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Anti-Ulcer Activity of Aqueous and Ethanolic Leaf Extract of Krishna Tulasi (*Ocimum sanctum*) in Albino Rats

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ABSTRACT

The Anti-ulcer effect of aqueous and ethanolic leaf extract of *Ocimum sanctum* **(**Krishna Tulasi**)** was investigated in pylorus ligation, cold restrains stress and forced swimming endurance models in wistar albino rats. The anti-ulcer activity was assessed by determining and comparing the ulcer index in the test drug group with that of the distilled water (-ve) control group and Ranitidine 20mg/kg was used as a reference standard. OSa and OSe 400mg/kg orally produced significantly inhibited gastric lesions in pyloric ligation, cold restrain stress and forced swimming endurance model. The extract (400 mg/kg) shows significant ($P<0.05$) reduction in gastric volume, free acidity, total acidity, combined acidity and ulcer index as compared to control. This present study indicates that *Ocimum sanctum* aqueous and ethanolic *leaf* extract have potential anti ulcer activity which could be either due to cytoprotective action of the drug.

Keywords: *Ocimum sanctum* Krishna Tulasi OSk)*Ocimum sanctum* Leaf Extract Aqueous (OSa), *Ocimum sanctum* Leaf Extract Ethanol (OSe),UI-ulcer index.

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INTRODUCTION

The patho-physiology of gastric ulcer involves an imbalance between offensive (acid, pepsin, and *H. pylori)* and defensive factors (mucin, prostaglandin, bicarbon-ate, nitric oxide and growth factors [1,2].Most of the commonly used drugs such as H2– blockers, M1– blockers, and proton pump inhibitors are ulti-mately balancing aggressive factors and defensive factors [3]. But they suffer grossly from incidence of relapses, adverse effect and dangers of drug inter-action during ulcer therapy. Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects. There is always a need for better agent which can enhance ulcer protection, avoid side effects and expenses.Krishna tulasi (*Ocimum sanctum*) has been shown to possess many curative properties in traditional medicine and its several medical applications are well recognized [4,5]. The Ocimum sanctum L(two main morphotypes cultivated in India and Nepal are purple-leaved (Krishna *tulsi*) and green-leaved (Sri or Lakshmi *tulsi*).

The fresh leaves are taken as Prasad by millions of Indians for many years. A tea prepared with the leaves of Krishna tulsi is commonly used in cough, cold, mild indigestion, diminished appetite and malaise. From our laboratory we have already reported the antiulcer property of Neem in Rats [6].OS is also effective against Gastric Ulcer. The literature survey reveals no enough reports on antiulcer activity of the leaves extract of Krishna tulasi (*Ocimum sanctum).* This prompted us to investigate the anti-ulcer activity of leaves extract of Krishna tulasi.

MATERIALS AND METHODS

Experimental animals

Healthy Wister albino rats of either sex weighing between 180-200 gm from our breeding stock were used for study. Animals were housed at 26 ± 2^0 in polypropylene cages. Then the animals were brought to lab conditions at 12 h/12 h light and dark cycle. Animals were fed with standard pellet diet and with water *ad libitum*. The test was carried out especially under identical experimental conditions at the same time of the day, to maintain uniformity of the study. The institutional animal ethical committee (Regd.no- 926/ab/06/ CPCSEA) has approved the experimental protocol (Approval No. 41/Chair-man- IAEC. RIPS, Berhampur-10).

Drugs and Chemicals

Ranitidine (Glaxo SmithKline Pvt Ltd. Mumbai) was used as the standard drug. The tests drug Krishna tulasi aqueous & ethanolic leaf extract was standardized and sup-plied by Quality control Laboratory M/s Natural Remedies, Bangalore The phytochemical examination of the extract was performed by the standard methods[7,8].

