Research Article

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EVALUATION OF PREVENTIVE EFFECT OF *WITHANIA SOMNIFERA* ROOT EXTRACT AGAINST ETHANOL INDUCED ULCERS IN RATS

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Abstract: Peptic ulcer is a serious gastrointestinal disorder required to be treated with various drugs. According to Ayurvedha, *Withania somnifera* was used to treat various diseases including peptic ulcer. The present study was designed to evaluate the antiulcer activity of plant in ethanol induced gastric ulcer. Wister Albino rats were selected and divided into five groups. Group-I (normal saline), group-II (Sucraflate) , group-III (Alpha Lipoic acid powder), group-IV & group-V administered graded doses of plant extract. The drug was given to rats prior to administration of ethanol. Rats were scarified and examined for severity of ulceration. Rats pretreated with standard drug and plant extract showed significant decrease in the average number of ulcers, scoring of ulcer and ulcer incidence and decreased ulcer indexation. The standard drugs and plant extract significantly inhibit the ulcer formation. There are several targets and drugs required to treat the peptic ulcer. Currently increased uses of plants are in treatment of this disease. So the present study was done to evaluate the preventive effect of *Withania somnifera* antiulcer activity in ethanol induced ulcer. This study observations proved, that this plant has anti-ulcer activity and can be used as an alternative and adjuvant in the treatment of ulcer disease. There is a requirement of further studies to find the active component and dosage of the plant extract.

Keywords: Peptic ulcer, Ulcer Index, Ethanol, Alpha Lipoic acid

INTRODUCTION

Peptic ulcer is due to imbalance between protective and affective agents and it is also due to *H. Pylori* infection. Several factors like smoking, alcohol, drugs like aspirin can cause ulcers[1]. Inhibition of gastro protective methods by aggressive factors leads to increase in the gastric acid production by stimulation of proton pump leading to gastric ulcers [2]. There are many drugs are used to treat peptic ulcer like H_2 blockers, proton pump inhibitors, ulcer protective which decrease HCL production. Antacids can neutralize the acids. Sucralfate protects the gastric mucosal membrane [3]. The conventional chemistry and pharmacology produced effective synthetic drugs to treat gastric ulcers but these drugs produce undesirable effects in the patients [4]. To overcome this drawback, use of herbal medicine is the best choice because there is no adverse effects with this medicine *Withania somnifera* called Ashwagandha. This plant parts are widely used in the treatment of several diseases in ayurvedic medicine. It can be used to treat arthritis, gastric ulcers, anxiety, sexual problems, diabetes, to increase immunity and for anti-oxidant protective effects [5,6,7]. The root is used in treatment of anorexia, anti-tumor and rejuvenation properties [8,9,10,11]. Plant root contains various phytochemicals and have various clinical uses in the treatment of various diseases[12]. Oxidative stress plays a major role in the pathogenesis of gastro intestinal diseases. Studies proved increased free radicals and lipid peroxidation which are involved in the pathogenesis of peptic ulcer. Ethanol is an agent that can increases the formation of oxygen free radicals and stimulates the lipid peroxidation in the gastric mucosa and causes acute gastric ulcers [13]. This study aims to investigate anti-ulcer activity of *Withania somnifera* in ethanol induced ulcer in rats.

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MATERIALS AND METHODS

Chemicals: Omeprazole (Atoz pharma), Alpha lipolic acid (Sigma Aldrych), Sucralfate (Zydus Alidac),

Collection of *Withania somnifera* roots: The roots were purchased from the local shop and identified by Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh.

Preparation of root extract: The roots were allowed to dry under sunlight. The roots were grounded into fine powder using domestic blender [14]. The 500gm of root powder was subjected for

extraction by Soxhlet apparatus by using ethyl alcohol solvent. The filtrate was transferred to an aerated oven present at 40° C and allowed to completely dry[15]. The obtained extract was stored and used.

Animals: Healthy adult albino rats of male sex weighing 180-230g obtained from central animal house Gandhi Medical college, Secunderabad. All the animals were housed under standard laboratory conditions like light/dark at 12/12 hr cycle, free access to water and fed with pellets [16]. The study was carried out according to CPSCEA guide lines. Study was approved by the Institutional Animal Ethics Committee (Gandhi Medical College).

