights on from 7.00 h to 19.00 hrs) at 23°C prior to the experiments. All the experiments were carried out under the guidance of Ethical Committee of Rural College of Pharmacy (129/99/CPCSEA).

Experimental design

Treatment of Animals.

Thirty healthy albino rats were equally divided into 5 groups containing 6 rats each and treated as follows:

Group -I: Control Vehicle (5% w/v Acacia, 5 ml./kg. body weight)

Group -II: Famotidine (reference drug) (20 mg/kg. body weight)

Group -III: Methanol extract of *Neregamia alata* whole plant (MENA) dissolved in 5% w/v Acacia (125 mg/kg. body weight)

Group -IV: Methanol extract of *Neregamia alata* whole plant (MENA) dissolved in 5% w/v Acacia (250 mg/kg. body weight)

Group -V: Methanol extract of *Neregamia alata* whole plant (MENA) dissolved in 5% w/v Acacia (500 mg/kg. body weight)

Gastric ulcer induced by pylorus ligation (PL) in rats.

Albino rats (Pregnancy was excluded) were housed in individual cages and fasted (water allowed) for 48 hours prior to pyloric ligation, care being taken to avoid coprophagy. Under light ether anesthesia the abdomen is opened by a small midline incision below the xiphoid process; pyloric portion of the stomach is slightly lifted out, and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach is replaced carefully, and the abdominal wall closed by interrupted sutures. The drugs are administered orally two hours prior to pyloric ligation. The animals were deprived of both food and water during the postoperative period, and were sacrificed at the end of six hours after operation. Stomach is dissected out and the contents are drained into a tube and this is subjected to gastric secretion study, analysis for pH and for free and total acidity. The stomach is then cut open along the greater curvature and the inner surface is examined for any ulceration. The mean ulcer size was calculated by dividing the total length (in mm) of ulcers for all the animals divided by total number of animals (7).

Gastric secretion study

The volume of gastric juice obtained by pyloric ligation was expressed in terms of ml/100g of body weight. Total acidity, free acidity and dissolved mucous substances of gastric juice were measured.

Total Acidity: A volume of 2 ml diluted gastric juice was titrated with 0.01 N sodium hydroxide run from a micro burette using phenolphthalein as indicator and the acidity was expressed as mg HC1/100g body weight of rat.

Free Acidity: It is determined in similar manner using Topfer's reagent as indicator and sodium hydroxide was run until canary yellow color was observed.

Hypothermic restraint stress-induced ulcer lesions (HRS)

The experiment was performed by the method of Levine et al (8), and Toma et al (9). After 24 h of fasting, the animals received an oral administration of drug or vehicle. One hour after treatment, rats were immobilized in a restraint cage at 4° C for 3 h to induce gastric ulcer. The animals were killed and the stomach were removed and opened along the greater curvature to determine the ulcer index.

Statistical analysis

The results were expressed as mean ± S.D. The data were also analyzed by ANOVA (one-way analysis of

variance) using SPSS package. The statistical analysis was performed using Dunnet's't' multiple comparison test for all parameters. The values were considered significant at the levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS

Phytochemical investigation

The results of preliminary phytochemical investigation of the methanolic extract of *Neregamia alata* whole plant (MENA) are given in Table-1

Effect of MENA on gastric ulcer induced by pylorus ligation (PL)

The MENA showed significant anti-ulcer effect against ulcers induced by pylorus ligation in a dose dependent manner. In PL induced ulcer model, MENA at a dose of 125, 250 and 500 mg/kg body weight showed protective effect of 36.47, 45.86 and 75.02.%, respectively, where as famotidine showed protection index of 81.20% at a dose of 20 mg/kg body weight (Table-2).

Effect of MENA on acid secretion studies

The effect of MENA on different offensive factors like free and total acidity, which play a crucial role in the pathogenesis of gastric ulcers, was studied by collecting gastric juice from stomach in PL model. As shown in Table-2, treatment with MENA at a dose 125, 250 and 500 mg/kg body weight has significantly reduced the free and total acidity ($P < 0.01$ and $P < 0.001$) respectively when compared to famotidine, the reference drug.

