

## ANTI -ULCER ACTIVITY OF METHANOLIC EXTRACTS OF WATTAKAKA VOLUBILIS AND TABEBUIA ROSEA IN RATS

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### ABSTRACT

*Wattakaka volubilis* and *Tabebuia rosea* are plants, reported for their variety of ethnic medicinal uses. Hence we have planned to screen anti ulcer activity of leaves of these plants with the methanolic extracts. Leaf powder successively extracted with petroleum ether and methanol was subjected for phyto - chemical screening to identify different phytoconstituents. LD50 studies for alcoholic extracts were conducted upto the dose level of 5 g /kg by following OECD up and down method of guidelines No.425. Anti ulcer activity was evaluated in various animal models like aspirin induced and ethanol induced ulcer models in rats. Preliminary phyto - chemical screening of the methanolic extract of *Wattakaka volubilis* and *Tabebuia rosea* revealed the presence of tannins, flavanoids, alkaloids, saponins, terpenoids, phenolic compounds and sugars. No mortality was observed with methanolic extracts of *Wattakaka volubilis* and *Tabebuia rosea* up to the maximum dose level of 5 g/kg. Further methanol extracts at 500mg/kg (*Wattakaka volubilis* and *Tabebuia rosea*) per oral doses significantly ( $P < 0.01$ ) reduced the ulcer score, ulcer number and ulcer index in aspirin induced and ethanol induced ulcer models in rats. The present study revealed the antiulcer activity of leaf extracts of *Wattakaka volubilis* and *Tabebuia rosea* and the activities are due to the presence of phyto - chemical constituents[1][2][3] such as saponins, flavonoids, as these phyto - chemical constituents were already reported for the above mentioned effects.

**Keywords:** *Wattakaka volubilis*, *Tabebuia rosea*, Ethanol, Aspirin, Anti ulcer activity.

### INTRODUCTION

Gastric ulcers are most common disease and are a very common global problem today. Peptic ulcer is a lesion of the gastric mucosa and duodenal mucosa occurs at a site where the mucosal epithelium is exposed to acid and pepsin. Peptic ulcers occur due to imbalance between the gastric acid secretion and gastric mucosal integrity. The aggressive and protective factors in the stomach are acid pepsin secretion, mucosal barrier, blood flow, cellular regeneration, prostaglandins and epidermal growth factors. Sometimes the gastric mucosa is continuously exposed to potentially injurious agents such as pepsin, bile acids, food ingredients, bacterial products and drugs. Factors such as stress, smoking, nutritional deficiency and ingestion of NSAID'S all can increase the incidence of gastric ulcers. It is reported that prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation. Physical, chemical and psychological factors may lead to gastric ulceration in humans and experimental animals. Reactive oxygen species (ROS) are reported in the pathophysiology of human diseases such as neurodegenerative inflammation, viral infections autoimmune gastro intestinal inflammation and gastric ulcers.

Treatment of peptic ulcer aims at

1. Inhibition of gastric acid secretion.
2. Reinforcement of gastric mucosal production.

Antiulcer drugs like H<sub>2</sub> -receptor blockers, proton pump inhibitors, antimuscuranic drugs produce adverse reactions such as hypersensitivity, arrhythmia, impotence and haemopoietic changes with a possibility of increased rate of ulcer recurrence within one year after cessation of the treatment. Because of the above mentioned demerits reported with the current antiulcer therapy there is a need for the search of newer therapeutic antiulcer agents from plant sources from the alternative therapy of Ayurvedha medicine. Some plant extracts produce promising and favorable reasons in the treatment of gastric ulcers. Several plants and herbs are advocated for the treatment of gastrointestinal disorders including gastric ulcers. No scientific data is available in support of gastroprotective effect of *Wattakaka volubilis* and *Tabebuia rosea*

The plant *Wattakaka volubilis* Linn (Family- Asclepiadaceae), is a Traditional medicinal plant used to treat various diseases in Indian traditional system of medicine. Particularly, the plant material used in folk medicine for the treatment of various diseases such as antifungal, antibacterial <sup>4</sup>, hypoglycemic <sup>5</sup>, anti-inflammatory,

analgesic and anti-lipid peroxidative <sup>6</sup>, protection against selenite induced cataract in rat lens<sup>7</sup>, *in vitro* anti-leishmanial and antitumour <sup>8</sup>, hepatotoxicity <sup>9</sup>, prevention of proteolysis in rat lens <sup>10</sup>, and apoptosis inducing potential <sup>11</sup>.

