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Research Article

Evaluation of Anti-Ulcer Activity of Methanol Extract of *Dioscorea oppositifolia* Tubers in Adult Wistar Rats

***M. JHANSI RANI, [1]S. MOHANA LAKSHMI, [1]A. SARAVANA KUMAR**

[*,1]Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh, India.

ABSTRACT

In the traditional system of medicine, the *Dioscorea oppositifolia (*dioscoreaceae*)* have been employed for treating ulcer, fever, diarrhoea leprosy, piles. The present study was undertaking to investigate the anti ulcer effect of *Dioscorea oppositifolia* in ethanol and indomethacin induced gastric ulcer in wistar rats. The methanol extract of *Dioscorea oppositifolia* (MEDO) at the doses of 200 and 400 mg/kg, p.o. were administered for 14 days to the all group of animals except disease control. The dose of MEDO 200mg/kg and 400 mg/kg orally once daily was administered to rats for 14 days omeprazole (20mg/kg p.o.) was used as a standard drug. The severity of gastric mucosal damage induced by absolute ethanol (90%) was analyzed in terms of ulcer index value. Significantly decreased the ulcer index value in the animal groups which are treated with MEDO 200mg/kg, 400mg/kg and omeprazole (20mg/kg p.o.) control animals in ethanol and indomethacin induced ulcer. It can be concluded that *Dioscorea oppositifolia* has showed anti ulcer activity in adult wistar rats.

Keywords: *Dioscorea oppositifolia* anti-ulcer activity, ethanol-induced gastric ulcer, indomethacin induced gastric ulcer.

INTRODUCTION

Recently there has been a rapid progress in the understanding of peptic ulcer most of the studies focus on newer and better drug therapy. This has been the rationale for the development of new anti ulcer drugs which include the herbal drugs. A number of anti ulcer drugs such as H_2 receptor antagonist proton pump inhibitor and cytoprotectents are available for ulceration all these drugs have side effects like arrhythmias, gynaecomastia, entero chromaffin like cell, hyperplasia and hematopoietic [1]. Therefore the present study to evaluate the anti ulcer activity of *Dioscorea oppositifolia.*

The *Dioscorea oppositifolia* is a perennial twining vine belong to the family Dioscoreaceae. The leaves are arranged oppositely with heart shape. It is distributed worldwide, the perennial tuber of *Dioscorea oppositifolia* is well known, due to the triterpenoid compound [2]. The tuber contains about 20% starch, 75% water, 0.1% vitamin B_1 and 10 to 15mg vitamin C. It also contains mucilage, amylase, amino acids and glutamine [3]. The tubers are sometimes used as an herbal tonic. It stimulates the stomach and spleen and has on effect on lungs kidneys. The tubers have been eaten for the treatment of poor appetite, chronic diarrhea, asthma and dry cough [4]. Hence the present work is carried out to evaluate the effect of methanol extract of *Dioscorea oppositifolia* an adult wistar rats with anti ulcer activity by ethanol and Indomethacin induced gastric ulcer.

MATERIAL AND METHODS

Plant Material

The tubers of *Dioscorea oppositifolia* were collected from Talakona forest, Chittoor District of Andhra Pradesh, India, in the month of November, 2009. The plant was authenticated by Prof. P. Jayaraman, Director of National

***Corresponding Author M.Jhansi Rani E mail: jhansi.m39@gmail.com**

Institute of Herbal Science, West Tambaram, Chennai. The voucher specimen (PARC/2009/430) of the plant was deposited at the college for further reference.

Preparation of extraction

The tubers of *Discorea oppositifolia* was dried in shade and pulverized in grinder-mixer to obtain a coarse powder. It was then passed through 40 mesh sieve. Weight quantity to continuous hot extraction with methanol in soxhlet apparatus for 48 hours. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to given an extract methanol extract of *Dioscorea oppositifolia* 12.85% w/w.