Study design: The selected rats were divided into 5 groups and 6 rats for each group.

Group-I: Normal Saline (5ml/kg/orally)
Group-II: Sucralfate (100mg/kg/orally) [17]
Group-III: Alpha Lipoic Acid powder (100mg/kg/ orally) [18]
Group-IV: *Withania somnifera root* extract (250mg/kg/orally)
Group-V: *Withania somnifera root* extract (500mg/kg/orally) [19]

Ethanol-induced ulcer: The rats were divided and administered drugs according to study design. After one hour all the group of animals received 80% of ethanol (1ml/kg/orally). Then rats were scarified under chloroform anesthesia after 1 hour. Rat abdomen was incised along the greater curvature and washed gently under running tap water. The stomachs were placed on the watch glass and examined for the severity of ulceration according to the following scale[20].

Ulcer scoring:

0 = Normal gray colored stomach,
0.5 = Pink to red colored stomach,
1 = Spot ulcer,
1.5 = Hemorrhagic streak,
2 = Number of ulcers< 5,
3 = Number of ulcers > 5 and
4 = Bleeding ulcers [21].

Ulcer Index: The ulcer index was calculated by below formula [22].

$$UI = \frac{\text{Mean ulcer index control group} - \text{Mean ulcer index test group}}{\text{Mean ulcer index control group}} \times 100$$

Histopathological study: The stomach tissue was fixed in 10% formalin and processed with paraffin wax. 5 micrometer sections were taken and stained with hematoxylin and eosin by standard procedure. The extent and depth of ulcer were evaluated by microscopic study[23].

Statistical analysis: The data was analysed using SPSS 16.0 software. One way ANOVA followed by PostHoc (Duncan"s) test applied to find significance between groups. P values < 0.05 were considered statistically significant [24].

RESULTS

It is evident from Table-1 that decreased average number of ulcers pretreated with Sucralfate (2.83±0.74), Alpha Lipoic Acid powder (4.57±1.42), low and high dose of plant (4.29±0.38, 3.58±1.12) compared to control group (7.11±1.52). The average scoring also significantly decreased pretreated groups compared to non-drug treated group. There is a significant difference observed in ulcer incidence, ulcer index and ulcer inhibition compared to control with drug administered groups (Table.2). The high dose plant extract administered group showed same effect like standard drugs. Control group stomach showed hemorrhagic erosions, damage to lining epithelial cells and damage to mucosa (Figure.1). Pretreatment with standard drugs and plant extract showed mild hemorrhage, edema and changes in gastric epithelium (Figure.2 &3).

Tabel.1: Effect of *Withania somnifera* on ethanol induced number of ulcers and average scoring

GROUPS	Drug and Dose administration	Average number of ulcers (MEAN±SD)	Average scoring (MEAN±SD)
Group-I	Normal saline (5ml/kg/orally)	7.11±1.52	3.28±0.31
Group-II	Sucralfate (100mg/kg/orally)	2.83±0.74*	1.21±0.83*
Group-III	Alpha Lipoic Acid powder (100mg/kg/orally)	4.57±1.42*,†	2.80±0.34*,†
Group-IV	*Withania somnifera* extract (250mg/kg/orally)	4.29±0.38*,†	2.82±0.52*,†
Group-V	*Withania somnifera* extract (500mg/kg/orally)	3.58±1.12*	2.26±0.12*

(*P<0.05 significant compared group-I with other groups, †P<0.05 significant compared group-II with other groups)

Table.2: Effect of *Withania somnifera* on ethanol induced ulcer incidence, ulcer index and ulcer inhibition

GROUPS	Ulcer Incidence (%)	Ulcer Index	Ulcer Inhibition (%)
Group-I	100	20.31	0
Group-II	38.23*	6.23*	79.57*
Group-III	66.66*,†	13.97*,†	31.12*,†
Group-IV	64.58*,†	11.24*,†	36.05*,†
Group-V	50.00*,†,‡	10.78*,†,‡	46.92*,†,‡

(*P<0.05 significant compared group-I with other groups, †P<0.05 significant compared group-II with other groups, ‡P<0.05 significant compared group-III with others)