*Tabebuia rosea* (Bertol.) DC. Commonly known as "Pink Trumpet Tree" can grow up to 15 meter and well known for its beautiful flowers. The timber is widely used for general construction and carpentry in many European countries. The *Tabebuia roseae* ethanolic leaf extract is said to have remarkable antimicrobial activity against a wide range of gram positive and gram negative bacteria<sup>12</sup>. The essential oil of *Tabebuia rosea* leaf and bark is reported to be cytotoxic effects <sup>13</sup>.

Hence the present study was planned to evaluate antiulcer activity of alcoholic extracts of leaves of *Wattakaka volubilis* and *Tabebuia rosea* in ethanol induced and aspirin induced gastric ulcer model in experimental animals, rats. NSAID'S are reported with gastro duodenal ulceration because of their effect on prostaglandin synthesis (PGE and PGI<sub>2</sub>). Prostaglandin protects the mucosal layer of stomach from the corrosive effect of gastric acid. In the stomach prostaglandins play a protective role such as stimulation of HCO<sub>3</sub> ions secretion, maintenance of mucosal blood flow and regulation of mucosal cell turnover and repair. Suppression of prostaglandin synthesis results with the increased susceptibility of mucosal injury and gastro duodenal ulceration. Prostaglandins relax the circular muscle of gastric mucosa and cause flattening of the folds, increasing the mucosal area exposure to necrotic agents and reduce volume of gastric irritants. It was reported that decreased gastric motility exerts a gastro protective action. In the present study NSAID'S i.e., aspirin induced gastric ulcer model, the methanolic extracts of leaves of *Wattakaka volubilis* and *Tabebuia rosea* significantly reduced the number of ulcers and ulcer index.

This study reports the antiulcer activity of leaf extracts of *Wattakaka volubilis* and *Tabebuia rosea* using experimental models in rats.

### METHODS AND MATERIALS

#### Plant materials

The leaves of *Wattakaka volubilis* and *Tabebuia rosea* were procured from Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri venkateshwara University, Tirupati, Andhra Pradesh, India, in June 2010<sup>14</sup>. The plant was identified by a Botanist, and

voucher specimen was deposited in Sri Venkateshwara University, Department of Botany and a copy has been preserved for the future reference at the herbarium of the institute TRR College of Pharmacy (1447/PO/a/11/CPCSEA). After authentication, the leaves were cleaned and shade dried and milled into coarse powder by a mechanical pulverizer.

#### Preparation of the plant extract

The leaves of these plants were dried under shade at room temperature (27- 30°C) for 15-30 days, after which the leaves of the plant were chopped and grounded into coarse powder. The powdered material (2 kg) was defatted with petroleum ether (60-80°C) in a Soxhlet extraction apparatus and marc was extracted with methanol (1000 ml). Overnight, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. The crude extract was dissolved in 1% Tween 80 to required concentrations and used for the experiments.

#### Extract was subjected to preliminary Phyto - chemical evaluation

##### Test for carbohydrates

**Molisch's test:** To 2-3 ml of extract few drops of Molisch's reagent (alpha naphthol solution in alcohol) was added. The test tube was shaken well and concentrated sulphuric acid was added along the sides of the test tube. Formation of violet ring at the junction of two liquids was observed. This clearly indicates the presence of carbohydrates.

##### Test for reducing sugars

**Fehling's test:** In a test tube 1 ml of Fehling's A and 1 ml of Fehling's B solution were added. These mixed solutions were boiled for a minute. Then equal amount (2ml) of test solution was added. Brick red precipitate was observed which confirmed the presence of reducing sugars.

