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STUDY OF ANTIULCER POTENTIAL OF HYDROALCOHOLIC LEAVES EXTRACT OF *STROBILANTHES KUNTHIANA*

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ABSTRACT

Over the past 200 years, peptic ulcer disease—which includes both stomach and duodenal ulcers has posed a serious threat to the world's population due to its high morbidity and significant mortality. The incidence and frequency of this disease and its consequences have exhibited notable geographic variations according to epidemiological data. Plants have given the human race a variety of incredible medical agents and natural goods that serve as the basis for all pharmaceuticals. Even if many plants are studied for their potential as medicines, more understudied plants need to be investigated for the same goal. It is commonly recognised that Neelakurinji (*S. kunthiana*) has both decorative and therapeutic qualities. It is evident from animal models that *Strobilanthes kunthiana* leaf extract exhibits strong anti-ulcer action. When compared to the reference medicine omeprazole, it possesses stomach antisecretory and muco-protective properties. Even when present in quite high amounts, the extract is not hazardous. The presence of flavonoids is likely what causes the anti-ulcer action. To describe and investigate the biological activity of the chemicals found in the extract, more research is being done.

Keywords: *Strobilanthes kunthiana*, Hydroalcoholic extract, Antiulcer activity.

INTRODUCTION

Peptic ulcer disease embraces both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality. Epidemiological data for this disease and its complications have shown striking geographical variations in incidence and prevalence. Development of ulcer disease and death from it has been associated with the birth of urbanisation and was interpreted as a birth-cohort event with the peak of disease in those born during the late 19th century [1-2].

Our understanding of the disease changed greatly with the discovery of Campylobacter pyloridis (renamed *Helicobacter pylori* in 1989 because of a revised taxonomic classifi cation) in 1982 by Warren and Marshall [3-4].

This discovery switched the notion from an acid-driven disease to an infectious disease, opening a huge area for intensive research that resulted in the reconciliation of previously suggested mechanisms of pathogenesis. The fall of the acid dogma in peptic ulcer disease, which had found its undisputed acceptance during and after the introduction of histamine H2-receptor antagonists, led to the present therapeutic principle. Maintenance acid suppressive therapy for duodenal ulcer, which followed decades of dominance of surgical interventions (subtotal gastric resections, several forms of vagotomy), was replaced with a short-term antibiotic regimen targeting eradication of *H. pylori* infection [5-6].

H. pylori eradication as cure of peptic ulcer received its full recognition when the Nobel Prize for Medicine and Physiology was awarded to Warren and Marshall in 2005. This recognition has not, however, closed the chapter on peptic ulcers. The management of ulcer disease and its complications remains a clinical challenge. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin are an increasingly important cause of ulcers and their complications even in *H. pylori*-negative patients. Other rare causes of ulcer disease in the absence of *H. pylori*, NSAIDs, and aspirin also exist.

NSAIDs, including low-dose aspirin, are the most important cause of ulcer complications in developed countries where prevalence of *H. pylori* infection is falling. In patients who develop uncomplicated peptic ulcers while on NSAIDs, more than 90% of gastric or duodenal ulcers heal with 8 weeks of standard-dose H2-receptor antagonists (eg, ranitidine 150 mg twice a day), provided that NSAIDs are discontinued. However, healing of gastric ulcers will be greatly impaired if patients continue to take NSAIDs. A descriptive review of head-to-head trials suggested that PPIs might be better than a standard dose of ranitidine in healing gastric ulcers in patients receiving continuous NSAIDs [7].

Plants have provided different amazing medicinal agents, natural products as the source of all drugs to the human race. Though lots of plants are explored for medicinal purposes, some underutilized plants still need to be explored for the same purpose. Neelakurinji (*S. kunthiana*) is well known to possess both ornamental and medicinal properties.

Gastrointestinal bleeding and ulceration are the most recurrent and formidable problems linked with NSAID. Because of these side effects, researchers are in dire need to develop safer compounds. The gastric mucosal lesions caused by ethanol, were reported as by prying with the gastric defensive

mechanisms. While there are many products used against gastric ulcers, most of these drugs generate several adverse reactions. The plant *S. kunthiana* is a rich source of pharmacological constituents and can act as herbal alternatives for various disorders. The present study aim to evaluate antiulcer activity of leaves extract of *S. kunthiana*.

MATERIAL AND METHODS

Extraction (By Maceration Method)

Strobilanthes Kunthiana leaves were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (300 gm) and packed in (1 liter) air tight glass Bottle. The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant drugs were subjected to extraction by ethanol and Water (ethanol: water; 70:30) as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated[8].

