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COMPARATIVE STUDY OF ANTIULCER ACTIVITY OF METHANOLIC EXTRACTS OF *WATTAKAKA VOLUBILIS* (LINN.F.) STAF AND *TABEBUIA ROSEA* (BERTOL.) DC IN RATS

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ABSTRACT: The aim of this study was to compare the antiulcer activity of methanolic extracts of *Wattakaka volubilis* (Linn.f.) Staf and *Tabebuia rosea* (Bertol.) DC in rats. The anti-ulcer effect was evaluated using Anti secretory model (chemical induced pylorus ligation). Ranitidine were used as standard drugs for ulcer studies. The extracts were administered orally at 500 mg/kg. The result of the present study indicates oral administration of *Wattakaka volubilis* and *Tabebuia rosea* produced significant inhibition of the gastric lesions induced by Anti secretory model (Chemical induced pylorus ligation). Comparison was made between two plant extracts and methanolic extract of *Wattakaka volubilis* than Methanolic extract of *Tabebuia rosea*. Preliminary phytochemical screenings indicated the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, glycosides and sugars in both the extracts. This study confirmed the antiulcer properties of this plant as it is used in traditional medicine.

INTRODUCTION: Ulcer is defines as erosion in the lining of the stomach or duodenum and is caused by the disruptions of the gastric mucosal defense and repair systems ¹.

Ulcer in the stomach is called gastric ulcer and in the duodenum is called duodenal ulcer and together peptic ulcer. Gastric ulcers, one of the most widespread disorder ².

When the gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acid, bacterial products (*Helicobacter pylori*) and drugs, the gastric ulcer prevalence increases ³.

These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis, and cell proliferation growth, diminished gastric blood flow and gastric motility ⁴.

Peptic ulcer therapy has undergone many studies over past years and a number of synthetic drugs are now available for the treatment. Reports on clinical evaluation of these drugs show that there are incidences of relapses and several adverse effects and danger of drug interaction during drug therapy ^{5&6}.

The development of new anti-ulcer drug from medicinal plants is an attractive proposition because diverse chemical compounds have been isolated from medicinal plants with anti-ulcer activity ⁷ and have been shown to produce promising results in the treatment of gastric ulcers ⁸.

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MATERIALS AND METHODS:

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, Tirupathi, Andhra Pradesh, India. 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 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 (1000ml) 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:

1. Test for Carbohydrates:

- a. **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 test tube. Formation of violent ring at the junction of two liquids was observed. This clearly indicates the presence of carbohydrates.

2. Test for Reducing Sugars:

- a. **Fehling's Test:** In a test tube 1ml of Fehling's A and 1ml 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.

3. 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 concentrated sulphuric acid was added along the sides of the test tube slowly and shaken well. Greenish yellow fluorescence appeared. This confirmed the presence of terpenoids.

4. Test for Steroids:

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

5. 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. **Drangendroff's Reaction:** 2 to 3 ml of filtrate was taken in a fresh test tube. To this few drops of dragendroff'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 found which confirmed the presence of alkaloids.

6. Test for Tannins:

- a. **Ferric chloride solution Test:** Little quantity of extract was taken in a test tube. To this, 2ml of ethanol was added and mixed well followed by the addition of 1ml of 5% ferric chloride reagent. Deep blue color 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 2ml lead acetate was added. White precipitate formed which inferred the presence of tannins.
- c. **Bromine Test:** 2ml of extract was taken in a test tube followed by the addition of bromine water. Discolouration of solution was observed which inferred the presence of tannins.

7. Test for Flavonoids:

- a. **Shinoda Test:** Little quantity of extract was taken in a test tube. To this, 5ml of 95% ethanol was added followed by the addition of 2ml concentrated 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 flavonoids.
8. **Test for Saponins: Foam Test:** 0.5g of plant extract was shaken with 10-20ml of distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence of the presence of saponins.
- a. **Haemolysis Test:** A drop blood on slide was mixed with few drops of plant extract, RBC was ruptured which inferred the presence of Saponin.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF WATTAKAKA VOLUBILIS AND TABEBUIA ROSEA LEAVES

S. No.	Constituent	Methanolic extract of <i>Wattakaka volubilis</i>	Methanolic extract of <i>Tabebuia rosea</i>
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Glycosides	+	+
4	Phytosterols	+	-
5	Saponins	+	+
6	Tannins	+	+
7	Proteins and Amino acids	+	+
8	Flavonoids	+	+
9	Diterpene	+	+

Anti-Ulcer Activity:

Chemical Induced Pylorus Ligation Ulcer Model:

Wistar albino rats weighing between (100-130g) were divided into 5 groups of 6 rats in each. They were under fasting for 24hrs with water *ad libitum* prior to experiment in individual cages with measures taken to avoid caprophagy. Group 1 was served as normal control given with vehicle only. Group 2 with standard drug ranitidine (8mg/kg) & ethanol and Groups 3 and 4 were treated with doses of MEWV and METR (500mg/kg) respectively, Group 5 with standard drug ranitidine only.

