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Examination of anti ulcer activity of azadirachta indica leaves.

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ABSTRACT

The cause of ulceration in patients is mainly due to hypersecretion of gastric juice and also due to hypersecretion of pepsin. In traditional system of medicine a number of herbal preparations have been used for the treatment of peptic ulcers. There are various medicinal plants has been used for the treatment of gastrointestinal disorders. In view of this, in present study we have to evaluate the anti-ulcer activity of *Azadirachta indica*. Study was carried out, by using three methods i.e., alcohol, paracetamol and stress induced ulcers in rats pretreated with the doses of 250 mg/kg AQAI and ALAI, 10mg/kg Omeoprazole and 50 mg/kg Ranitidine. The antiulcer activity of aqueous and alcoholic extracts of *Azadirachta indica* leaves (AQAI and ALAI) at 250 doses using different experimentally induced gastric ulcer models in rats. Gastric ulcers were induced in rats by 80% alcohol, paracetamol and forced immersion stress induced methods. In alcohol induced ulcer model, paracetamol induced ulcer model and stress induced model the ulcer index was determined. Where as in stress induced ulcers stress plays an important role in ulcerogenesis. In alcohol-induced ulcers, AQAI and ALAI were effective in reducing lesion index and increasing the gastric mucus content. It was also effective in decreasing ulcer index in paracetamol-induced ulcers. All the results obtained with *Azadirachta indica* were dose dependent. The results suggest that AQAI and ALAI possesses significant and dose dependent antiulcer activity. The antiulcer activity of AQAI and ALAI can be attributed to its cytoprotective and antisecretory action.

Keywords: *Azadirachta indica*, antisecretory, cytoprotective, gastric ulcer, alcohol induced ulcers, paracetamol-induced ulcers and stress induced ulcers.

INTRODUCTION

Gastrointestinal (GI) tract

In order to digest food, absorb nutrients and excrete unabsorbed waste products, the GI tract has to perform a no of coordinated activities and the tract has to provide the whole body with a continual supply of water, electrolytes and nutrients[1]. In order to achieve these objects, several organs have to integrate with each other and are regulated by nervous rand hormonal systems, as well as the central nervous system[2]. Thus for fully understanding physiological functions of GI tract and its various pathological states including peptic ulcer, diarrhea and constipation, etc., a brief description of some important headings is given below.

Control and Co-ordination of GI Tract

In addition to the main function of assimilation of food, the GI tract has to perform endocrine function and also gut has its own integrative neuronal network, the enteric nervous system(ENS) that shares about the same number of neurons as those of spinal cord. Many of the neurotransmitters or neuromodulators and hormones in GI tract are peptides. The smooth muscles, blood vessels and glands (exocrine, endocrine and pancrine) are main elements under the neuronal and hormonal control.

Neuronal Control

The gut wall contains millions of enteric neurons, which are organized into two separate plexuses (myoenteric plexus

also called Auerbach's plexus) in between the outer or longitudinal and the middle or circular muscles layers and the Meissner's plexus (submucous plexus) on the luminal side of the circular muscle layer. The former is continuous from the upper oesophagus to the anus while the latter is located continuously only in small and large intestines[3,4]. These plexuses are interconnected and their ganglia are innervated with preganglionic parasympathetic fibres from vagus nerve that are mostly chol,inergic and often excitatory. The sympathetic fibres are mainly postganglionic which have endings in the plexuses where they inhibit the secretion of acetylcholine. The enteric nervous system has not only the sympathetic and parasympathetic neurons but also non-adrenergic and non-cholinergic neurons that secrete norepinephrine, acetylcholine, serotonin(5-HT), purines and certain peptides including substance-p, Vasoactive Intestinal Peptiode(VIP), somatostatin, enkephalins, bombesin, cholecystokinin and neurotensin[5,6]. In addition, Angiotensin II may also be secreted in its function. The sensory neurons that respond to chemical and mechanical stimuli are also present in enteric plexus. Therefore, both local reflexes as well as reflexes mediated through the celiac plexus and central nervous system (CNS) are involved to regulate activity of GI tract. The mucosal glands have also been found to possess many peptides, for example gastrin[7,8,9].

