INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

ANTIULCER ACTIVITY OF METHANOLIC AQUEOUS EXTRACT OF FLOWERS OF *CANTHIUM DICOCCUM* AGAINST THE DICLOFENAC SODIUM INDUCED GASTRIC ULCERATION ANIMAL MODEL OF RATS

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ABSTRACT

The Antiulcer Activity of Methanolic aqueous extract of flowers of *Canthium dicoccum* at a dose of 200, 400 mg/kg b.w p.o was investigated in Diclofenac induced ulcers in Albino Wistar rats (180-230 gm). In the model common parameter determined was ulcer index. There was no mortality up to a dose of 2000 mg/kg b.w p.o indicating the safety of the plant. Preliminary Phytochemical studies showed the presence of Carbohydrates, Flavanoid, Glycosides, Tannins, Protein, Terpenes and Volatile Oils. Methanolic aqueous extract of *Canthium dicoccum* at a dose of 200,400 mg/kg b.w p.o produced significant reduction of ulcers in Diclofenac induced induced ulcer Model as compared to control group. % ulcer protection of Methanolic aqueous extract of *Canthium dicoccum*w as calculated and maximum ulcer protection was found at a dose of 400 mg/kg for Diclofenac induced ulcer The ulcer protective effect of extract was comparable with that of the standard drugs. Results of study suggest that Methanolic aqueous extract of *Canthium dicoccum* posses Mucoprotective effect which may be due to the presence of flavonoids in the extract as it has an astringent property.

Key words: *Canthium dicoccum*, Antiulcer Activity, Methanolic aqueous extract

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INTRODUCTION

A peptic ulcer is an open crater or sore that develop in the inner lining (mucosa) of the stomach or the duodenum. A coating of mucus and other chemicals normally shields the stomach and duodenum from digesting themselves. When these protective mechanisms are disrupted, powerful digestive acids can erode into the lining of these organs and cause peptic ulcer. Peptic ulcer is a major gastro-intestinal disorder caused by an imbalance between offensive (gastric acid, pepsinogen secretion) and defensive(mucus secretion, gastric mucosal integrity) factors. It is a round or oval sore where the lining of the stomach or duodenum has been eaten away by stomach acid and digestive juices. A peptic ulcer, also known as peptic ulcer disease is an ulcer of an area of

the gastrointestinal tract that is usually acidic and thus extremely painful. If it is in the stomach, it is referred to as a gastric ulcer. If it is in the duodenum, it is called a duodenal ulcer. Duodenal ulcers are more common than gastric ulcers and usually occur in people aged under 50. Gastric ulcers are more common in people aged over 50. 5-10% of people worldwide suffer from peptic ulcer at least once in their lifetime. Peptic ulcers affect both men and women. The term peptic ulcer refers to chronic ulcerative disorder of the upper gastro intestinal tract, which have a common participation of acid and pepsin in their pathogenesis. It includes duodenal ulcer and gastric ulcer, as well as, ulcer associated with Zollinger- Ellison syndrome. It is a physiologic marvel that gastric juice can easily digest the swallowed pieces of meat but normally has no corrosive action on the gastric mucosa itself. Several factors seen to be involved in the protection of the gastric mucosa from auto digestion. Endogenous prostaglandins stimulate secretion of gastric mucus as well as gastric and duodenal mucosal bicarbonate. They also participate in the maintenance of gastric mucosal blood flow and integrity of mucosal barrier and promote epithelial cells renewal in response to mucosal injury. A breakdown of the balance between the corrosive action of acid-pepsin and the mucosal resistance results in peptic ulcers. In duodenal ulcer or ulcers due to Zollinger-Ellison syndrome, evidence of an absolute or atleast relative gastric hyper secretion can be demonstrated. In contrast, defective mucosal resistance seems to be major contributory factor in gastric ulcers (1-3).