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EXPERIMENTAL GROUPS

Animals were divided into five groups:
(Each animal model containing six Albino rats)
Group 1: Albino rats were given only distilled water /Tween 80 each day up to 7 days.
Group 2: Albino rats were given Ranitidine (20mg/kg/day, bw, p.o.) up to 7 days.
Group 3: Albino rats were given Test extract (100mg/kg/day, bw, p.o.) up to 7 days.
Group 4: Albino rats were given Test extract 200mg/kg/day, bw, p.o.) up to 7 days.
Group 5: Albino rats were given Test extract (400mg/kg/day, bw, p.o.) up to 7 days.

Pylorus Ligation-induced Gastric Ulcer Model

Wister strain albino rats were housed in individual cages and fasted (water allowed ad-libidum) for 24hrs prior to pyloric ligation. Proper care was taken to avoid coprophagy. Under light ether anesthesia, a midline abdominal incision was made extending from the xiphoid process for a distance of about 1cm. The stomach was identified and tracing it downwards the pylorus was reached. The pyloric ligature was placed with sterilized cotton thread, care being taken that neither damage to the blood supply nor traction to the pylorus occurred. Grasping of the stomach with instruments was meticulously avoided, as it was liable to develop ulceration at those points. The abdomen wound was cleansed thoroughly with normal saline, dried and covered with a solution of beta dine. The animals were sacrificed 19 hours after pyloric ligation by a cervical dislocation. The pyloric end of stomach was dissected out by clamping the lower end of esophagus. The glandular portion of stomach was observed for ulcer index. The gastric content was drained into tubes, centrifuged and subject to analysis for various biochemical parameters. The volume and P^H of gastric juice were measured. The gastric content was titrated against 0.01N Na OH to find out the free acidity and total acidity [9,10].Free acidity: The first titration to about P^H 4.0 measures the amount of free HCL Present, i.e. free acidity. Total acidity: The complete titration is said to give total acidity .Some protein hydrochloride and any organic acids are titrated. Proteins present include mucin in gastric secretion. Results of Titration are expressed as ml of 0.1N HCL.Normal values of free acid: 0 to 30 mEq/L and Total acid: 10 to 40 mEq/L (Table1 & 2).

Cold Restraint Stress in Rats

Albino rats were housed in individual cages. Food and water were withdrawn 24 h before the experiment. Proper care was taken to avoid coprophagy. After oral administration of the test compound, distilled water and ranitidine solution, the animals were slightly anesthetized with ether. Both lower and upper extremities were fixed together and the animals were wrapped in wire gaze. They were placed supine at 4 ^{0}C for 03 h in a freeze. Then the rats were sacrificed and stomachs were removed. The stomachs were examined by magnifying glass. For severity of the growth of the ulcer an arbitrary scoring system was used to determine 'Ulcer Index' (Hanson and Brodie, 1960 and Bonfils et al. 1966(Table3 & 4).

% Protection = [(UI control – UI treated) /UI control] x 100

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Forced swimming endurance test

The rats were subject to swimming stress by keeping them in propylene tank of dimension (37×37×30 cm),filled with water to a height of 25cm.Extract were given to rats ,once daily for a period of 6th days. On 7th Day the rats were allowed to swim till complete exhaustion and the end point was taken when the animal started drowning. Then mean ulcer index for each group was calculated (Table 5 & 6).

Analysis

The results were recorded in the pre-designed case record form (CRF) and fed into the computer for data analysis. The results were analyzed by suit-able statistical method in using spss software in a PC .The discrete data were analyzed by Chi-squares test and continuous data were analyzed by one way ANOVA, results with $p < 0.05$, which was accepted as significant.

RESULTS AND DISCUSSION

The aqueous and ethanol extract of Krishna tulasi (400 mg/kg bw) shows significant ($P<0.05$) reduction in gastric volume, free acidity, total acidity, combined acidity and ulcer index as compared to control pyloric ligation, cold restraint stress and forced swimming endurance ulcer induced model and showing anti-secretary mechanisim [11,12].Hence efforts are on to find a suitable treatment from Natural product source for the prevention and treatment of various diseases and for the promotion of good health by various cultures worldwide [13,14,15,16].Tulasi aqueous and ethanolic leaf extract is effective as that of the standard synthetic drug such as ranitidine. So, has therapeutic potential for the control of Gastric Hyperacidity. However further studies are required to established its exact mode of action and active.