Effect of MENA on gastric ulcer induced by hypothermic-restraint stress

In the gastric ulcer induced by hypothermic-restraint stress, MENA at a dose of 125, 250 and 500 mg/kg body weight showed again significant activity. MENA at a dose of 125, 250 and 500 mg/kg body weight showed dose-dependent protective effect of 34.36, 50.34 and 75.45% respectively, where as famotidine showed protection effect of 80.50% at a dose of 20 mg/kg body weight, in both the above models. (Table-3)

Table-1: Phytochemical screening for the whole plant methanolic extract of Neregamia alata

Phytoconstituents	Methanol extract
Alkaloids	+
Carbohydrates & Glycosides	+
Phytosterols	™
Fixed oils and Fats	™
Tannins & Phenols	+
Proteins & Free amino acids	™
Gums & Mucilage	™
Flavonoids	+
Volatile Oils	™
Lignin	™
Saponins	+

Table-3: Effect of methanolic extract of Neregamia alata whole plant (MENA) Hypothermic restraint stress (HRS) induced Gastric ulcer in Rats.

Group	Ulcer Index	Protection (%)
Control (5% w/v Acacia, 5 ml. / kg.b.w)	8.72 + 2.3	™
MENA(125mg/kg b.w)	5.13 + 0.4*	34.36
MENA(250mg/kg.b.w)	4.33 + 0.8*	50.34
MENA(500mg/kg b.w)	2.14 + 0.6**	75.45
Famotidine (20mg/kg b.w)	1.70 + 0.5**	80.50

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test.
**P < 0.01 and*
***P < 0.001 as compared to control (n = 6 in each group).;*
MENA = Methanolic extract of Neregamia alata whole plant;
B.W=Body weight

Table-2. Effect of methanolic extract of Neregamia alata W&A.whole plant (MENA) on free and total acidity in pylorus ligation induced ulcer model.

Group	Free acidity (µequiv./ml)	Total acidity (µequiv./ml)	Ulcer Index	Protection (%)
Control (5% w/v Acacia,5 ml. / kg.b.w)	52.45 ± 2.634	59.62 ± 6.034	18.14 + 0.22	-
MENA(125mg/kg b.w)	38.58 ± 1.043*	42.13 ± 2.164*	11.36 + 0.23*	36.47
MENA(250mg/kg.b.w)	31.83 ± 3.062*	38.17 ± 5.417**	9.82 + 1.03*	45.86
MENA(500mg/kg b.w)	18.93 ± 4.035**	31.14 ± 4.313**	4.53 + 0.21**	75.02
Famotidine (20mg/kg b.w)	12.67 ± 2.021**	28.42 ± 3.657**	3.41 + 0.32**	81.20

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test.
**P < 0.01 and*
***P < 0.001 as compared to control (n = 6 in each group).*
MENA = Methanolic extract of Neregamia alata whole plant
B.W=Body weight

DISCUSSION

The plant was taken for our studies to screen and submit a report on Anti-ulcer activity of methanolic extract of *Neregamia alata* whole plant (MENA). The preliminary phytochemical investigation shows the presence of alkaloids, steroids, triterpenoids, flavonoids, carbohydrates, glycosides, tannins and saponins.

Pyloric ligation induced ulcers caused due to imbalance between offensive and defensive mucosal factors (10) are ideal model to infer the mechanism by which a drug works as an anti-ulcerogenic agent. PL-induced gastric ulcers occur because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion (10).

A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment in repair. Hence estimation of acid secretion and mucus secretion is a valuable part of the study to clarify the mechanism of action of the drug under trial.

In the present study, we found that MENA at dose of 125, 250 and 500 mg/kg body weight significantly showed protection effect of 36.47, 45.86% and 75.02%, respectively, which were comparable to standard drug famotidine (81.20%).

Famotidine antagonized pentagastrin, histamine and carbachol induced hyper acidity in gastric rats (11). It protects experimental animals from gastric ulceration induced by stress (12).

Gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats (7). The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric acid secretion (13). The current data clearly demonstrated that, MENA in a dose-dependent manner decreased hydrogenionic concentration suggesting that the pharmacological mechanism has a relationship to antisecretory activity.