Diclofenac– induced ulcers in rats

The animal in all the groups were kept for 24 h. fasting after that animal of all groups' received diclofenac sodium (NSAIDs, 20 mg/kg). The oral feeding of water and diclofenac sodium was continued for 3 days, the animal of II, III and IV were administered with ranitidine (13.5mg/kg), flower extract (200mg/kg), flower extract (400mg/kg) respectively after 3 h. of diclofenac administration. On 4th day the animals were sacrificed, stomach were removed and cut along the greater curvature to measure the ulcer index (4, 5).

Aim of the study is to evaluate the Anti Ulcer activity of Methanol aqueous flower extract of *canthium dicoccum* Linn.in the male Wistar albino rats.

MATERIALS AND METHODS

Collection of plant material

The Flowers of Canthium dicoccum used for the present studies was collected from Chitoor district of Andhra pradesh. The plant was identified, confirmed and authenticated by comparing with voucher specimen available at Survey of medicinal plants and collection unit, Department of Botany, Sri Venkateswara University, Tirupathi by Field Botanist Dr. Madhav shetty. The bark was cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanichal grinder. The resulting powder was then used for extraction.

Preparation of Methanolic Extract

The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 1500 ml of methanol for seven days. The extract was concentrated and dried using Rotary flash evaporator. It was kept in dessicator until used

Experimental animals

Swiss Albino rats adult of either sex were obtained from Mahaveer enterprises, Hyd(169/CPCSEA/1999). The rats were divided randomly into 4 groups of 5 rats each for each model. Each rat that weighed between 180-200 gm was housed separately (Four rats per cage). The animals were left for 48 hrs to acclimatize to the animal room conditions. They were maintained in standard laboratory conditions of temperature 22±2^{o}c, humidity, 12 hours light and dark cycles fed with standard pellet diet (Hindustan lever, Bangalore) and adequate tap water.

The rats are divided into different groups each comprising of minimum of five rats as detailed below **Group I –** Control rats (Diclofenac +tween 80); **Group II** – Diclofenac induced ulcer rats orally treated with Ranitidine (13.5 mg/kg b.w); **Group III** – Diclofenac induced ulcer rats orally treated with formulation (Canthium dicoccum) at the dose of 200 mg/kg b.w (SINGLE DOSE); **Group IV** – Diclofenac induced ulcer rats orally treated with formulation (DOUBLE DOSE). The animal in all the groups were kept for 24 h. fasting after that animal of all groups' received diclofenac sodium (NSAIDs, 20 mg/kg). The

oral feeding of water and diclofenac sodium was continued for 3 days, the animal of II, III and IV were administered with ranitidine (13.5mg/kg), flower extract (200mg/kg), flower extract (400mg/kg) respectively after 3 h. of diclofenac sodium administration. On 4th day the animals were sacrificed, stomach were removed and cut along the greater curvature to measure the ulcer index.

Collection of Gastric Juice (6, 7)

The stomach was excised carefully keeping the esophagus closed, opened along the greater curvature and the gastric contents were removed. The gastric contents were collected in plain tubes and centrifuged at 3000 rpm for 5 min; the volume of the supernatant was expressed as ml/100 gm body weight. The mucosa was flushed with saline and observed for gastric lesions using a dissecting microscope, ulcer score was determined.

Free acidity and Total acidity (8, 9)

Centrifuge the gastric contents at 1000 rpm for 10 min, note the volume. Pippete out 1 ml of supernatant liquid and dilute it to 10 ml with distilled water. Note the P^H of the solution with the help of P^H meter. Titrate the solution against 0.01N NaOH using topfers reagent as an indicator.(It is Dimethyl-amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid and total acidity in gastric fluids) Titrate to end point when the solution turns to orange colour. Note the volume of NaOH which corresponds to free acidity. Titrate further till the solution regains its pink colour. Note the total volume of NaOH which corresponds to the total acidity.

RESULTS AND DISCUSSION

Preliminary Phytochemical studies showed the presence of Carbohydrates, Flavanoid, Glycosides, Tannins, Protein, Terpenes and Volatile Oils.

Effect of Gastric Volume

Administration of the extract significantly decreased the gastric volume in comparison with rats treated with Ranitidine. Comparing the gastric volume and gastric acidity, the gastric volume gets decreased, simultaneously the gastric acidity also decreased significantly (Table-1).