Table:-1 Effect of Aqueous Leaf Extract of *Ocimum sanctum* (OSk) On Various Parameter in Pyloric Ligation Induce Gastric Ulcer in Albino Rats.

Group	Treatment	Mean ulcer index	Protection (%)	pH of gastric juice	Volume of gastric Content in ml	Free acidity Meq/ltr	Total acidity Meq/ltr	Combined Acidity Meq/ltr
I	Control (Distilled Water)	3.83± 0.17	-	3.12± 0.49	12.67± 1.77	36.83± 4.48	66.17± 4.54	29.33± 4.51
II	Ranitidine (20mg/kg bw)	0.83± 0.4	78%	5.16± 0.69	4.69± 1.01	18.67±2. 01	33±4.8	14.33± 3.40
III	OSk (100mg/kgbw)	1.33± 0.20	65%	4.66± 0.33	5.83± 0.47	23.83±1. 62	48.83±1. 23	25.0± 1.42
IV	OSk	1.34±	74%	5.83±	5.0± 0.36	20.66±1.		23.51

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	(200mg/kgbw)	0.36		0.30		51	4.17±1.22	±1.37
V	OSk (400mg/kgbw)	1±0.36	78%	5 ± 0.26	4.5± 0.43	20.0±1.57	39.33±0.88	19.33±1.23

Values are express as mean ±SEM of 6 observations, statistically comparisons as follows: Significant at*p<0.05 Compared to Control.

Table:-2 Effect of Ethanolic Leaf Extract of *Ocimum sanctum* (OSk) On Various Parameter in Pyloric Ligation Induce Gastric Ulcer in Albino Rats.

Group	Treatment	Mean ulcer index	Protection (%)	pH of gastric juice	Volume of gastric Content in ml	Free acidity Meq/ltr	Total acidity Meq/ltr	Combined Acidity Meq/ltr
I	Control(Tween-80)	3.83± 0.17	-	3.12± 0.49	12.67±1.77	36.84± 4.48	66.17± 4.54	29.33± 4.51
II	Ranitidine(20 mg/kg bw)	0.83± 0.4	78%	5.16± 0.69	4.69±1.01	18.67± 2.01	33±4.8	14.33± 3.40
III	OS(100mg/kgbw)	1.16± 0.17	70%	5.46± 0.69	5.0±0.58	25.33± 1.33	35.55±2.89	10.17± 2.11
IV	OS(200mg/kgbw)	1.0± 0.0.26	74%	5.06± 0.6	4.33±0.22	20.83± 1.20	31.83±1.19	11±1.19
V	OS(400mg/kgbw)	0.83± 0.17	78%	4.67± 0.3	3.83±0.17	19.66± 0.71	34.16±0.6	14.5±0.66

Values are express as mean ±SEM of 6 observations, statistically comparisons as follows: Significant at*p<0.05 Compared to Control.

Table:-3 Effect of Aqueous Leaf Extract of *Ocimum sanctum* (OSk) On Cold restrain stress (CRS) Induce Gastric Ulcer in Albino Rats.

Group	Treatment	Mean ulcer index	Protection (%)
I	Control(Distilled Water)	3.83±0.17	-
II	Ranitidine(20mg/kg)	1.50±0.22	61%
III	OS(100mg/kg)	2±0.5	48%
IV	OS(200mg/kg)	1.51±0.61	62%
V	OS(400mg/kg)	0.83±0.17	78%

Values are express as mean ±SEM of 6 observations, statistically comparisons as follows: Significant at*p<0.05 Compared to Control.

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Table: 4 Effect of Ethanolic Leaf Extract of *Ocimum* sanctum (OSk) On Cold restrain stress (CRS) Induce Gastric Ulcer in Albino Rats.

