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PHYTOCHEMICAL SCREENING AND ANTI-ULCER ACTIVITY OF ETHANOLIC EXTRACT OF CISSAMPELOS PAREIRA LINN PENNEL LEAVES ON WISTAR ALBINO RATS

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Abstract:

Indomethacin induced ulcers in wistar rats was the main model of investigation of the anti-ulcer activity of the ethanolic extract of Cissampelos pareira Linn Pennel leaves in which ulcer index was used as the primary parameter. Inhibition of gastric lesions of the ethanolic extract of the leaves was observed at doses of 175mg/kg and 350 mg/kg when taken orally. At these doses, notable reduction in the gastric volume, free acidity and the ulcer index were observed when compared to the control group. From the results obtained, it can be noted that the ethanolic extract of Cissampelos pareira Linn Pennel leaves have anti-ulcer activity which can also be called as anti-secretory activity.

KEY WORDS: Cissampelos pareira Linn Pennel, anti-ulcer activity, ulcer index, Indomethacin

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INTRODUCTION:

Herbal plants are the ones which possess medicinal properties along with other uses such as in cosmetic preparations etc. These plants are said to have minimal or no side effects. Since prehistoric times, herbs are being used in traditional system of medicine and are believed to prevent and cure various ailments. Many of the ancient manuscripts described the uses and benefits of herbs. These herbs have gained lot of importance these days and many scientists are conducting intensive researches on the properties of various parts of herbal plants. These include the data related to the uses, efficacy, adequate dose, safety parameters and toxic doses. These herbs are now achieved significance for their use in pharmaceutical preparations. There are many examples of such drugs which gained popularity in treating various serious diseases which include Vincristine that is been used as anti-tumor agent, Ephedrine in treating bronchospasm and Digitalis in treating cardiac problems. As a result, beliefs on use of herbal products in curing diseases has attained immense popularity and is becoming a demand to prepare herbal drugs which are safer than synthetic ones.

The plant Cissampelos pareira Linn Pennel which is also known by the names 'Patha', 'Velvet Leaf Tree' is commonly found throughout the subtropical and tropical regions of the Himalayas. The leaves of this plant are triangular and the stem also gained importance in Ayurveda for its uses. It is used in the prevention and treatment of various health conditions such as chronic non-healing ulcers, sinuses, chronic skin diseases. It is also used in the treatment of poisonous animal bites. It has a remarkable anti-inflammatory property. The chemical composition of this plant includes alkaloids like hayatidin, hayatinin, hayatin and cycleanine that are isolated from this plant.

Gastric ulcers are a common problem for everyone these days which may be caused due to various reasons such as hemorrhagic surgical shock, infection due to H. pylori or due to use of some drugs that cause stomach ulcers as a side effect (Rao et al.,2004). This study is done to evaluate and assess the anti-ulcer properties of Cissampelos pareira Linn Pennel Linn.

Collection and extraction:

Required quantity of leaves were collected from Cissampelos pareira Linn Pennel Linn and they were shade dried at room temperature. These shade dried leaves were made into coarse powder by using mechanical grinder and this powder was preserved in an air tight container. Then extraction process was carried out by using 70 grams of the powder prepared which was mixed with 1000ml of distilled water. This mixture was heated sufficiently till the solvent got separated. This solvent was filtered using a muslin cloth and the extract was taken for centrifugation process at a speed of 1000rpm. After the completeion of the centrifugation process, the marc was collected and dried. Then it was weighed which yielded a weight of 14 grams.

Preliminary phytochemical screening of extracts: Various tests were performed for the obtained ethanolic

extract for the identification of phytochemical constituents (Kokate, 2002). These tests revealed the presence of various compounds such as phenols, flavonoids, steroids, tannins and saponins.

Animals: For this study, healthy adult wister albino rats were taken which weighed between 150-200 grams. These rats were stored in polypropylene cages which maintained standard laboratory environments i.e., 25° C±2°C, 12-hour light and dark cycles. They were fed with required amount of the standard food and water. Ethical approval was taken from the institutional animal ethical committee prior to performing the study.

Acute oral toxicity studies: Acute oral toxicity studies were performed according to OECD guideline 425 for ethanolic extract *of Cissampelos pareira Linn Pennel* leaves.

Indomethacin induced ulcer: Male rats were grouped into 4 categories each containing 6 animals. These rats were made to fast for 24 hours before conducting the study to prevent the situation of coprophagy.

Group I – control group receiving 20 mg/kg body weight of Indomethacin

Group II – standard group receiving 20mg/kg Omeprazole orally

Group III – group receiving 200mg/kg ethanolic extract of *Cissampelos pareira Linn Pennel* leaves orally

Group IV – group receiving 400mg/kg ethanolic extract of *cissampelos pareira linn pennel* leaves orally

Group I was considered as control group without any treatment. The animals were injected with Indomethacin at a dose of 20mg/kg to induce ulcers. They received ethanolic extract of *Cissampelos pareira Linn Pennel* leaves orally at doses of

200mg/kg and 400mg/kg. After 4 hours, the animals were sacrificed and the stomach was cut and opened to analyze the percentage of ulcer inhibition (Kannappan *et al.*, 2008, Panda *et al.*, 1993, Parmar NS *et al.*, 1991, Pati K.S. *et al.*, 2008).

