



## Antiulcer and Anxiolytic Effect of *Durenta repens* Linn in Experimental Animal Model

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### ABSTRACT

The Petroleum ether and Chloroform extract of *Durenta repens* leaves was investigated for its potential to protect gastric mucosa against pylorus ligation induced ulcer and to find out the anxiolytic action in elevated plus maze model. Chloroform extract at the dose of 200mg/kg protected the gastric mucosa in the pylorus ligation ulcer induction significantly ( $p < 0.001$ ) when compared with that of the standard drug famotidine (10mg/kg) and acts as a potent antiulcer effect. Elevated plus maze results were significant in alleviating the anxiety in the animals' results in increased time spent and entries into the open arm compared with the standard drug diazepam (1mg/kg).

**Keywords:** *Durenta repens*, methanolic extract, pylorus ligation, famotidine, diazepam.

### INTRODUCTION

A number of plants have been identified and evaluated by various researchers for gastro protective activity and CNS actions.<sup>1</sup> The present study is influencing gastroprotective and anxiolytic activity of *Durenta repens*<sup>2</sup> leaves in suitable animal models. Ulcer is induced by the method described by Shay et al i.e) pylorus ligation method and the anxiolytic action was screened by elevated plus maze method. *Durenta repens* Linn belonging to the family Verbenaceae is commonly known as Duranta traditionally used in various diseased conditions like malarial fever, hepatoprotective, antihistaminic etc. Phytochemical analysis was confirmed the presence of carbohydrates, steroid, cardiac glycosides, saponins, flavonoids, phytosterols and tannins etc.

### MATERIALS AND METHODS

The leaves of *Durenta repens* were collected from campus of Vijaya College of Pharmacy, in Hyderabad and authenticated by Dr. N. Sivaraj, Principal Scientist, National Bureau of Plant Genetic Resources Regional Station, Hyderabad. The leaves were shade dried until free from moisture. Then they were subjected to size reduction to get coarse powder of desired particle size. The powdered drug was subjected to extraction with petroleum ether and chloroform in a Soxhlet extractor, temperature was maintained on an electric heating mantel with thermostat control<sup>3</sup>. The extracts were then concentrated to 3/4th of their original mass using rotary vapour apparatus. The concentrated extract were then transferred to a china dish and evaporated on a thermostat controlled water bath till it formed a thick paste. The thick mass was vacuum dried in a desiccator till it is free from moisture. The pet ether and chloroform extracts were administered orally as a suspension by triturating with 5% Tween 80.

### Animals

Normal healthy male wistar albino rats and mice (180-240g) were housed under standard environmental conditions at temperature ( $25 \pm 2^\circ$  C) and light and dark (12: 12 h). They were fed with standard pellet diet and water *ad libitum*.

### Phytochemical Test

Phytochemical tests on the extract and fractions were performed using standard procedures.<sup>3</sup>

### Acute toxicity studies

The acute toxicity studies were performed to study the acute toxic effects and to determine the minimum lethal dose of the drug extracts as per the guideline OECD 423. Swiss albino mice of either sex weighing between 18-25gm were used for the study. The pet ether and chloroform extracts of *Durenta repens* were administered orally to different groups of overnight fasted mice at the dose 30, 100, 300, 1000 and 2000mg/kg body weight. After the administration of the extracts, animals were observed continuously for the first 8hrs for any toxic manifestation. Thereafter observations were made at regular intervals for 24hrs. Further the animals were under investigation upto a period of one week.<sup>4</sup>

### Pharmacological screening

#### Antiulcer activity by pylorus ligation method

Adult albino rats of either sex weighing between 100-130 gm were divided into 3 groups of 6 animals. The animals were deprived of food for 24 hours before the commencement of experiment but water was allowed *ad libitum*. The drugs were given orally 2 hours prior to pylorus ligation<sup>5</sup>, which was carried out according to the technique reported. Group I received acacia suspension 1ml/kg, Group II and Group II received the chloroform and pet ether extract 200mg/kg and Group IV received



Ranitidine 10mg/kg respectively. The animals were sacrificed six hours after pyloric ligation to observe gastric lesion. The gastric juice was collected, centrifuged and its pH was determined. Free and total acidity were estimated titrimetrically using 0.01N NaOH solution. The data concerning the pH, acid secretion and ulcer analysed by one way followed by Tukey multiple comparison test.

#### Anxiolytic activity by elevated plus maze model

The plus maze apparatus consists of two open arms (35X5cm<sup>2</sup>) crossed with two closed arms (35X5X20 cm<sup>3</sup>). The arms were connected together with a central square (5X5cm<sup>2</sup>).<sup>6</sup> The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Animals were divided into 3 groups of 5 animals each, Group I control received distilled water (1ml/kg, p.o), Group II received Diazepam (1mg/kg, p.o) and Group III and IV received pet ether and chloroform extract (200mg/kg, p.o). After 30 minutes they were placed individually in the center of the apparatus, facing the closed arm. The time spent in both the open and closed arms was recorded for 5 minutes. The numbers of entries into open and closed arms were also counted during the test. An entry was defined as having all four paws within the arm.