Preliminary Phytochemical Screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of leaves of ***Strobilanthes Kunthiana***, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids[9].

Pharmacological activity

Wistar rats (180-200 g) and Swiss albino mice (males; 20–25 g) were used in the present study. The animals were procured from College of Veterinary Science and Animal Husbandry Mhow, Indore (M.P), India. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee[10].

Acute toxicity study:

Five groups (n = 5) of male albino mice were used in the acute toxicity study of *Strobilanthes Kunthiana* Hydroalcoholic extract. Animals from all groups were fasted overnight and administered (p.o) with single dose (250, 500, and 2000mg /kg) of the extract. A group of animals which received equal volume of PBS served as control. Changes in the behavior of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days[11].

Anti-ulcer (ulcer-preventive) activity study:

Five groups (n = 6) of Wistar rats were used to study the anti-ulcer activity of *Strobilanthes Kunthiana* Hydroalcoholic extract. PBS, Hydroalcoholic extract, omeprazole and ethanol were administered to the animals per orally (p.o). Group 1 received PBS (10 mL/ kg) all over the experimental period (11 days) and served as control. Group 2 received PBS (10 mL/ kg) for 10 days and on the 11th day received absolute ethanol (5 mL/ kg) and served as ulcer control. Group 3 and 4 were respectively administered with 250 and 500 mg/kg *Strobilanthes Kunthiana* Hydroalcoholic extract, group 5 with omeprazole (8 mg /kg) for 10 days. All the groups were fasted for 24 h and again administered with the extract or drug at respective dose. After 30 min of this treatment, animals of groups 2-5 were administered with 5 mL/ kg ethanol to induce ulcer. After 15 min of ethanol administration, all the animals were sacrificed using anesthetic ether. Gastric volume was measured by pylorus ligation method. Each stomach was opened along the greater curvature and examined macroscopically for gastric erosions under a dissecting microscope (20 X). The length and width (mm) of ulcer on the gastric mucosa were measured by plane glass square (10×10 mm). The Ulcer Area (UA) was calculated. The % of protection (P %) availed to the animals through various treatments were calculated using the formula [12]:

$$P\% = \frac{\text{UA ulcer control} - \text{UA treatment}}{\text{UA ulcer control}} x100$$

Grouping of animals:

Group-I Control Received PBS 10 ml/kg, p.o

Group-II Ulcer Control Received PBS 10 ml/kg, p.o up to 10 days and on 11th day receives absolute alcohol 5 ml/kg, p.o

Group-III Received HASK 250 mg/kg, p.o

Group-IV Received HASK 500 mg/kg, p.o

Group-V Received Omeprazole 8 mg/kg, p.o

RESULTS AND DISCUSSION

Medicinal plants since ancient time are lauded for their diverse pharmacological actions which could be attributed to the presence of secondary plant metabolites such as alkaloids, flavonoids, glycosides, tannins, steroids etc. some of these plants are important source of natural antioxidants that have been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heart diseases and stroke by scavenging free radicals which are implicated in the pathogenesis of many diseases. The present study indicated that the Hydroalcoholic extract of leaves of *Strobilanthes Kunthiana* have Excellent amount of total phenolic and Flavonoid.

Ulcer has long been recognized as one of the most important gastrointestinal problem. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer. *Strobilanthes Kunthiana* has several pharmacological properties including anti-inflammatory and anti-diarrhoeal. In spite of its uses in the traditional medicine against various ailments, this plant has so far not been screened for anti-ulcer activity. We report on the anti-ulcer activity of Hydroalcoholic leaves extract of *Strobilanthes Kunthiana.* The results of the present study have shown that Hydroalcoholic leaves extract of *Strobilanthes Kunthiana* possess gastro protective activity, as evidenced by its significant inhibition in the formation of ulcers induced by ethanol.

The protective effect was confirmed by histological examination showing prevention of mucosal lesions and sub-mucosal edema. As flavonoids have been identified in the methanolic extract, we believe that the anti-ulcer activity of this extract is probably due to the antioxidant activity of the extract. Antioxidant activities of flavonoids have been well documented in the literature. Moreover, flavonoids have been reported for their anti-ulcerogenic activity and gastric protection already. Sub-acute toxicological studies have revealed that the methanolic extract of Hydroalcoholic leaves extract of *Strobilanthes Kunthiana* show slight CNS depression for a few hours after treatment at the dose of 2000 mg /kg. However, there was no sign of toxicity or mortality up to 14 days indicates that the extract is relatively safe. Any substance that is not toxic at 2000 mg /kg is considered relatively safe.