##### Test for Terpenoids

**A) Salkowski reaction:** 2ml of extract was taken in a test tube. To this 2ml of chloroform was added. Then 2ml of conc. Sulphuric acid was added along the sides of the test tube slowly and shaken well. Greenish yellow fluorescence appeared. This confirmed as the presence of terpenoids.

##### Test for steroids:

**A) Liebermann's reaction:** About 1 ml of extract was taken in a fresh clean test tube. To this 1 ml of acetic acid was added. This solution was heated and cooled. Then few drops of conc. sulphuric acid were added along the sides of the test tube. Blue colour was observed. This confirmed the presence of sterols in *Wattakaka volubilis* and *Tabebuia rosea*.

##### Test for Alkaloids

Little quantity of extract was taken in a test tube. To this 2ml of dil.HCL was added. The solution was shaken well and filtered. This filtrate was used to perform the following tests:

**A) Dragendorff's reaction:** 2 to 3 ml of filtrate was taken in a fresh test tube. To this few drops of Dragendorff's reagent was added. Orange brown precipitate was observed. This inferred the presence of alkaloids.

**B) Mayer's test:** 2 to 3 ml of filtrate was taken in a test tube followed by the addition of Mayer's reagent. A white precipitate was formed which confirmed the presence of alkaloids.

##### Tests for Tannins

**A) Ferric chloride solution test:** Little quantity of extract was taken in a test tube. To this, 2ml ethanol was added and mixed well followed by the addition of 1ml of 5% ferric chloride reagent. Deep blue colour was observed which inferred the presence of tannins.

**B) Lead acetate test:** 2ml of extract was taken in a test tube followed by the addition of alcohol and shaken well. To this 2 ml lead acetate was added. White precipitate formed which inferred the presence of tannins.

**C) Bromine water test:** 2ml of extract was taken in a test tube followed by the addition of bromine water. Discoloration of solution was observed which inferred the presence of tannins.

##### Tests for Flavonoids

**A) Shinoda test:** Little quantity of extract was taken in a test tube. To this, 5ml 95% ethanol was added followed by the addition of 2ml conc. HCl along the sides of the test tube slowly. Then 0.5g magnesium turnings were added. Appearance of pink colour confirmed the presence of flavonoids.

**B) Lead acetate test:** Small quantity of residue was taken in a test tube to which lead acetate solution was added. Yellow colour precipitate formed which inferred the presence of flavanoids.

##### Test for Saponins

**A) Foam test:** 0.5 gm of plant extract was shaken with 10-20 ml of distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

**B) Haemolysis test:** A drop blood on slide was mixed with few drops of plant extract, RBC was ruptured which which inferred the presence of saponin<sup>15</sup>.

##### Anti-ulcer Activity

###### Aspirin induced gastric ulcers<sup>16</sup>

Albino rats of either sex weighing between (160-200 g) each group containing 6 animals were divided into 4 groups. Group A: Normal animals treated with vehicle only; Group B: Standard Ranitidine (50 mg/kg p.o)<sup>17</sup>; Group C: MEWV (500 mg/kg); Group D: METR (500 mg/kg).

###### Experimental Procedure

Wistar rats of either sex weighing between (160-200 g) were divided into 4 groups of 6 rats in each. Group A was served as normal control given with vehicle only. Group B with standard drug, and groups C and D treated with doses of MEWV and METR respectively. After 30 min aspirin was administered at a dose of 250 mg/kg p.o, and after 6 h rats were sacrificed by using anesthetic ether and their abdomen was opened for determination of gastric lesions, washed in warm water and examined for ulcers. The ulcer index was scored as and percentage protection was also reported.

###### Ethanol (EtOH) induced gastric ulcer<sup>18</sup>

Albino rats of either sex weighing between (160-200 g) each group containing 6 animals were divided into 4 groups. Group A: Normal animals treated with vehicle only; Group B: Standard Ranitidine (50 mg/kg p.o); Group C: MEWV (500 mg/kg); Group D: METR (500 mg/kg).

