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Research Article

EVALUATION OF ANTI-ULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF THE *TERMINALIA ARJUNA* BARK [ROXB]

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ABSTRACT

The anti-ulcer activity of hydro alcoholic extract of ***Terminalia arjuna*** bark Roxb. Family (Combretaceae). HAETA was investigated in Ethanol induced and Stress induced ulcer models in wistar albino rats. In both models the most common parameter determined was ulcer index. HAETA at doses 100, 400 mg/kg p.o produces significant inhibition of gastric lesions induced by ethanol induced gastric ulcer & stress induced gastric ulcers. The extracts 100, 400 mg/kg showed significant P<0.01 reduction in ulcer index, pH, free acidity and total acidity as compared to control, omeprazole 20 mg/kg were employed as reference drug in anti-ulcer studies respectively. The present study suggests that HAETA was found to possess anti-ulcerogenic as well as ulcer healing properties, which might be due to anti-secretory activity.

Keywords: *Terminalia arjuna* Roxb bark, Ethanol & Stress induced ulcer, Ulcer index Omeprazole 20 mg/kg.

INTRODUCTION

Peptic ulcer is the erosion in lining of stomach or duodenum. The word 'peptic' refers to pepsin, a stomach enzyme that breaks downs proteins. Peptic ulcer located in stomach is known as gastric ulcer, it is believed that the ulcers are mainly due to imbalance between protective factors (mucous and bicarbonates) and aggressive factors (acid and pepsin) in stomach. [1] Such factors could range from natural causes (gastric cancer) infections (H.*pylori*), life style (usage of drugs like non steroidal anti-inflammatory agents, alcohol, stress & cigarette smoking.)[2] These agents have been implicated in the process of 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. [3] Current treatment of ulcers in developing countries has been largely based on suppression of pain, with little (or) no strategy aimed to cure the disease. Ulcers are mainly treated with combinations of different kind of anti-biotics, acid reducers and H_2-receptor blockers, proton pump inhibitors etc, which are expensive to a common man and have prolong side effects also. Herbal medicine is used as fast emerging alternative treatment of ulcers, [4] possible due to low cost, availability, fewer adverse effects and perceived effectiveness. Hence effort is to find a suitable treatment from natural product as a source i.e. hydro alcoholic extract of *Terminalia arjuna* bark.

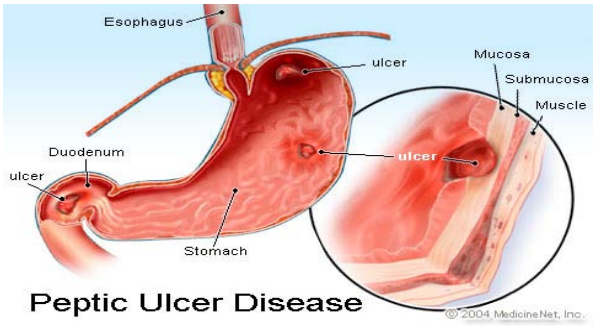

Fig. 1: Various locations of ulcers

Among medicinal plants *Terminaila arjuna* Roxb has been recommended for several disorders in folk medicine. Indian Materia & Medica describes the use of *Terminaila arjuna* in treatment of various ailments. It is a large tree, often with buttressed trunk, leaves are usually oblong (or) elliptic-long, flowers are yellowish-white, and fruits are ovoid. It is common on the rivers, streams and distributed throughout India. [5]The bark of the plant is known to contain a crystalline compound such as arjunine, arjunetin, essential oils & reducing sugars, etc. The plant extensively used to treat anti-dysentric, antipyretic, astringent, cardiotonic, lithotriptic, diuretic, cirrhosis of liver, ulcers, cancer. [6, 7] The bark powder is also given with honey in fractures and contusions with ecchymosis etc. An ointment made from bark by mixing with honey is used to cure acne while the ashes of the bark are prescribed in scorption stings.[8] The present study was undertaken to evaluate the HAETA for its anti ulcer activity.

MATERIALS AND METHODS

Plant materials

The bark part of *Terminalia arjuna* was collected form chengalpattu, kanchipuram district, Tamilnadu, India and identified by Dr. P. Jayaraman, Director of national institute of herbal sciences, west Tambaram, Chennai, India. The bark was cut, air dried and ground into powder.

Fig. 2: *Terminalia arjuna* bark

Preparation of Extract

The powdered bark material (500g) was macerated at room temperature (24-26^{0}c) with methanol (1500ml) in 1:3 ratios for 4 days with occasional shaking followed by re-maceration with the same solvent for 3 more days. The macerates were combined, filtered and distilled off in reduced pressure. Finally brown colour shine crystals are obtained.