Experimental Procedure: Five groups (n=6) of wistar rats were used to study the anti-ulcer activity of methanolic extracts of *Wattakaka volubilis* and *Tabebuia rosea*. 5% acacia mucilage (5ml/kg),

methanolic extracts, ranitidine and ethanol are administered to the animals per orally (p.o).

- Group 1 received 5% acacia mucilage (5ml/kg) all over the experimental period (11 days) and served as control.
- Group 2 received 5% acacia mucilage (5ml/kg) for 10 days and on 11th day received absolute ethanol (5ml/kg) and served as ulcer control.
- Group 3 and 4 were respectively administered with 500mg/kg MEWV and METR.
- Group 5 received Ranitidine (8mg/kg) for 10 days. All the groups were fasted for 24hrs and again administered with the extract or drug at respective dose.

After 30 min of this treatment, animals of groups 2-5 were administered with 5mL/kg ethanol to induce ulcer⁹. After 15 min of ethanol administration, under light ether anesthesia, the abdomen was opened and the pylorus ligation performed and then sutured. 4 h after pylorus ligation all the animals were sacrificed with excess of anesthetic ether and the stomach of each rat was dissected out.

Gastric juice collected into centrifuge tubes was centrifuged at 1000 rpm for 10 min and volume was noted. The pH of the gastric juice was recorded by pH meter. The gastric content was subjected for analysis of free and total acidity. The stomachs were washed under running tap water and then focused under microscope to note the ulcers in the glandular portion. The number of ulcers per stomach was scored and the scoring is done as per standard procedure¹⁰.

Scoring of ulcer

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.

$$\text{Ulcer index} = \text{UA} + \text{US} + \text{UP} / 10$$

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100 \text{ meq /lt/ } 100\text{g}}{0.1}$$

Statistical Analysis: The results were shown in the Table No.1. The values expressed as mean \pm 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 $P < 0.05^{**}$ were considered significant.

RESULTS:

Chemical Induced Pylorus Ligation Ulcer Model: In Chemical Induced Pylorus Ligation Ulcer Model in rats, a significant increase in ulcer index (4.137 ± 0.170) is noted. In the same model a

Where, UA=Average number of ulcers per animal, US=Ulcer severity score, UP=Percentage of animals with ulcers ($\text{UP} = \frac{\text{Total ulcers in a Group}}{\text{total number of animals}} \times 100$).

The formula for calculation of Percentage ulcer inhibition;

$$\text{Percentage inhibition} = \frac{\text{UIC} - \text{UIT}}{\text{UIC}} \times 100$$

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

Methods for biochemical estimation of free and total acidity collection of gastric juice: Gastric content collected from pylorus ligated rats was centrifuged and the volume of gastric juice as well as pH of gastric juice was noted. The gastric juice was subjected to biochemical estimations as follows;

Determination of free and total acidity: 1 ml of gastric juice was pipette into a 100 ml conical flask, 2 or 3 drops of Topfer's reagent was added and titrated with 0.01N Sodium hydroxide until all traces of red color disappears and the color of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then, 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted now this volume corresponds to total acidity.

Acidity was calculated by using the formula;

significant increase in gastric volume (3.5 ± 0.24 ml), free acid (41.27 ± 1.41 mEq/L) and total acid (83.05 ± 1.8 mEq/L) are noted. Standard i.e., Ranitidine (8 mg/kg) treatment has significantly reduced ulcer index (1.55 ± 0.108), gastric volume (1.85 ± 0.19 ml), free acid (6.36 ± 0.96 mEq/L) and total acid (12.12 ± 1.02 mEq/L).

Standard + absolute ethanol administration has shown ulcer index (3.90 ± 0.23), gastric volume (2.96 ± 0.26 ml), free acid (40.38 ± 0.42 mEq/L), and total acid (78.90 ± 0.98 mEq/L). In Chemical induced pylorus ligation ulcer model, the extracts MEWV, METR have significantly reduced the

ulcer index (2.398 ± 0.152 , 3.425 ± 0.240). Similarly a significant reduction in gastric volume (2.5 ± 0.11 , 2.7 ± 0.09 ml), free acid (22.14 ± 1.6 , 35.82 ± 1.6 mEq/L), and total acid (39.70 ± 1.66 , 76.60 ± 1.46 mEq/L) is noted. It is represented in **Table 2**.