Group	Treatment	Mean ulcer index	Protection (%)
I	Control(Distilled Water)	3.83±0.17	-
II	Ranitidine(20mg/kg)	1.50±0.22	61%
III	OS(100mg/kg)	1.67±0.21	57%
IV	OS(200mg/kg)	1.51±0.61	61%
V	OS(400mg/kg)	0.83 ±0.17	78%

Values are express as mean ±SEM of 6 observations, statistically comparisons as follows: Significant at*p<0.05 Compared to Control

Table:-5 Effect of Aqueous Leaf Extract of *Ocimum sanctum* (OSk) On Forced Swimming Endurance Test Induce Gastric Ulcer in Albino Rats.

Group	Treatment	Mean ulcer index	Protection (%)
I	Control(Distilled water)	3.17±0.30	-
II	Ranitidine(20mg/kg)	0.67±0.33	79%
III	OS(100mg/kg)	1.67±0.20	47%
IV	OS(200mg/kg)	1.33±0.21	58%
V	OS(400mg/kg)	0.83±0.31	73%

Values are express as mean ±SEM of 6 observations, statistically comparisons as follows: Significant at*p<0.05 Compared to Control.

Table:6 Effect of Ethanolic Leaf Extract of *Ocimum sanctum* (OSk) On Forced Swimming Endurance Test Induce Gastric Ulcer in Albino Rats.

Group	Treatment	Mean ulcer index	Protection (%)
I	Control(Distilled water)	3.17±0.30	-
II	Ranitidine(20mg/kg)	0.67±0.33	79%
III	OS(100mg/kg)	1.33±0.21	58%
IV	OS(200mg/kg)	0.34±2.1	82%
V	OS(400mg/kg)	0.65±2.0	89%

Values are express as mean ±SEM of 6 observations, statistically

REFERENCES

[1] Tripathi KD. Essentials of medical Pharmacology. 6th ed. Medical Publishers (P) Ltd: New Delhi, India;2008.

[2] Hardman JG, Limbird LE, Goodman Gilman A. Goodman Gilman's; The Pharmacological Basis of Therapeutics. 11th ed .The McGraw Hill Companies, Inc: New York; 2007.

[3] Korrapati Vishali, Kuttappan Nair Valsalakumari Kavitha, Venugopalan Rajesh and Perumal Perumal. J Pharm Res 2011: 4(2); 391-392.

[4] Sudhakar Pemminati, Gopalakrishna HN, Akshaya Alva, Pai MRSM, Seema Y ,Vishnu Raj, Durga Pillai. J Pharm Res 2010; 3(3): 624-626.

[5] Sudhakar Pemminati, V Nair, P Dorababu, HN Gopalakrishna. Indian J Pharmacol 2007; 39(2): 87-89.
[6] Bharat Bhusan Mohapatra, Mangala Charan Dasb, Subas Chandra Dindac, KEV Nagojia. Journal of Pharmacy Research 2012; 5(3): 1571-1573.
[7] Kokate CK, AP Purohit and SB Gokhale. Pharmacognosy 2007, 13th edn., Nirali Prakashan Publisher,ISBN:8185790094,pp:635.
[8] Evans WC, Trease and Evans Pharmacognosy, 15th Ed, Elsevier, Printed in New Delhi, 2008, 193, 336.
[9] Ashoka Shenoy M, Shastry CS, Sridevi, Gopkumar P. J Pharm Res 2009; 2(7): 1218-1220.
[10] Ganguly AK, Bhatnagar OP. Canadian J Physiol Pharmacol 1973; 51: 748-50.
[11] Kulkarni SK, Handbook of Experimental Pharmacology, Edn 3, Vol I, Vallabh Prakashan, New Delhi, 1999,148-50.
[12] Goel RK, Bhattacharya SK. Indian J Expl Bol 1991; 29: 701-14.
[13] Nagaraju B, Puranik DS,Patan Fayaz, Padmavathi GV, Dattathreya G and Sharma YK. J Pharm Res 2011; 4(11): 3994-3996.
[14] The Ayurvedic Pharmacopoeia of India, Part I, Vol III, Govt of India, New Delhi,2001, 27-28
[15] Suffredini IB, Bacchia IM, Sertie JAAA. J Ethnopharmacol 1999; 65: 217.
[16] Gupta SK, Prakash J, Srivastava S. Indian J Exp Biol 2002; 40: 765-773.