To further confirm its anti-ulcerogenic effect we have evaluated the efficacy of MENA against hypothermic-restraint stress -induced ulcer model. Stress has been reported to have an important role in etiopathology of gastro-duodenal ulceration, increase in gastric motility, vagal over activity (14) mast cell degranulation (15), decreased gastric mucosal blood flow (16) and decreased prostaglandin synthesis. Any of these factors could play a role in genesis of stress-induced ulcers (17). Methanolic extract of *Neregamia alata* Wight & Arn. whole plant (MENA) significantly reduced ulcer index in a dose dependent manner in hypothermic-restraint stressed rats, which further supports anti-ulcerogenic effect of MENA.

Further, our results fortify the ethanopharmacological importance of MENA as an anti-ulcer agent. Etiology of ulcers produced in different ulcer models is diverse. Since MENA has been found effective in both the models depicting its anti-ulcerogenic activity, MENA and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence, however more experimentation and detailed phytochemical and experimental analysis are required for a definitive conclusion.

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REFERENCES

1. Warrier P.K., Nambiar V.P., Ramankutty C. Indian medicinal plants a compendium of 500 species. Vol-1 Orient Longman Pvt Ltd. pp 10–14 (1993).
2. Khare C. P. Encyclopedia of Indian Medicinal plants: Rational Western Therapy, Ayurvedic and other traditional Usage Botany. Berlin, New York. Springer. pp. 3–5 (2004).
3. Plummer D.I. An Introduction to Practical Biochemistry, ed 2, Tata Magraw-Hill Publishing Co. Ltd., New Delhi. pp 136,143(1985).
4. Pollock J.R.A. and Stevens R.. Editors. *Dictionary of Organic Compounds*, vol.-**5**, ed 4, London, Eyre and Spottiswoode. (1965).
5. Trease G.E. and Evans W.C. Pharmacognosy, Ed 12, (ELBS Publication, Baillier Tindall, East Bourne) pp 344, 539(1996).
6. Tyler V.E., Brady L.R. and Robbers J.E. *Pharmacognosy*, 9th 9. Philadelphia, PA: Lea and Febiger. Botanical medicine: efficacy, quality assurance, and regulation. New York Mary Ann Liebert, pp59–64 (1993).
7. Shay H., Kamorow S.A., Fele S.S., Meranz D., Gruenstein H. and Siplet H. Gastroenterology. **5**: 43(1945).
8. Levine R.J.. A method for rapid production of stress ulcers in rats. In: Pfeiffer C.J. (Ed.), *Peptic Ulcer*. Munksgaard, Copenhagen,: pp 92–97(1971).
9. Toma W., Hiruma-Lima C.A., Guerrero R.O. and Souza Brito A.R.M.. Preliminary studies of Mammea americana L. (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice. *Phytomedicine*. **12**(5):345–350(2005).
10. Goel R.K. and Bhattacharya S.K. Gastroduodenal mucosal defense and mucosal protective agents. *Indian Journal of Experimental Biology*. **29**: 701–714(1991).
11. Satyanarayana S., Kumar Prasanna and Viswesaram D. Antiulcer activity of Agni tundirasa and its comparison with Cimetidine in Shay rat. *Ancient Science of Life*. **3**:207–211(1980).
12. Ram P., Rastogi B.N., Mehrotra. Compendium of Indian Medicinal Plants. Volume I, :pp228(1960–1969).
13. Baggio C.H., Freitas C.S., Rieck L. and Margues M.C.A. *Gastroprotective effects of a crude extract of Baccharis illinita DC in rats*, *Pharmacological Research*. **47**: 93–98(2003).
14. Cho C.H., Ogle C.W. and Dai S. Acute gastric ulcer formation response to electrical vagal stimulation in rats. *European Journal of Pharmacology*. **35**: 215–219(1976).
15. Cho C.H. and Ogle C.W. Cholinergic-mediated gastric mast cell degranulation with subsequent histamine H_1 and H_2-receptor activation in stress ulceration in rats. *European Journal of Pharmacology*. **55**: 23–33(1979).
16. Hase T. and Moss B.J. Microvascular changes of gastric mucosa in the development of stress ulcer in rats. *Gastroenterology*. **65**: 224–234(1973).
17. Singh S. and Majumdar D.K. Evaluation of the gastric activity of fixed oil of Ocimum sanctum (Holy Basil). *Journal of Ethnopharmacology*. **65**:13–19(1999).