Hormonal Control

Gastrointestinal hormones include the peptides synthesized by mucosal cells e.g. gastrin and gastric inhibitory peptide (GIP). Gastric mucosa and wall of whole GI tract release paracrine secretions or local hormones of upper GI tract, which stimulate secretions and motility[10]. Neurotensin, peptide YY and enteroglucagon are general inhibitors of the secretion and motility that are released from ileum and colon in response to the nutrients (chime) into distal intestine. The glucose homeostasis is regulated by release of insulin and glucagon from pancreas[11,12,13].

Although pharmacologically impotent functions of GI tract include gastric secretions, motility of bowel, emesis (vomiting) and exreation of bile but gastric secretions are more relevant to the present work and have, therefore, been described in details on the following

MATERIALS AND METHODS

Cimetidine was gift sample from Taj pharmaceuticals Ltd, Omeoprazole was gift sample from Aurobindo pharma Ltd.,Ranitidine was gift sample from Aurobindo pharma Ltd, Alcohol was gift sample from ChangshuYangyuan Chemicals, China.

RESULTS

Acute toxicity study

Administration ofthe *Azadirachta indica* extracts *in* rats at doses of 250 mg/kg by oral gavage did not reveal any adverse effects or signs of toxicity.

Observations twice daily for fourteen days also did not reveal any drug related observable changes or mortality. Accordingly, the acute oral LD50 of the extractives was concluded to exceed 2000 mg/kg bodyweight, the highest dose tested in the study.

Effect on alcohol induced gastric ulcers

Oral administration of 80% alcohol produced hemorrhagic gastric lesions in glandular portion of stomach. Pretreatment with AQAI and ALAI at the dose of 250 mg/kg and omeprazole (10 mg/kg) significantly ($p<0.001$) protected the gastricmucosa as shown by reduced values of lesion index (19.3 ± 0.35and 27.47± 0.75 respectively) against alcohol challenge as compared to solvent control (26.14 ± 0.24).

Table 1: Effect of *Azadirachta indica* at various doses on alcohol induced gastric ulcer in rats.

Treatment (n=6)	Dose mg/kg(p.o.)	Lesion index	% Inhibitionof ulcer	Mucus content (μg Alcian blue/g wet tissue)
1% CMC	-	26.14 ± 0.24	-	0.50 ± 0.01
Ulcer control	-	35.94±0.36	-	0.57±0.02
Omeprazole	10	27.47± 0.75	20.12	0.66 ± 0.01
AQAI	250	30.21 ± 0.43	7.63	0.51 ± 0.02
ALAI	250	19.3 ± 0.35	45.01	0.86 ± 0.01

Values are mean ± S.E.M. n=number of animals in each group.Significant differences with respect to solvent control group were evaluated byStudent's *t* – test. ($p<0.05$, $p<0.01$ and $p<0.001$).