Preliminary Phyto Chemical Screening

The freshly prepared crude methanol extract of *Dioscorea oppositifolia* was qualitatively tested for the presence of major phytochemical constituents are Aminoacids, carbohydrates, triterpeniodes [5].

Acute toxicity study

The acute toxicity of methanol extract of *Dioscorea oppositifolia* leaves was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study [6].

Animals used

Healthy adult wistar rats (150-250gm) were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh. The animals were maintained in a well ventilated room with 12:12 hour light or dark cycle in polypropylene cages. The animals were fed with standard pellet fed (Hindustan Lever Limited, Bangalore) and water was given *ad libitum.* Ethical committee clearance was obtained from institutional animal ethics committee (IAE) of CPCSEA (Ref. No./IAEC/XIII/05/SVCP/2009-2010)

Histopathology

After collection the gastric content and measuring the ulcer area, small pieces of stomachs from each group were embedded in paraffin wax. Sections of 5µm thick were cut in a microtome and mounted on glass slides using standard techniques. After staining the tissues with hematoxylin-eosin stain, the slides were viewed under a light microscope equipped for patho graphy.

Ethanol-induced gastric ulcer

All the animals were fasted for 24 hours before administration of ethanol. The animals were divided into five groups each consisting of six rats. Group I represented the control group which received distilled water orally. Group II received ethanol, Group III & IV received methanol extract of *Dioscorea oppositifolia* 200mg/kg, 400mg/kg respectively for 14 days and Group V treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14^{th} day, Gastric ulcers were induced with absolute ethanol 90% (1ml/200g) orally. They were kept in specially constructed cage to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 hour later with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored by following method [7].
Ulcer index was calculated by

$$\%I = \frac{US_C - US_t}{US_C} x100$$

Where, US_C – ulcer surface area in control
US_t – Ulcer surface area in treated animals.

Indomethacin-induced gastric ulcer

All the animals were fasted 36 hours before administration of indomethacin. The animals were divided into five groups each consisting of six rats. Group I disease control group which received distilled water or normal saline. Group II received indomethacin (20mg/kg p.o.). Group III & IV group pretreated MEDO (400 mg/kg p.o.) for 14 days. Group V as standard drug omeprazole (20mg/kg p.o.). The rats were anaesthetized with ether 1 hour latter the stomach was incised through the grater curvature and examined for the number of lesion under the dissecting microscope [8] by titrating with 0.01N NaOH using phenolphthalein as an indicator gastric juice estimated [9] pepsin [10] sialic acid and fructose [11].

Statistical Analysis

Results are expressed as mean ± standard error of mean. The significance of difference between means of control and treated group were determined by student's t-test using ANOVA followed by the result regarded as significant at $P<0.001$.

RESULTS

Acute toxicity study

Acute toxicity study in which the animals treated with the MEDO at a higher dose of 2000mg/kg did not manifest any significant abnormal signs, behavior changes, body weight changes or macroscopic findings at any tine of observation. There was no mortality in the above mention dose at the end of the 14 days of observation.

Phytochemical screening

The result of preliminary phytochemical screening of methanol extract of *Dioscorea oppositifolia* (MEDO) revealed that presence of carbohydrates, amino acids and triterpenoids absence of phenol and steroids.

Ethanol-induced gastric ulcer

In control animal oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated banks of thick, black and dark red lesion. MEDO has shown significant inhibition of ulcer at the doses of 200mg/kg and 400mg/kg respectively in comparison to control. Omeprazole as referred standard drug was inhibition of ulcer significantly.

Indomethacin induced gastric ulcer

The result obtained show that the ulcer surface area and mean ulcer index were significantly reduced in groups treated with the methanol extract of *Dioscorea oppositifolia* compared to their respective control group. Therefore, it can be through that the MEDO may stimulate the secretion of prostaglandins or possess prostaglandins like-substances preventing gastric ulcers in order to probe the effectiveness of MEDO.