The pictures of the excised stomach and the graph indicating the ulcer indices of all groups are represented in **fig. 1 and 2**.

TABLE 2: EFFECT OF METHANOL EXTRACTS OF WATTAKAKA VOLUBILIS AND TABEBUIA ROSEA LEAVES ON CHEMICAL INDUCED PYLORUS LIGATED RATS

S. No.	Groups	Volume of gastric juice	pH	Total acidity	Free acidity	Ulcer index
1	Pylorus control	3.5 ± 0.24	3.57 ± 0.04	83.05 ± 1.8	41.27 ± 1.41	4.137 ± 0.170
2	Standard drug + Absolute ethanol	2.96 ± 0.26	4.32 ± 0.03	78.90 ± 0.98	40.38 ± 0.42	3.90 ± 0.23
3	Standard drug (Ranitidine 8mg/kg)	$1.85 \pm 0.19^*$	$4.55 \pm 0.120^*$	$12.12 \pm 1.02^*$	$6.36 \pm 0.96^*$	$1.55 \pm 0.108^*$
4	MEWV (500mg/kg)	$2.5 \pm 0.11^*$	$2.80 \pm 0.061^*$	$39.70 \pm 1.66^*$	$22.14 \pm 1.6^*$	$2.398 \pm 0.152^*$
5	METR (500mg/kg)	$2.7 \pm 0.09^{**}$	$3.252 \pm 0.04^{**}$	$76.60 \pm 1.46^{**}$	$35.82 \pm 1.6^{**}$	$3.425 \pm 0.240^{**}$

The values are Mean \pm SEM (n=6). Statistical significant test for comparison was done by one way ANOVA followed by Dunnett's '-t' test. Symbols statistical significant: *P < 0.01 and **P < 0.05 Vs control.

