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Research Article

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

A. SUVARCHALA KIRANMAI*[1], HEMAMALINI.K[2], AND DR.UMA VASIREDDY[3]

[1, 2]Department of Pharmacology, TRR College of pharmacy, Meerpet, Hyderabad. [3]IPA Treasurer.

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ABSTRACT

Objective: 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 and to conduct Preliminary Phytochemical screening.

Method: The anti-ulcer effect was evaluated using immobilization induced stress and chemical induced stress ulcer models using rats. Ranitidine were used as standard drugs for ulcer studies. The extracts were administered orally at 500 mg/kg and Standard drug ranitidine was administered orally at 8mg/kg.

Results: The result of the present study indicates oral administration of *Wattakaka volubilis* and *Tabebuia rosea* produced significant inhibition of the gastric lesions induced by Immobilization induced stress ulcers and Chemical induced stress ulcer models, Upon comparing both the extracts Methanolic extract of *Wattakaka volubilis* exhibited significant Anti- ulcer activity 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.

Conclusion: This study confirmed the antiulcer properties of this plant as it is used in traditional medicine. Among both extracts Methanolic extract of *Wattakaka volubilis* is more potent compared to Methanolic extract of *Tabebuia rosea*.

Keywords: *Wattakaka volubilis* and *Tabebuia rosea*, Anti-ulcer activity, Immobilization induced ulceration, Chemical stress induced ulcers.

INTRODUCTION

Ulcer is defined 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 under gone 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].

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 authentification, the leaves were cleaned and shade dried and milled into course powder by a mechanical pulverizer.

Preparation of Plant Extract

The leaves of these plants were dried under shade at room temperature (27-30ºC) for 15-30 days, after which the leaves of the plant were chopped and grounded into coarse powder. The powdered material (2 kg) was defated with petroleum ether (60-80ºC) 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 filterate was concentrated at 30ºC under reduced pressure in a rotary evaportor. 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 napthol 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 teh junction of two liquids was observed. This clearly indicates the presence of carbohydrates.

Test for Reducing Sugars

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 equla 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 concentrated sulphuric acid was added along the sides of the test tube slowly and shaken well. Greenish yellow fluorosence appeared. This confirmed the presence of terenoids.

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 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 filterate was used to perform the following tests:

A) Drangendroff's Reaction: 2 to 3 ml of filterate 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 filterate 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.

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 colour was observed which inferred the presence of tannins.

B) Lead acetate Test: 2ml of extract was taken in a test tube foloowed 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.

Test for Flavonoids

A) Shinoda Test: Little quantity of extarct 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.

Test for Saponins

A) 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.

B) Haemolysis Test: A drop blood on slide was mixed with few drops of plant extarct, RBC was ruptured which inferred the presence of saponins.

Anti-Ulcer Activity

Immobilization induced Stress ulceration [9, 10, and 11]

Wistar albino rats of either sex weighing between 75-100g were used for the screening. They were divided into 4 groups of 6 animals each. Then the animals were marked for their identity and left for overnight starvation. Animal of group 1 receive normal saline, group 2 receive standard drug, group 3 and 4 receive methanolic extracts of plants Wattakaka volubilis and Tabebuia rosea(500mg/kg). The physical stress for one set rats were induced by tying the limbs of the rats and placed upside down to a wooden board for a period of 5hrs. The values obtained for the test was compared with the control values.

The ulcer indexes of each group animals were calculated using the formula:

(UI/10) + (Avg ulcer/group) + (Avg ulcer/stomach)

Where UI= ulcer incidence

Table 1: Determination of ulcerogenic indices

Type of ulcer	core
Minute sporadic punctuate lesion	0.5
Hemorrhagic strokes	1.0
One lesion of large extension or multiple moderate size lesions	2.0
Several large lesions	3.0

Statistical Analysis

The results were shown in the Table No.2. 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 P<0.05**were considered significant.

Chemical stress induced ulcer [12]

Wistar albino rats of either sex weighing between 75-100g were used for the screening. They were divided into 4 groups of 6 animals each. Then the animals were marked for their identity and left for overnight starvation. Animal of group 1 receive normal saline, group 2 receive standard drug, group 3 and 4 receive methanolic extracts of plants Wattakaka volubilis and Tabebuia rosea(500mg/kg). Chemical stress was induced to set of rats by oral administration of aspirin at a dose of 200mg/kg body weight. After 5hrs all the animals were killed using anesthetic ether and opened the abdominal cavity and the stomach was excised. The excised stomach was opened along the greater curvature and cleaned the interior by normal saline and examined for the degree of ulceration. The ulcerogenic indices were determined according to the pattern showed in immobilization induced ulceration.

Statistical Analysis

The results were shown in the Table No.3. 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 P<0.05**were considered significant.