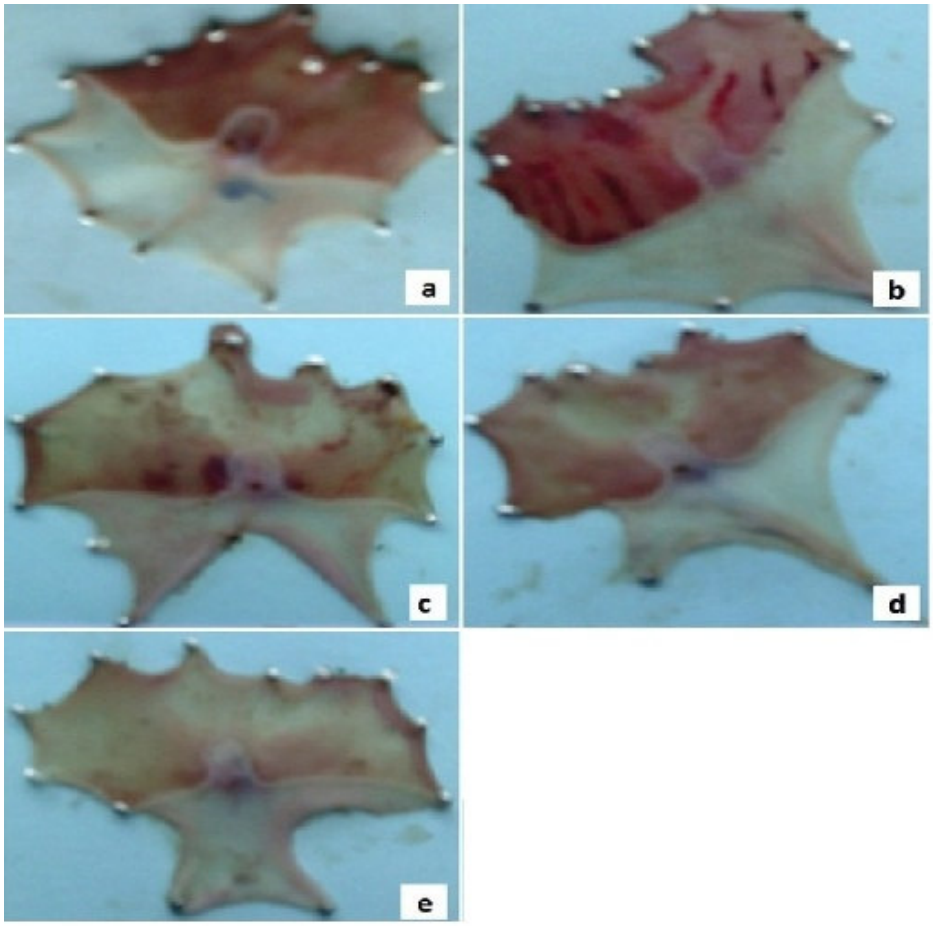

Fig-1: Effect of *Azadirachta indica* on alcohol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQAI*(250 mg/kg) treated (d) *ALAI* (250 mg/kg) treated (e) Omeprazole (10 mg/kg treated)

Effect on Paracetamol induced gastric ulcers

In *Azadirachta indica* treated groups (250 mg/kg), the ulcer index values (0.43 ± 0.02respectively) weresignificantly reduced ($p<0.001$) when comparedto solvent control (0.70 ± 0.02), while the ulcer index for ranitidine treated group was 0.25 ± 0.02 ($p<0.001$). The %inhibition of ulcer showed by AQAI and ALAI (250mg/kg) and ranitidine was 51.3%, 37.2% and 53.2% respectively.

Table 2.Effect of *Azadirachta indica* at various dose levels on paracetamol induced gastric ulcer in rats.

Treatment(n=6)	Dosemg/kg (p.o.)	Ulcer index	% Inhibition of ulcer
1% CMC	-	0.70 ± 0.02	-
Ulcer control	-	0.84±0.01	--
Ranitidine	50	0.25 ± 0.02	51.3
AQAI	250	0.43 ± 0.02	37.2
ALAI	250	0.30 ± 0.02	53.2

Values are mean ± S.E.M. n=number of animals in eachgroup; Significant differences with respect to solvent controlgroup were evaluated by Student's *t* - test. ($p<0.001$).