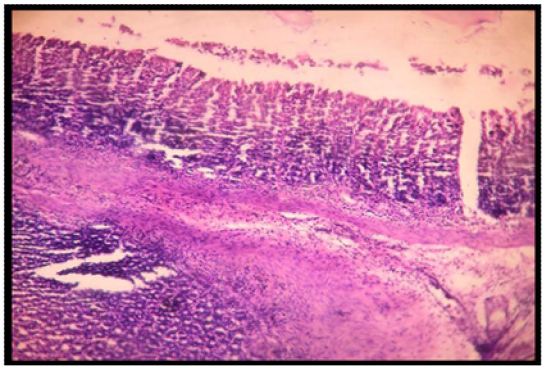

Figure.1: Stomach histology of control group

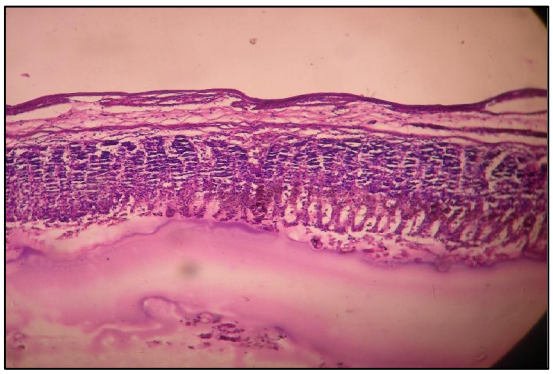

Figure.2: Stomach histology of Alpha Lipoic Acid powder group

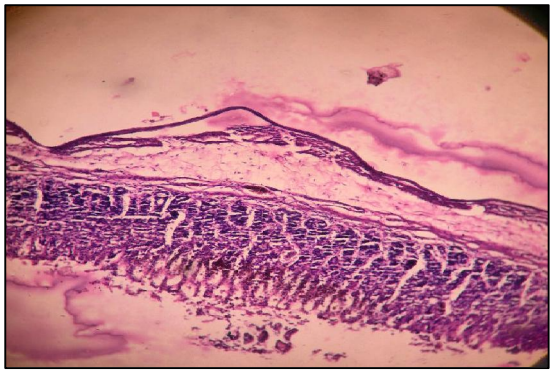

Figure.3: Stomach histology of *Withania somnifera* root extract group

DISCUSSION

There are several causes for development of ulcers. In general there is an imbalance between protective and affective agents in the gastric mucosa. HCL secretion can be stimulated by acetylcholine, histamine, stress and other factors. Drugs like NSAIDs inhibit the bicarbonate production and cause formation of ulcers. Some of the agents are using to induce ulcers experimentally in animals. Induction of ulcers with ethanol is old and easy method. It is known that administration of ethanol orally causes development of hemorrhagic ulcers in gastric mucosa. Studies proved that ethanol damages gastric mucosa extensively. The mucosa develops vascular injury, edema, hemorrhagic spots, epithelial thinking and necrotic lesions, but development of these changes depend on the dose of ethanol. The basic mechanism of development of ulcer due to increased oxidative free radicals in ethanol model. *Withania somnifera* roots have several pharmacological properties and uses. The results of present study have shown that *Withania somnifera* root extract prevents ethanol induced ulcers. Pretreatment with root extract reduces the average number of ulcers, average scoring, ulcer incidence, ulcer index and increases ulcer inhibition in ethanol induced gastric ulcer. Gastric ulcer preventive efficacy of root extract is nearly same like standard drugs. This protective effect was proved and confirmed by histopathological examination. Root extract treated group gastric mucosa showed less ulcers, lesions and sub-mucosal edema. This anti-ulcer activity may be present in flavonoids and antioxidant effect in root extract.

CONCLUSION

From this study, it's clear that *Withania somnifera* root extracts have significantly decreased the ulcers induced by ethanol administration in rats. It has gastro protective and ulcer protective activity when compared with standard drugs Sucralfate, Alpha Lipoic Acid powder. This gastro protective activity is due to presence of flavonoids and anti-oxidant in the extract. Further animal and clinical trials are required to characterize and find the effective dose of biologically active compounds present in the extract.

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