###### Experimental Procedure

Wistar rats of either sex weighing between 160 – 200 gm were divided into 4 groups of 6 animals each and kept under fasting for 24 hrs with water ad libitum prior to experiment. The animals of group 1 were pretreated with vehicle and the animals of group 2 were treated with standard i.e. Ranitidine (50 mg/kg p.o). Similarly the animals of group 3 and 4 were pre-treated with methanolic extracts of MEWV and METR respectively. Ethanol (100% 1ml/200 g. p.o) was administered to all the animals after 60 minutes. The animals were sacrificed by cervical dislocation after one hour of EtOH administration and stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored and percentage protection was also reported.

###### Scoring of ulcer<sup>19</sup>

0 = Normal stomach,  
0.5 = Red coloration,

1 = Spot ulcers,  
 1.5 = Hemorrhagic streaks,  
 2 = Ulcer > 3 mm but > 5 mm,  
 3 = ulcers > 5 mm.  
 Ulcer index=UA+US+UP/10

Where,

UA=Average number of ulcers per animal,  
 US=Ulcer severity score,  
 UP=Percentage of animals with ulcers.  
 UP=Total ulcers in a group/total number of animals x 100.

The formula for calculation of Percentage ulcer inhibition:

Percentage inhibition=UIC-UIT/UIC X 100

Where

UIC=Ulcer index of control group,  
 UIT= Ulcer index of test group.

**RESULTS**

The results were shown in the Table No.1. The values expressed as mean ± SEM from 6 animals. The results were subjected to statistical

analysis by using one way ANOVA followed by Dunnett's -'t'- test to verify the significant difference, if any among the groups. P< 0.01\* and 0.001\*\* were considered significant.

**Anti-ulcer activity**

**Aspirin induced ulcer model in rats**

In aspirin induced ulcer model a significant rise in ulcer index (11.49±0.04) are noted. Standard drug ranitidine 50 mg/kg treatment has significantly reduced ulcer index (0) similarly the extracts MEWV and METR have significantly reduced the ulcer index (3.38±0.03 and 5.25±0.11) and the ulcer formation (70.58% and 54.30%) is significantly reduced.

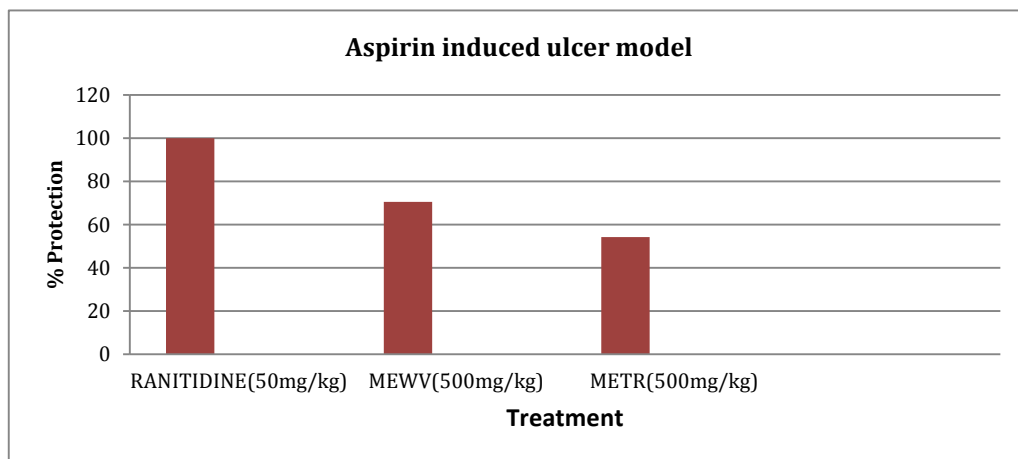
**Ethanol induced ulcer model in rats**

In ethanol induced ulcer model a significant rise in ulcer index (13.14±0.21) are noted. Standard drug ranitidine 50 mg/kg treatment has significantly reduced ulcer index (1.68±0.06). Similar to the extracts MEWV and METR have significantly reduced the ulcer index (5.09±0.04 and 6.78± 0.07) and the ulcer formation (61.26% and 48.40%) is significantly reduced.