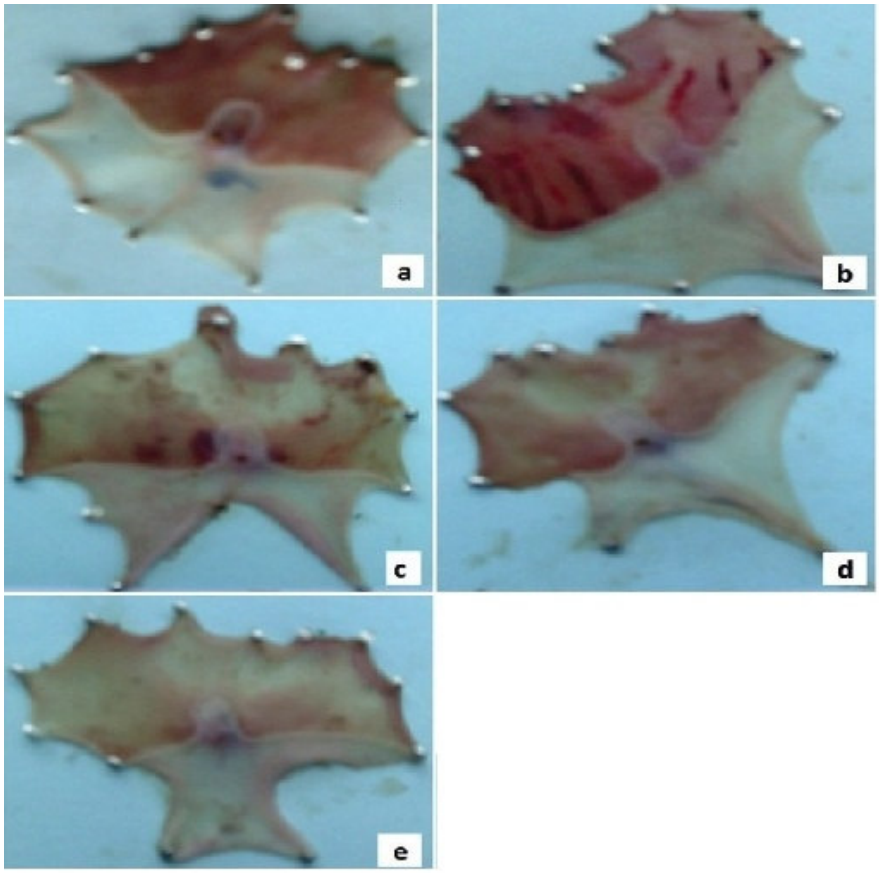

Fig-2: Effect of *Azadirachta indica* on paracetamol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQAI*(250 mg/kg) treated (d) *ALAI*(250 mg/kg) treated (e) Ranitidine (50 mg/kg treated)

Stress-induced ulcers

In water immersion stress induced ulcers, the mean score value of ulcer inhibition was found to be significant (P<0.001) for 250 mg/kg of the extract. The percentage ulcer inhibition was 75.29 and 84.55 for 250 mg/kg for both aqueous and alcoholic extracts, and that of the standard was found to be 91.42.

Table 3.Effect of *Azadirachta indica* at various dose levels on Stress induced gastric ulcer in rats.

Group	Ulcer index	Percentage inhibition
Normal Control	00.00±0.00	-----
Ulcer control	22.73±4.31	------
Standard	2.86±0.13	91.42
AQAI	6.90±3.02	75.29
ALAI	4.34±2.87	84.55

Values are mean ± S.E.M. n=number of animals in eachgroup; Significant differences with respect to solvent controlgroup were evaluated by Student's *t* - test. (p<0.001).