Table-1 Effect of Formulation on Gastric Volume

Groups	Body wt. of rats	Drugs given	Gastric volume
GROUP I	177.2 ± 1.15	Diclofenac sodium + 2% Tween 80	1 ± 0.04
GROUP II	161.2 ± 2.15	Ranitidine + Diclofenac sodium	0.8 ± 0.05
GROUP III	172.5 ± 4.45	Herbal formulation + Diclofenac sodium	0.6 ± 0.03*
GROUP IV	164.4 ± 1.16	Herbal formulation (double dose) + Diclofenac sodium	0.5 ± 0.04**

Values are expressed in terms of mean ± SEM of 5 rats (ANOVA) P values:
****< 0.001 - Highly significant, * <0.05 - Significant, N S: Non Significant**

Effect of Free Acidity and Total Acidity

The free acidity and total acidity was determined based on the titre values. The free acidity and total acidity of extract on albino rats decreased significantly in comparison with the standard group treated with Ranitidine (Table-2).

Table-2 Effect of Formulation on Free Acidity and Total Acidity

Groups	Body wt. of rats	Drugs given	Free Acidity	Total Acidity
GROUP I	177.2 ± 1.15	Diclofenac sodium + 2% Tween 80	14.70 ± 0.29	29.6 ± 0.69
GROUP II	161.2 ± 2.15	Ranitidine + Diclofenac sodium	8.8 ± 0.31	15.56 ± 0.69
GROUP III	172.5 ± 4.45	Formulation + Diclofenac sodium	7.3 ± 0.32*	12.56 ± 0.68*
GROUP IV	164.4 ± 1.16	Formulation (double dose) + Diclofenac sodium	4.6 ± 0.42**	9.5 ± 0.59**

Values are expressed in terms of mean ± SEM of 5 rats (ANOVA) P values:
****< 0.001 - Highly significant, * <0.05 - Significant, N S: Non Significant**

Ulcer index

The ulcer index was calculated by taking the mean ulcer score of each groups.Then the mean ulcer score graph was plotted with groups on x-axis and ulcer index on y-axis.The histograms of different groups were then interpolated by comparing the ulcer index of group I with group II, III and IV. It was noticed that the ulcer index of Dose group (Dose-III&IV) was significantly less when compared to the standard group (Group-II) treated with Ranitidine (Table-3 and Fig-1-4)).

Table-3 Effect of Formulation on Ulcer Index

Groups	Body wt of rats	Drugs given	Ulcer index
GROUP I	177.2 ± 1.15	Diclofenac sodium + 2% Tween 80	3.7 ± 0.14
GROUP II	161.2 ± 2.15	Ranitidine + Asprin	2.2 ± 0.10
GROUP III	172.5 ± 4.45	Formulation + Diclofenac sodium	2 ± 0.18*
GROUP IV	164.4 ± 1.16	Formulation (double dose) + Diclofenac sodium	1.5 ± 0.9**

Values are expressed in terms of mean ± SEM of 5 rats (ANOVA) P values:
****< 0.001 - Highly significant, * <0.05 - Significant, N S: Non Significant**

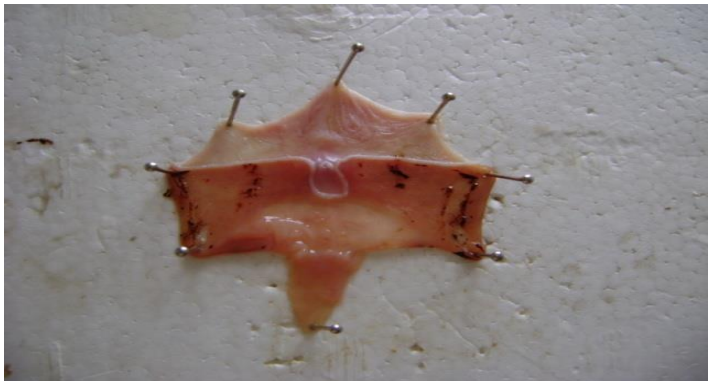

Fig-1 Group I (Control)