Preliminary phytochemical screening

A portion of residue from each extracts was subjected for phytochemical analysis in order to see the presence of Alkaloids, Triterpenoids, Tannins and Flavonoids, Saponins. [9]

Chemicals and reagents

Omeprazole 20 mg/kg served as standard, Ethanol 95% (ulcer inducing agent), Methyl orange reagent & Phenolphthalein

(Indicator), Phosphate buffer (buffer), H_2O_2 solution (vehicle) were used, the dose of the hydroalcoholic extract of *Terminalia arjuna* Roxb (HAETA) was selected randomly as 100, 400 mg/kg body wt, of the animal. A suspension of HAETA in 5% (w/v) Carboxy Methyl Cellulose was prepared for oral administration.

Animals

A healthy, adult wistar rat of either sex (180-220g) was obtained from the Central animal house facility from Vels University, Chennai. The animals was kept in a well ventilated room and the animals was exposed to 12 hrs day and night cycle with a temperature between 20±3^{0}C and fed with standard animal pellet feed (Hindustan lever limited) and water *ab-libitum.* The protocol was approved by animal ethics committee constituted for purpose of animal experimentation as per CPCSEA guidelines. (XII/VELS/PCOL/47/2000/CPCSEA/IAEC/11.03.11)

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Stress induced ulcer model

Albino wistar rats of either sex are divided into four groups of each containing six animals in each group.First Group represented the control group, which received distilled water orally. Second &Third Groups received Hydro alcoholic extract of *Terminalia arjuna* bark at the dose of 100 and 400 mg/kg. Standard drug Omeprazole was administered at the dose of 20 mg/kg orally for Fourth group. The gastric ulcers were induced in rats by, Cold resistance stress (CRS) induced ulcer to 18 hours fasted rats, Cold resistance stress is produced by strapping the rats on a wooden plank and keeping them for 2 hours at 4^o - 6^{o}c. Ulcer scoring will be done after sacrificing the animal by cervical dislocation. [10]

Ethanol induced ulcer model

The ulcer was induced by administering ethanol (1ml/200g). All the animals were fasted for 36 hours before administration of ethanol .The animals were divided into five groups, each consisting of six rats. One Group represented the control group, which received distilled water orally. Second group receive ethanol, Third & Fourth Groups received hydroalcoholic extract of *Terminalia arjuna* bark in the dose of 100 and 400 mg/kg and, Omeprazole, in the dose of 20 mg/kg were administered orally for Fifth group as reference standard drug. The gastric ulcers were induced in rats by administrating absolute ethanol (95%) (1ml/200g) Orally, after 45 min of HAETA and Omeprazole treatment .They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1h latter with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored, similar to pyloric ligation induced ulcer model. [11]

In vivo anti-oxidant estimation from tissue homogenate:

Super oxide dismutase (SOD) activity: Inhibition of reduction of nitro blue tetrazolium (NBT) to blue colored Formosan in presence of phenazine methasulphate (PMS) and NADH is measured at 560 nm using n-butanol as blank. One unit of enzyme activity is defined as the amount of enzyme that inhibits rate of reaction by 50% in one min under the define assay condition and the results have been expressed as unit (U) of SOD activity/mg protein. [12]

Ulcer index [13]

The stomach was opened along the greater curvature and fixed on a cork plate. The number and severity of ulcers was registered using the following scores.

Severity Score:

0 = Normal colored stomach

0.5 = Red coloration

1 = Spot ulcer

1.5 = Hemorrhagic streaks

2 = Ulcers ≥ 3 but ≤5

3 = Ulcers > 5.

Ulcer index was calculated as: **UI = UN+US+ UP X 10-1**

Where,

UI = ulcer index,

UN= average of number of ulcers per animal,

US= average of severity score

UP =percentage of animals with ulcer.

Statistical analysis

The values expressed as mean ± S.E.M (standard error of the mean). Dunnett- test was used for the evolution of data and p<0.01 accepted as significant.

RESULTS

Plant studies

Terminalia arjuna bark was extracted by using 70% methanol and 30% water by cold maceration method and the yield was found to be 2.1%. A qualitative phytochemical study of the extract was performed by using suitable chemicals and reagents to confirm the presence of alkaloids, flavonoids, steroids and tannins, etc.

Stress induced ulcer

Induction of stress by cold resistance method to control group clearly produced the characteristic mucosal lesions, and which may be due to free radical generation. Pre-treatment with HAETA, orally at doses of 100 and 400 mg/kg, showing protection index 51% and 61% and significant reduction in ulcer index, free acidity, total acidity, P_H and inhibition of free radicals generation, the positive control (Omeprazole 20 mg/kg), showed 72% reduction of ulcers.