Table 1: Summary of preliminary qualitative phytochemical tests for *Strobilanthes Kunthiana* (Leaves) extracts

Phytoconstituents	*Strobilanthes Kunthiana* (HE)
i) Primary Metabolites	
Carbohydrates	(+)Present
Amino acids	(-)Absent
Proteins	(-)Absent
Fats and oils	(-)Absent
ii) Secondary metabolites	
Steroids	(+)Present
Triterpenoids	(+)Present
Volatile oils	(-)Absent
Gums and mucilage	(-)Absent
Glycosides	(-)Absent
Saponins	(+)Present
Flavonoids	(-)Absent
Tannins & Phenolics	(+)Present
Alkaloids	(-)Absent

HE = Hydroalcoholic extract; '+' = Present; '-' = Absent

Table 2: Total Phenolic Content of Hydroalcoholic extract of leaves of *Strobilanthes Kunthiana*

Sample	Total phenolic content GAE mcg/ml
Hydroalcoholic extract 100µg/ml	22.54± 0.101

n=3, values are given in SEM

Table 3: Total Flavonoid content of Hydroalcoholic extract of leaves of *Strobilanthes Kunthiana*

S. No.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mcg/ml
1	Hydroalcoholic extract (100µg/ml)	16. 9571 ± 0.220

n=3, values are given in SEM

Table 4: Effect of the Ethanolic leaves extract of *Strobilanthes Kunthiana* on Ethanol Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg b.w)	Gastric Volume(ml)	Ulcer area(mm^2)	Protection (%)
Control	PBS 10 ml/kg	0.82+0.04	0.00+0.00	NA
HASK	250 mg/kg	2.54+0.20	612.21+11.00	30.57%
HASK	500 mg/kg	1.87+0.27	324.58+0.27	62.76%
Omeprazole	8 mg/kg	1.93+0.22	191.24+12.65	78.29%

Data are expressed as mean SEM., n=6 in each group. *p<0.05, **p<0.01 - One way ANOVA followed by Dunnett's test

Table 5: Effect of Hydroalcoholic extract of *Strobilanthes Kunthiana* on Antisecretory Parameters

Treatment	Dose (mg/kg b.w)	pH	Free Acidity (mEq/l/ 100g)	Total Acidity (mEq/l/ 100g)
Control	PBS 10 ml/kg	1.93±0.12	63.01±3.1	80.84±3.8
HASK	250 mg/kg	3.02±0.22	43.32±0.22	39.42±2.66
HASK	500 mg/kg	3.66±0.44	58.32±3.56	42.42±2.46
Omeprazole	8 mg/kg	5.32±2.55	23.13±3.5	31.93±3.5

Values are mean ± S.E.M, n=6, NS-not significant, *p < 0.05 and **p < 0.01 Vs control, One way ANOVA followed by Dunnett's test

Table 6: Effect of Hydroalcoholic extract of *Strobilanthes Kunthiana* on Total Proteins and C/P

Treatment	Dose (mg/kg b.w)	Total proteins (µg/ml)	C/P
Control	PBS 10 ml/kg	468.64±0.86	0.84
HASK	250 mg/kg	416.03±1.61**	1.6
HASK	500 mg/kg	314.05±1.53**	2.20
Omeprazole	8 mg/kg	248.50±1.11**	2.45

Values are expressed in terms of mean ± S.E.M, **p<0.01- One way ANOVA followed by Dunnett's test

Table 7: Effect of Hydroalcoholic extract of *Strobilanthes Kunthiana* on Total Carbohydrates

Treatment & Dose (mg/kg)	Total Carbohydrates (µg/ml)			
	Total Hexose	Hexosamine	Fucose	Sialic acid
Control	153.33±0.63	173.42±0.48	73.42±0.22	21.22±0.22
HASK	281.42±1.84**	233.80±1.24**	79.42±1.86**	40.42±1.46**
HASK	311.14±1.02**	262.22±0.82**	80.22±1.42**	34.64±1.40**
Omeprazole	479.31±0.22**	423.20±0.32**	161.24±0.24**	45.24±0.24**

Values are expressed in terms of mean ± S.E.M, **p<0.01 Vs control- One way ANOVA followed by Dunnett's test

CONCLUSION

From this study, it is clear that *Strobilanthes Kunthiana* leaf extract have significant anti-ulcer activity in animal models. It has muco-protective activity and gastric antisecretary when compared with that of reference drug omeprazole. The extract is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to the presence of flavonoids. Further studies are being carried out to characterize and explore the biological activity of the compounds present in the extract.

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