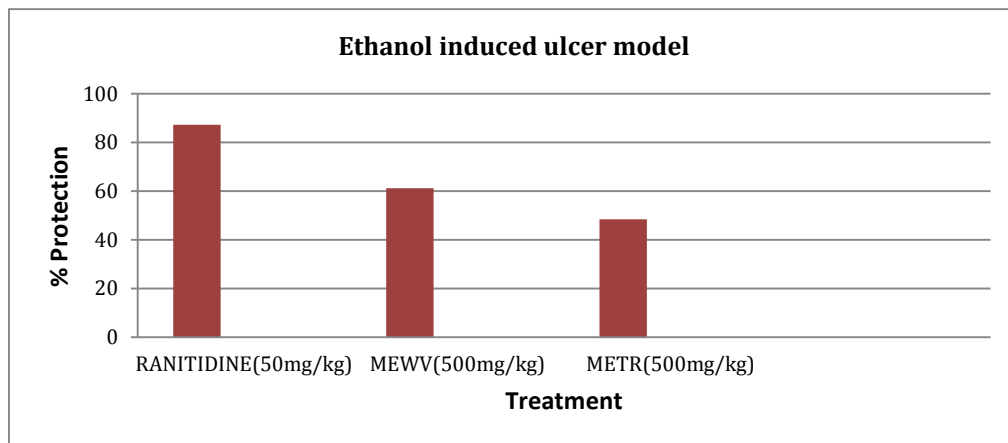
**Table No: 1 Antiulcer effect of MEWV and METR in different ulcer models in rats**

Treatment	Dose	ULCER INDEX		% of Protection	
		Aspirin	Ethanol	Aspirin	Ethanol
Control		11.49 ±0.04	13.14 ± 0.211	-	-
Ranitidine	50mg/kg BW	0**	1.68 ± 0.06**	100	87.21
MEWV	500mg/kg BW	3.38 ±0.03*	5.09 ±0.04*	70.58	61.26
METR	500mg/kg BW	5.25±0.11*	6.78 ± 0.07*	54.30	48.40

n = 6, Significant at P < 0.01\* and 0.001\*\*. MEWV-Methanolic extract of *Wattakaka volibilis*, METR- Methanolic extract of *Tabebuia rosea*.