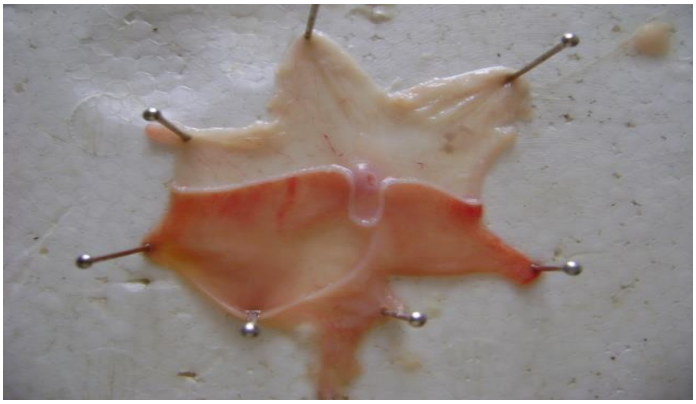

Fig-2 Group II (Standard)

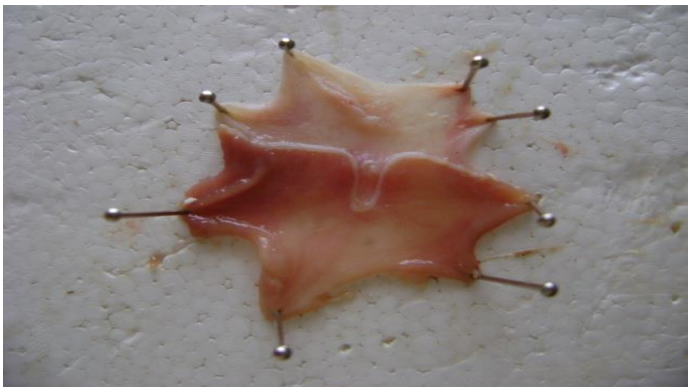

Fig-3 Group III (Single dose)

Fig-4 Group IV (Double dose)

It is evident from the result of the present investigation that the formulation of *Canthium dicoccum* possesses antiulcer activity in diclofenac induced acute ulcer model. It has shown a significant reduction in the gastric lesions in both the models. Although the etiology of gastric ulcer is not known in most cases, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents including plant extracts are used (in experimental animals) to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucus production, stabilizing the surface epithelial cells/or enhancing prostaglandin synthesis. Ranitidine the proton pump inhibitor play an important role in the reduction of gastric volume and total acidity and thus perform a cytoproective effect. The present results demonstrate that the formulation of *Canthium dicoccum* protect the rat gastric mucosa against hemorrhagic lesion produced by aspirin and ethanol. These inducing methods of gastric lesions are rapid and convenient way of screening plant extracts for antiulcer potency and cytoprotection in

macroscopically and microscopically visible lesions. Diclofenac induced gastric ulcers has been widely used for the experimental evaluation of antiulcer activity. Diclofenac induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury. It is of interest to note that administration of antioxidants inhibit aspirin induced gastric injury in the rats. *Canthium dicoccum* possess significant antioxidant activity. In conclusion, the antiulcer effects of the above plants have been reported earlier, but there are no studies reporting the combination of these herbals and their activity in these models are quite impressive. The antiulcer activity of the formulation *Canthium dicoccum* can be compared to the activity of the standard drug Ranitidine.

CONCLUSION

Canthium dicoccum possess the antiulcer activity against the Diclofenac sodium induced gastric ulceration animal model of rats. At the dose level tested it does not show any signs of toxic effects in treated mice as well as rats. Peptic ulcer is the most common disease. Many drugs are there in market to treat the ulcer, but they are having lot of adverse effects. In the present theory, using combination of herbal drugs have proved that these are the effective alternatives for chemical drugs. The Anti-ulcer Herbal formulation *Canthium dicoccum* is having significant activity in animals models used, as compared to the standard drug Ranitidine.

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