(Results are tabulated in Table-1&3)

Ethanol induced ulcer

The oral administration of Ethanol solution to the control group clearly produced the characteristic mucosal lesions. Ethanol solution induced long ulcer lesions after a relatively short time. Pre-treatment with HAETA, orally at doses of 100 and 400 mg/kg, showing protection index 66% and 76% significant reduction in ulcer index, free acidity , total acidity, P_H , the positive control (Omeprazole 20 mg/kg), showed 79% reduction of ulcers.

(Results are tabulated in Table-2)

Table 1: Effect of HAETA bark against Stress Induced gastric ulcer (Ulcer index, P^H , Free and Total acidity) rats

Group	Treatment	Ulcer index	% protection	P^H of gastric content	Free acidity (meq/lit)	Total acidity (meq/lit)
I	Stress	65.2±1.01	---------	2.54±0.12	22.1±2.01	65.0±2.01
II	HAETA *100mg/kg*	32.1±0.34*	51%	3.56±0.16**	16.2±0.56**	49.1±0.65**
III	HAETA *400mg/kg*	25.4±1.02**	61%	4.41±0.14*	13.1±1.13**	40.2±0.38*
VI	Stress+Omeprazole	18.80±3.26**	72%	4.71±0.22*	10.1±0.34**	36.2±0.24*

*P<0.01 Vs Control group; **P<0.05 Vs Control group. All values are represented as mean ± S.E.M and statistical significance using one way ANOVA, followed by Dunnett's test where P<0.05 was considered as moderately significant and P<0.01was considered as a statistically significant.

Table 2: Effect of HAETA bark against Ethanol Induced gastric ulcer (Ulcer index, P^H Free and Total acidity) rats

Group	Treatment	Ulcer index	% Protection	P^H of gastric content
I	Control (Distilled water)	4.54±0.42	--------	2.14±0.18
II	Ethanol	10.58±0.37	---------	2.54±0.12
III	HAETA *100mg/kg*	3.58±0.37**	66%	3.56±0.16**
IV	HAETA *400mg/kg*	2.5±0.44*	76%	4.41±0.14*
V	Stress+Omeprazole	2.16±0.25*	79%	4.71±0.22*

*P<0.01 Vs Control group; **P<0.05 Vs Control group. All values are represented as mean ± S.E.M and statistical significance using one way ANOVA, followed by Dunnett's test where P<0.05 was considered as moderately significant and P<0.01was considered as a statistically significant.

Table 3: Effect of HAETA aganist superoxidedismutase (SOD) activity in stress induced gastric ulcerative rats.

Group	Treatment	Dose mg/kg (p.o.)	SOD % inhibition of NBT reduction± SEM Stomach
I	Control	Vehicle	66.3±0.43
II	Stress(cold resistance)	-------	35.1±5.12
III	HAETA	100mg/kg	72.1±3.12**
IV	HAETA	400mg/kg	65.2±2.03*
V	Stress+ Omeprazole	20mg/kg	71.1±2.12*

*P<0.01 Vs Control group; **P<0.05 Vs Control group. All values are represented as mean ± S.E.M and statistical significance using one way ANOVA, followed by Dunnett's test where P<0.05 was considered as moderately significant and P<0.01was considered as a statistically significant.

DISCUSSION

The anti-ulcer activity of the hydroalcoholic extract of *Terminaila arjuna* bark against Stress & Ethanol Induced ulcers was established in this study. Stress can arise from prolonged anxiety, tension, and emotion, severe physical discomfort, hemorrhage, surgical shock, burns and trauma, thereby resulting in severe gastric ulceration. Recently research has shown that resistant cold stress can causes severe hemorrhage ulcer through disarrangement of the mucosal antioxidant enzyme such as superoxide dismutase and peroxides. Stress generate highly reactive OH* radicals that causes oxidative damage of the gastric mucosa and that the radicals is formed by metal catalyzed *Herber weiss* reaction between O_2 – and H_2O_2 following induction of the superoxide dismutase and oxidative damage of gastric peroxides, leads to formation of ulcer.

Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion are formed may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates into gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular 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.

CONCLUSION

The extract shows protection against characteristic lesions produced by Ethanol and stress induced ulcer. The antiulcer effect of HAETA may be due to presence Alkaloids, Triterpenoids, Tannins and Flavonoids, Saponins, which have been shown anti-ulcerogenic and anti-gastric activity.[14] However, until specific constituents are isolated and characterized, exact mechanism of action cannot be ascertained. Studies on the sub-acute and chronic toxicity of the extract are however in progress.

We have demonstrated in this study that the HAETA bark has ulcer healing property against experimentally induced ulcers, and this study confirms folkloric claims of the benefits of *Terminalia arjuna* bark in the treatment of ulcer.

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