**Fig.No.1: Antiulcer effect (% protection) of MEWV and METR in Aspirin induced ulcer model in rats.**



**Fig.No.2: Antiulcer effect (% protection) of MEWV and METR in ethanol induced ulcer model in rats.**

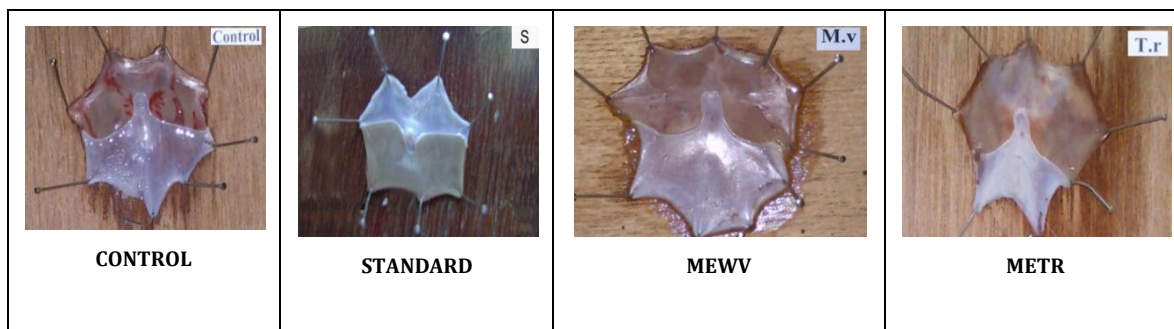


Fig.No.3: Effect of MEWV and METR on Aspirin induced ulcer model in rats

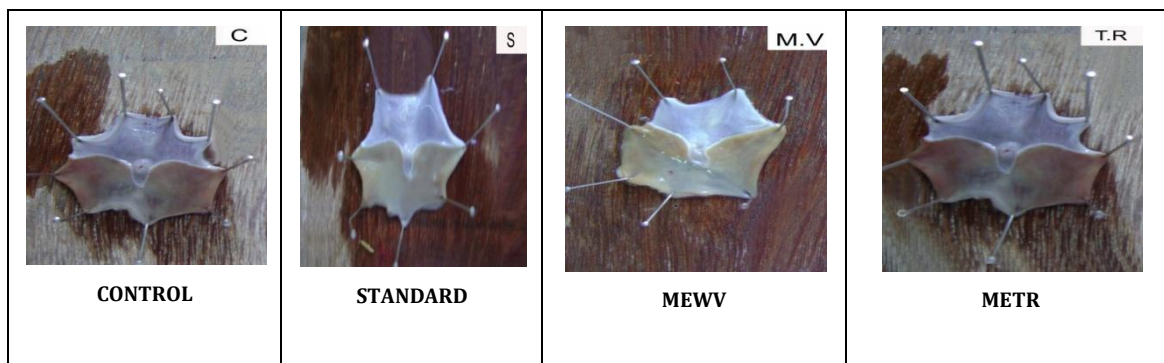


Fig.No. 4: Effect of MEWV and METR on Ethanol induced ulcer model in rats

## DISCUSSION

Etiologies to induce ulcers in human beings are several like stress, chronic use of anti-inflammatory drugs, continuous consumption of alcohol and spicy food<sup>20</sup>. In most of the cases, the exact causative factor of ulcer is unknown but it is generally accepted that it is the result of an imbalance in maintenance mucosal integrity through the several endogenous mechanism.<sup>21,22</sup>

The anti-ulcer activity shown by the methanolic extract in aspirin-induced ulcers suggests that the extract prevents change in permeability of gastric mucosa, prevents back diffusion of gastric acid, inhibits histamine release<sup>23</sup>, inhibits prostaglandin synthesis, inhibits lipid per-oxidation and activates cyclo-oxygenase<sup>24</sup>.

The anti-ulcer activity shown by the methanolic extract in ethanol-induced ulcers suggests that the extract has a cytoprotective effect, i.e. it protects the gastric mucosa by mechanisms other than gastric acid secretion<sup>25</sup>. Such mechanisms include inhibition of leukotrienes<sup>26</sup>, pepsinogen<sup>27</sup> and substance P<sup>28</sup>, free radical scavenging<sup>29</sup>, increasing gastric mucosal blood flow<sup>30</sup>, increasing the protective glycoprotein content and thereby strengthens the gastric mucosa<sup>31</sup> and prevention of oxidation of the mucosal xanthine dehydrogenase<sup>32</sup>.

The preliminary phyto-chemical analysis of *Wattakaka volubilis* and *Tabebuia rosea* extract showed the presence of tannins, flavonoids, alkaloids, saponins, terpenoids, phenolic compounds and sugars. The significant increase in the antiulcer activity of *Wattakaka volubilis* and *Tabebuia rosea* could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycosides and phenolic compounds. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen.<sup>33</sup> So the antiulcer activity of *Wattakaka volubilis* and *Tabebuia rosea* may be attributed to its flavonoids content.

## CONCLUSION

Leaf extracts of *Wattakaka volubilis* and *Tabebuia rosea* exhibited a significant anti-ulcer activity in experimental animals. Methanolic extract of *Wattakaka volubilis* exhibited relatively better anti-ulcer

activities than methanolic extract of *Tabebuia rosea*. The difference in the evaluated activities could be due to the number and also quantity of phyto-chemical constituents present in the extracts.

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