Animals of all the groups were made to fast for 36 hours after the treatment and were injected with anesthetic ether. Then the stomach was cut through an incision just below the xiphoid process without disturbing the blood supply. After examination, the stomach was sutured and the rats were left for recovery in their individual cases. They were avoided from consuming water during this period. After 4 hours, the rats were sacrificed by ether overdose. The stomach was removed cautiously to collect the required gastric contents. The collected gastric juice was subjected to centrifugation process at 1000rpm and gastric volume was measured. Total acidity and free acidity were determined using 0.01N sodium hydroxide and phenolphthalein indicator. The cut stomach was pinned onto a soft board to evaluate and calculate the ulcer index. (Vogel et al., 2002).

ULCER SCORING

- 0 Normal colored stomach
- 0.5 Red coloration
- 1 Spot ulceration
- 1.5 Hemorrhagic streak
- 2 Ulcers>3mm
- 3 ulcers>5mm(perforation)

Percentage inhibition:

Percentage of ulcer inhibition was calculated using the below formula. (Malairajan et al., 2007) Percentage protection = Control(M) — Test (UI)x100 control (UI)

Statistical studies:

The statistical evaluation of the results obtained were calculated using one way analysis of variance (ANOVA) and Dunnet's 't' test. The p values were calculated with the help of Prism Graph Pad software (Trail version).

RESULTS:

Phytochemical screening: The phytochemical screening of ethanolic extract of *Cissampelos pareira Linn Pennel* leaves revealed the presence of various constituents like phenols, flavonoids, steroids, alkaloids, tannins, proteins, saponins and amino acids.

Acute toxicity studies (LD₅₀): Animals were normal and there were no symptoms of toxicity observed during the entire study which helped to predict the maximum safe dose of the ethanolic extract to about 2000mg/kg. 175mg/kg was taken as low dose and 350mg/kg was taken as high dose in this study.

Indomethacin induced ulcer: Significant reduction of ulcer index was produced by Omeprazole. The percentage of protection from ulcer by Omeprazole and the extract at doses of 175mg/k and 350mg/kg was found to be:

Table No 1: Effect of Cissampelos pareira Linn Pennel Leaf extracton indomethacin induced ulcers

Treat	Dose	Ulce	%	Gastric	%inhi	Vol of
ment	(mg/	r	inhibition	acid	bition	gastric
	kg.b.	inde		output		juice
	wt)	X				
Conto	20	30.6±	-	99.67±	-	5.98±0
1		1.5		24.5		.117
Stand	20	12.6±	65.17%	36.83±	65.78	3.18±0
ard		0.8		15.3	%	.18
Test	200	18.88	41.19%	69.39±	22.32	5.78±0
dose		±1.53		8.75	%	.093
		8				
Test	400	10.16	76.53%	49.67±	52.04	4.15±0
dose		±3.1		14.7	%	.163

All values represent Mean ± SEM, n=6 in each group. ***P<0.001, **P<0.01, Control group is compared with standard and extract doses

Ulcers developed in (Control): group-1



Ulcers developed in (standard): group -2



Ulcer developed in (test dose-1):group-3



Ulcer developed in (test dose-2): group-4



DISCUSSION:

Recently, treatment using herbal products has be gaining popularity and has become one of the demanding sources for cure of various diseases. The phytoconstituents in the extracts of plants has shown various medicinal properties and comparatively more safe than synthetic drugs. In this study, the anti-ulcer activity of ethanolic extract of *Cissampelos pareira Linn Pennel* leaves was analyzed. Ulcers were induced in the wister albino rats with the help of Indomethacin at 20mg/kg orally.

Peptic ulcer disease is a condition where sores are formed on the lining of stomach and duodenal portion of the small intestine. Ulcers in the stomach damage the gastric lining. This may be due to excess gastric acid secretion in the stomach due to some drugs or may be due to an infection. Peptic ulcer disease requires such therapy that reduces gastric acid secretion the stomach and treats the signs and symptoms of the disease. The therapy includes usage of various classes of drugs such as proton pump inhibitors and H2 receptor antagonists.

In this model, we saw Indomethacin induced ulcers where the drug showed significant reduction of ulcers when compared with the standard group. The main parameter used in this study was ulcer index to analyze the ulcer. It should be noted that factors such as disturbance in balance of aggressive and defensive modes are responsible in causing ulcers.

CONCLUSION:

Herbal products are now a part of medicine to treat numerous diseases. In this study, we observed that the leaves of *Cissampelos pareira Linn Pennel* are to heal gastric ulcers in albino rats. The constituents in ethanolic extract of the leaves played a major role in showing anti-ulcer activity.

Further research enables us to learn more about the active constituents of this plant and their use in chronic ulcers as well as other uses and side effects. It also promotes us to acquire knowledge about the safe doses for use in treatment of various dieases.

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