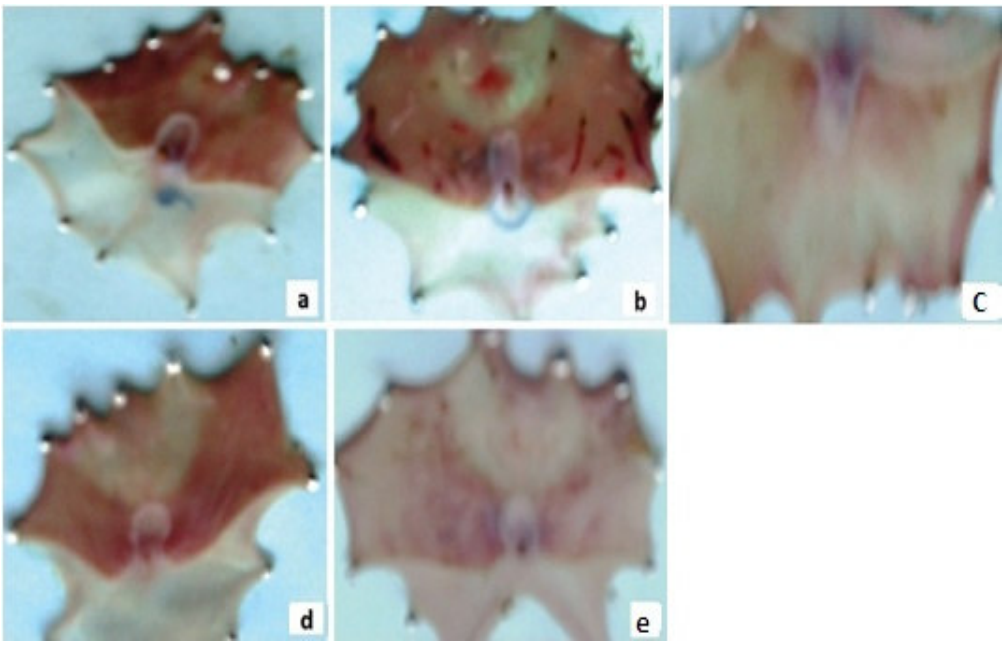

Fig-3: Effect of *Azadirachta indica* on stress induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) *AQAI*(250 mg/kg) treated (d) *ALAI* (250 mg/kg) treated (e) Omeprazole (10 mg/kg treated)

DISCUSSION

The anti-ulcer activity of *Azadirachta indica* was evaluated by employing alcohol/paracetamol/acetic acid/stress induced gastric ulcers in rats. Alcohol and paracetamol induced ulcer models were used because they represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving the increase of gastric acid output, vascular injury, depletion of gastric wall mucin, mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production.

Alcohol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation which causes damage to cell and cell membranes. *Azadirachta indica* has significantly protected the gastric mucosa against alcohol challenge as shown by reduced values of lesion index as compared to solvent control group suggesting its potent cytoprotective effect. This is further substantiated by increase in gastric mucus content produced by *Azadirachta indica* extract.

NSAID's like paracetamol, aspirin, indomethacin cause gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis. *Azadirachta indica* extract was significantly effective in protecting gastric mucosa against paracetamol induced ulcers at all the dose level studied. Hence *Azadirachta indica* extract affords effective protection to gastric mucosa against various insults by increasing gastric mucus content and decreasing the acid volume, free and total acidity in rats.

Stress plays an important role in ulcerogenesis. The pathophysiology of stress-induced gastric ulcers is complex. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production. The aqueous and alcoholic extracts of *Azadirachta indica* were effective in reducing the ulcers induced by stress.

The effects in all the 3 models studied were dose dependent. In conclusion, to the best of our knowledge for the first time, we have demonstrated that Hence *Azadirachta indica* extract has gastro protective activity against experimentally induced ulcers in rats. The mechanism of gastro protective action can be attributed to its antisecretory and cytoprotective property. However further experiments are required to establish and elaborate the molecular mechanism(s) of its Anti-ulcer activity.

CONCLUSION

The anti-ulcer activity of the plant *Azadirachta indica* was evaluated by employing paracetamol, alcohol and stress induced ulcer models. These models represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving, depletion of gastric wall, mucin mucosal damage induced by nonsteroidal anti-inflammatory drugs and free radical production.

NSAID's like aspirin and paracetamol causes gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis. Alcohol and Aqueous extract of the plant of *Azadirachta indica* was significantly effective in protecting gastric mucosa against paracetamol induced ulcers at all the dose level studied.

Alcohol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane. The extracts of the *Azadirachta indica* has significantly protected the gastric mucosa against alcohol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect. It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and

interference of gastric blood circulation are responsible for induction of ulceration.

The antiulcer activity of *Azadirachta indica* extracts in stress induced model is evident from its significant reduction in gastric volume, ulcer index and increase in pH of gastric juice. Because of animals treated with *Azadirachta indica* extracts significantly inhibited the formation of ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values.

It is suggested that *Azadirachta indica* extracts can suppress gastric damage induced by aggressive factors. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms. The excess gastric acid formation by prostaglandin (PG) includes both increase in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells.

The possible mechanism of antiulcer action of *Azadirachta indica* may be due to its flavonoid content. In this study we observed that *Azadirachta indica* provides significant anti-ulcer activity against gastric ulcers in rats.

On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *Azadirachta indica* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

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