#### RESULTS AND DISCUSSION

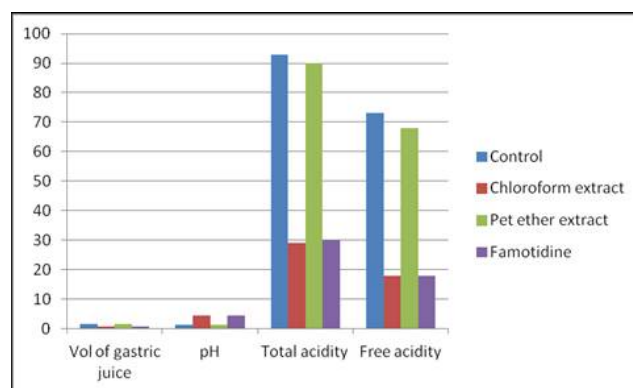
The extractive values for pet ether and chloroform extract was 20% and 25% which shows the solubility of the phytoconstituents in the particular solvent used. The phytochemical studies revealed the presence of carbohydrates, alkaloids, glycosides, reducing sugar, resins, flavonoids and terpenoids and the absence of tannins, saponins and acidic compounds. The toxicity study reveals that 2mg/kg as the therapeutic dose and up to 2mg/kg both the extracts were safe and not produced any toxicity symptoms. Pretreatment with pet ether and chloroform extracts reduced the incidence of ulcers in rats. There was no ulcer lesion in oral administration of acacia suspension and pretreatment groups. Gastric ulcer is believed to be due to an imbalance between acid and pepsin, and the weakness of mucosal barrier. Several mechanisms have been suggested for the effect of gastroprotective principles, including increasing hexosamine level and enhancing the strength of gastric barrier either physically or by blocking the H<sup>+</sup>, K<sup>+</sup> ATPase pump, stimulation of membrane stabilization by interference with Ca<sup>2+</sup> influx, scavenging oxygen generated free radical and inhibition of biological membranes.<sup>7</sup> *Durenta repens* exert its property by one or more of this proposed mechanisms However it should be pointed out that *Durenta repens* contain tannins and flavonoids to which the gastro protective effect could be attributed.<sup>8</sup> Further studies are required to isolate the active protective properties of *Durenta repens*. In elevated plus maze the animals spend greater time in the closed arms when placed in maze comprising of open and closed arms. Avoidance of the open arm portrays a manifestation of fear and anxiety. The results obtained showed chloroform had anxiolytic property by increasing

the cumulative time spent in the open arm. This effect was mainly due to modulation of GABA<sub>A</sub> – chloride channel receptor complexes. May also exert pharmacological action by increase in GABA content in the cerebral hemisphere.<sup>9,10</sup>

**Table 1:** Antiulcer effect of leaves extract of *Durenta repens*

Treatment	Volume of Gastric juice	pH	Total acidity (mEq/L)	Free acidity (mEq/L)
Control	1.6±0.08	1.2±0.04	93±5.8	73±4.1
Chloroform extract 2mg/kg	0.54±0.04	4.5±0.14*	29±2.8*	18±1.4*
Pet ether extract 2mg/kg	1.4±0.02**	1.3±0.03**	90±0.12**	68±1.2**
Famotidine 10mg/kg	0.59±0.03	4.3±0.07*	30±1.6*	18±1.3*

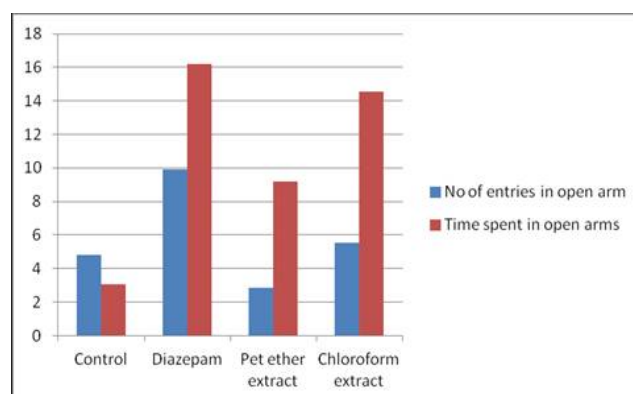
n=6, Values Mean±SEM, \*P<0.001, \*\*P<0.05



**Table 2:** Anxiolytic effect of *Durenta repens*

Treatment	Dose (mg/kg, p.o.)	Number of entries in open arms	Time spent in open arms (seconds)
Control	Vehicle (1ml/kg)	4.80±.62*	3.06 ± 0.63*
Diazepam	2	9.90 ± 1.66*	16.20 ± 0.58*
Pet ether extract	200	2.83±2.45**	9.20±0.10**
Chloroform extract	200	5.54 ± 0.68*	14.53 ± 0.71*

n=6, Values Mean±SEM, \*P<0.001, \*\*P<0.05



**CONCLUSION**

From the above study it was confirmed that *Durenta repens* can be safely used in the treatment of ulcer and anxiety disorders. Further studies were needed to confirm the exact molecular action and the specified pharmacological mechanism. Also, the active phytoingredient to be isolated for the further studies.

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