Table 1. Effect of *Dioscorea oppositifolia* tubers on various parameters in indomethacin induced gastric ulcer in rats

Group	Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Disease Control Water (10 ml/kg p.o.)	14 ± 0.36	---
II	Standard (Omeprazole 20 mg/kg p.o.)	4 ± 0.25***	71.42
III	MEDO (200 mg/kg p.o.)	10 ± 0.57***	28.57
IV	MEDO (400 mg/kg p.o.)	5.66 ± 0.33***	59.57

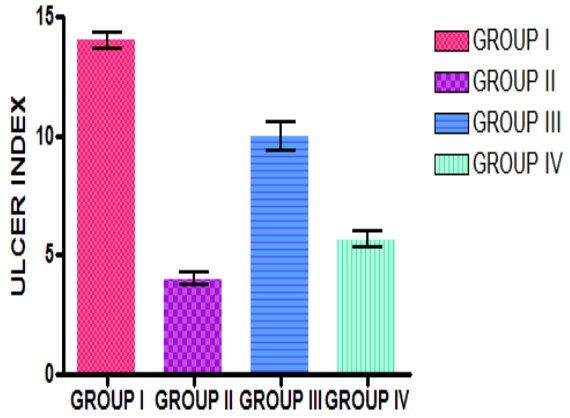
Values are expressed as mean ± SEM, ANOVA followed by student t-test in each group rats ***P<0.001, as compared to indomethacin induced group.

Table 1. Effect of *Dioscorea oppositifolia* tubers on various parameters in ethanol-induced gastric ulcer in rats

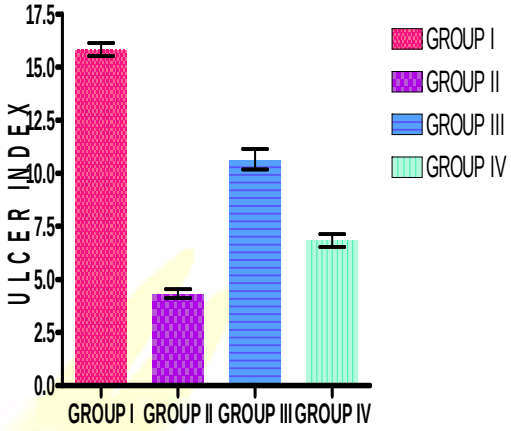
Group	Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Disease Control water (10 ml/kg p.o.)	15.83 ± 0.31	---
II	Standard (Omeprazole 20 mg/kg p.o.)	4.33 ± 0.21***	73.34
III	MEDO (200 mg/kg p.o.)	10.66 ± 0.50***	32.67
IV	MEDO (400 mg/kg p.o.)	6.83 ± 0.31***	56.54

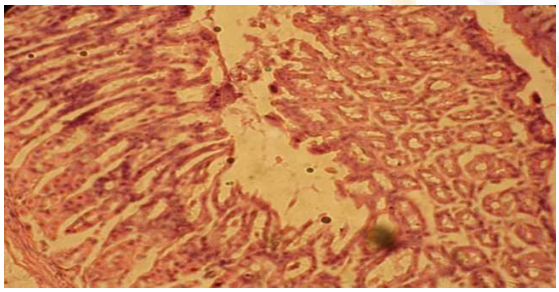
Values are expressed as mean ± SEM, ANOVA followed by student t-test in each group rats ***P<0.001, as compared to ethanol-induced group.