RESULTS

Immobilization Induced Stress Ulceration

In Immobilization induced stress ulcer model a significant rise in ulcer index (18.79 + 0.50) are noted. Standard drug ranitidine 10 mg/kg treatment has significantly reduced ulcer index (4.83+0.11) similarly the extracts of *Wattakaka volubilis* and *Tabebuia rosea* have significantly reduced the ulcer index (5.42 + 0.57 and 7.41 + 0.14) is significantly reduced. The values obtained are represented in Table no 2

Table 2: Effect of methanol extracts of *Wattakaka volubilis* and *Tabebuia rosea* leaves on immobilization stress induced ulceration

S. No.	Drug	Dose (ml/kg)	Average ulcer score/stomach	Average severity of ulcer/group	Ulcer incidence/ group	Ulcer index
1	Control	-	3.5±0.106	1.27±0.23	97.5±0.76	18.79±0.50
2	Standard drug	10mg/kg	0.650±0.095*	0.583±0.09*	45.67±1.45*	4.383±0.11*
3	MEWV	500 mg/kg	0.916±0.113*	0.216±0.04***	34.5±1.1***	5.42±0.57***
4	METR	500mg/kg	0.733±0.04**	0.50±0.05*	40.83±1.01*	7.413±0.143*

The values are Mean ±SEM (n=6). Statistical significant test for comparison was done by one way ANOVA followed by Dunnet's't' test. Symbols statistical significant: *P < 0.01 and **P < 0.05, ***P< 0.001 Vs control.

Chemical Induced Stress Ulceration

In Chemical stress induced ulcer model a significant rise in ulcer index (21.51 + 0.91) are noted. Standard drug ranitidine 10 mg/kg treatment has significantly reduced ulcer index (6.42+0.11) similarly the extracts of *Wattakaka volubilis* and *Tabebuia rosea* have significantly reduced the ulcer index (8.39 + 0.10 and 10.7 + 0.13) is significantly reduced. The values obtained are represented in Table no 3.

Table 3: Effect of methanol extracts of *Wattakaka volubilis* and *Tabebuia rosea* leaves on chemical stress induced ulcer

S. No.	Drug	Dose (ml/kg)	Aspirin	Average ulcer score/ stomach	Average severity of ulcer/group	Ulcer incidence/ group	Ulcer index
1	Control	-	200mg/kg	6.13±0.11	1.53±0.122	97.50±0.76	21.51**±**0.91
2	Standard drug	10mg/kg	200mg/kg	2.51±0.13*	0.50±0.09*	31.62±0.73*	6.42**±**0.11*
3	MEWV	500mg/kg	200mg/kg	3.55±0.11**	0.96±0.11*	42.25±1.01*	8.39**±**0.104**
4	METR	500mg/kg	200mg/kg	4.4±0.07*	1.1±0.13***	53.65±0.73***	10.7**±**0.134*

The values are Mean ±SEM (n=6). Statistical significant test for comparison was done by one ANOVA followed by Dunnet's-'t' test. Symbols statistical significant: *P < 0.01 and **P < 0.001, ***P< 0.05 Vs control.

DISCUSSION

The rsults 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* signiicantly inhibited the formation of ulcers against Stress induced ulcers i.e. both Immobilization induced stress ulcers and chemical stress induced ulcers**.**

Stress plays an important role in the etiology of gastro duodenal ulcers. Stress induced ulcers is probably mediated by the release of histamine which in turn increases gastric secretion and causes disturbances of gastromucosal macro circulation, alteration in motility and reduced production of mucous. Vagal activity has been suggested as the main factor in stress ulceration as it stimulates HCl in the stomach through muscarinic receptors. During stress induced vagal activity was up regulated, resulting with the production of more acid [13]. Further stress also causes mask cell degranulation and decreased synthesis of prostaglandins and complex neurochemical mechanisms are aslo involved like changes in the synthesis, action and degradation of neuromodulators. CNS also plays an important role in ulceration and regulation of plasma corticosterone.

In case of immobilization stress induced ulceration it was reported that immobilization creates stress that aggravates the severity of ulcers, lipid peroxidation and plasma corticosterone. Free radicals affects lipid by initiating peroxidation, superoxide, hydrogen peroxide and hydroxyl radical are important. Reactive oxygen species (ROS) are responsible for tissue damage. The higher lipid peroxidation and SOD levels are indication of the increased production of superoxide withn the tissue, the restraint effect cell degeneration through lipid peroxidation of membrane lipids, breaking od DNA strands and denaturation of cellular protiens. Where as in Chemical induced stress ulceration initially NSAID like Aspirin is used to induce ulcers. Non-Steroidal anti-inflammatory agents like Aspirin are known to induce gastric ulceration. Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion, and back diffusion of H^+ ions. In stomach, prostaglandins play avital role by stimulating secretion of HCO^{-3} and mucous, maintaining mucosal blood flow and deregulating mucosal cell turnover and repair. Thus the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastro duodenal ulceration [14].

The phytochemicals analysis showed the presence of Flavanoids in methanolic extract of *Wattakaka volubilis* and *Tabebuia rosea.* Flavanoids are the gastroprotective materials for which antiulcerogenic efficiency has been extensively confirmed. It is suggested that**,** these flavanoids would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with 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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