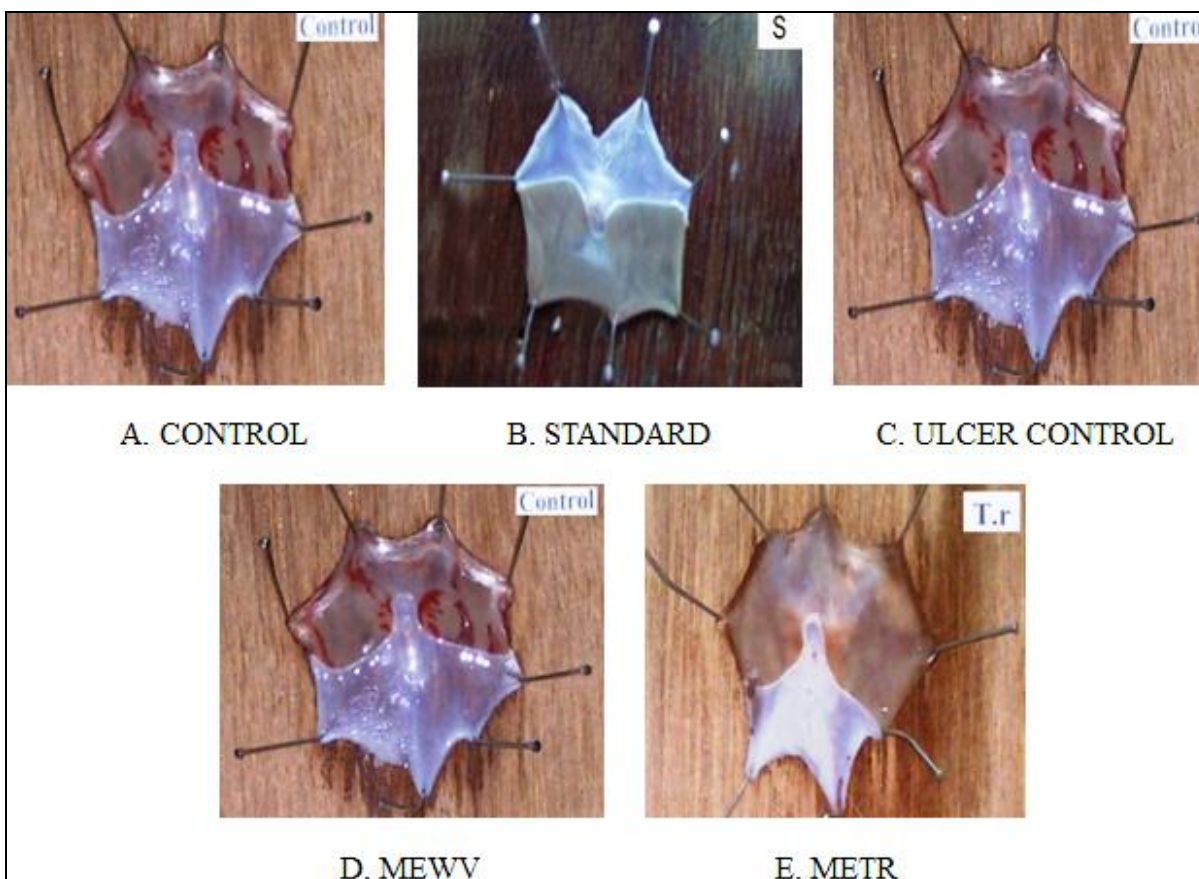


FIG 1 EFFECT OF WATTAKAKA VOLUBILIS AND TABEBUIA ROSEA LEAVES ON CHEMICAL INDUCED PYLORUS LIGATION ULCERS IN RATS. (A) Stomach of control rat; (B) Standard drug treated; (C) Ulcer Control; (D) Methanolic extract of *Watakaka volubilis*; (E) Methanolic extract of *Tabebuia rosea*

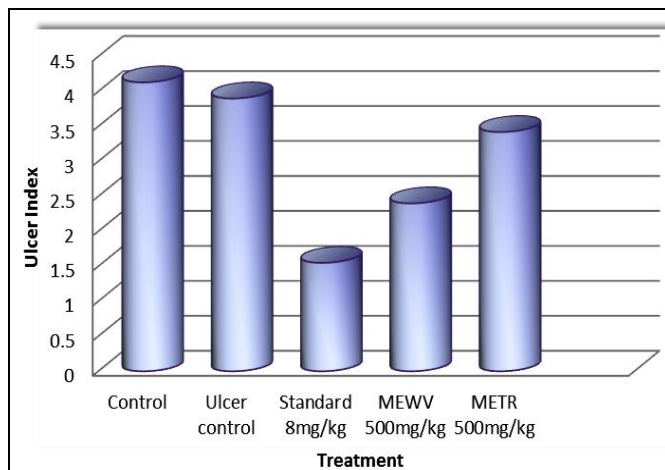


FIG. 2: EFFECT OF MEWV AND METR EXTRACT AND RANITIDINE ON CHEMICAL INDUCED PYLORUS LIGATION ULCER MODEL IN RATS

DISCUSSION: The results obtained in the study showed that the extract and fraction of *Wattakaka volubilis* and *Tabebuia rosea* possess anti-ulcer activity. The extract significantly reduced the ulcer index in rats. Methanolic extract of *Wattakaka volubilis* and *Tabebuia rosea* significantly inhibited the formation of ulcers against ethanol challenge followed by pylorus ligation. Narcotizing agents such as ethanol, when intragastrically to rats produce severe gastric hemorrhagic erosions. Ethanol causes disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production.

This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in gastric mucosa¹¹. After pylorus ligation gastric ulcers are due to over production of gastric acid or decrease in gastric mucous production. Pylorus obstruction may lead to acid-pepsin accumulation and subsequent digestion of mucosa.

The cause of gastric ulcers after pylorus ligation is due to an increase in gastric HCl secretion. Increased volume is an important factor involved in ulcer formation of the stomach which is exposed to the accumulated acid¹². Hence, the anti-ulcer activity of the methanolic extract of *Wattakaka volubilis* and *Tabebuia rosea* suggest the anti-secretory activity via antioxidant effects as their possible mechanism of action.

The phytochemicals analysis showed the presence of Flavonoids in methanolic extract of *Wattakaka volubilis* and *Tabebuia rosea*.

Flavonoids are the gastro protective materials for which antiulcerogenic efficiency has been extensively confirmed. It is suggested that, these flavonoids would be able to inhibit the vagus-vagal reflux which increases the acid secretion and counteract the deteriorating effects of reactive oxidants in gastrointestinal lumen.

In conclusion, the results indicated that the methanolic extract of *Wattakaka volubilis* and *Tabebuia rosea*, exhibited significant antiulcerogenic effects that support the evidence for its folkloric use, while these anti-ulcerogenic effects might possibly be due to the presence of flavonoids.

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