INDOMETHACIN INDUCED GASTRIC ULCER

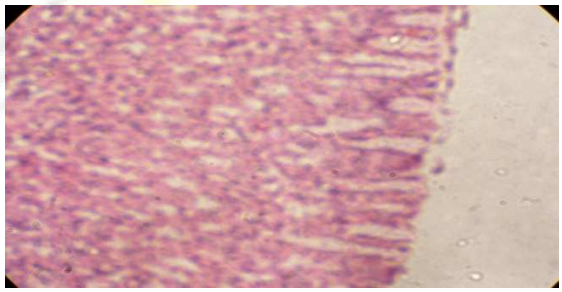


ETHANOL INDUCED ULCER

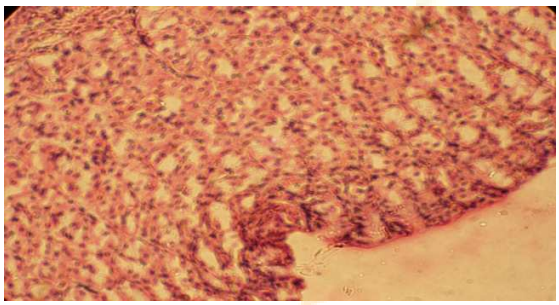




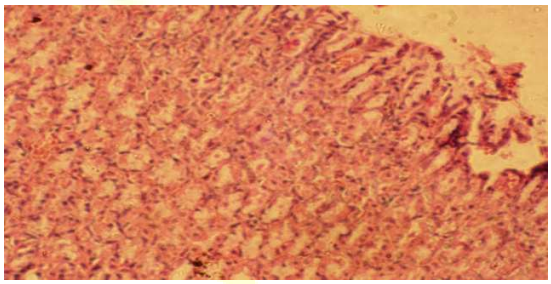
Group I: Disease Control



Group II: Standard



Group III: MEDO (200 mg/kg p.o.)



Group IV: MEDO (400 mg/kg p.o.)

DISCUSSION

In this present study ulcer was assessed by using ethanol induced gastric ulcer [7] and indomethacin induced gastric ulcer [8]. Ethanol and indomethacin induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be to stasis in gastric blood glow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates in gastric mucosa apparently causing cell and plasma membrane damage leading to increase the intracellular membrane permeability of sodium and water. The massive intra cellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [12].

Their anti-ulcerogenic potency was tested against indomethacin-induced ulcer. Indomethacin is a cyclooxygenase inhibitor which suppresses gastro duodenal bicarbonate secretion reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow in animals [13]. It is also well known that prostaglandin synthesized in large quantities by the gastrointestinal mucosa can prevent experimentally induced ulcers by ulcerogens. Thus when the gastric ulcer is induced by indomethacin, the cytoprotective effect of the anti-ulcer agent can be mediated through endogenous prostaglandins [14]. The result obtained show that the ulcer surface area and mean ulcer index were significantly reduced in groups treated with the methanol extract of *Dioscorea oppositifolia* compared to their respective control group. Therefore, it can be through that the MEDO may stimulate the secretion of prostaglandins or possess prostaglandins like-substances preventing gastric ulcers in order to probe the effectiveness of MEDO.

Phytochemical studies of the methanol extract of *Dioscorea oppositifolia* revealed the presence of flavonoids, alkaloids and triterpens which may be responsible for the anti-ulcer properties. Many compounds from these chemical classes such as nimbidine, ursolic acid, oleanolic acid, qualetin, diosmin, wogonin and sophoradine [15,16,17, 18,19] have been shown to possess anti-ulcer properties. The methanol extract of *Dioscorea oppositifolia* at a dose of 400mg/kg showed similar activity to that of omeprazole (a proton pump inhibitor, which is used to heal stomach and duodenal ulcers). The gastro protective effect of omeprazole is mediated through block of acid secretion by inactivation of H+/K+-ATPase [20]. This study reveals that the methanol extract of *Dioscorea oppositifolia* are potent inhibitors of gastric mucosal lesions caused by ethanol and indomethacin in rats.

CONCLUSION

In the present investigation methanol extract of *Dioscorea oppositifolia* showed significant anti-ulcer activity at two different doses 200mg/kg and 400mg/kg in ethanol and indomethacin induced ulcer in rats. Methanol extract of *Dioscorea oppositifolia* and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

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