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PROTECTIVE EFFECT OF *ANNONA SQUAMOSA* LINN. LEAF EXTRACT ON HCL-ETHONAL INDUCED GASTRIC ULCER IN ALBINO RATS.

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ABSTRACT

Peptic ulcer is a major health hazard both in terms of morbidity and mortality. It occurs due to imbalance between offensive versus impaired mucosal resistance. As an alternative to allopathic therapeutics, scientific research on plants used in traditional medicine is receiving growing interest as a way of identifying new agents for treating many ailments. In the present study *Annona squamosa* Linn. has been evaluated for its antiulcer potential by administering the aqueous leaf extract for 11 days on HCl-ethanol mixture (1ml/10g body weight; oral administration) induced ulcered rats. The rats were divided into five groups comprising of 6 rats each and treated respectively, I- normal control (saline), II - disease control (HCl-ethanol mixture), III & IV - leaf extract treated -*Annona squamosa* Linn. (250mg/kg & 500mg/kg body weight), V- standard drug (ranitidine 2.5mg/kg body weight). Ulcer induced rats showed marked alterations in gastric output, pH, biochemical and enzymatic parameters compared to normal control. In diseased animals ulcer index also increased. On treatment with test drug, gastric output, pH, activity of alkaline phosphatase, activity of enzymatic and non-enzymatic antioxidant such as superoxide dismutase, glutathione peroxidase, reduced glutathione, lipid peroxides were resumed to normal. The levels of hemoglobin and red blood cells were also in par with normal control. The results of present investigation revealed that the pretreatment with *Annona squamosa* Linn. leaf extract inhibits the ethanol-HCl induced congestion, hemorrhage, necrosis and ulceration which was evidenced from the resumption of various parameters. The data of the present study validate traditional claims of *Annona squamosa* Linn. which is reported to be beneficial in treating gastric ailments.

Keywords: Peptic ulcer, *Annona squamosa* Linn. HCl-ethanol mixture, Ulcer index, Gastric output, Alkaline phosphatase, Lipid peroxides, hemorrhages.

INTRODUCTION

Ulcer is a condition, where there is erosion in the lining of the stomach or duodenum and is caused by the disruptions of the gastric mucosal defense and repair mechanism [(1)]. Gastric ulcer is the ulcer of stomach and in the duodenum is called duodenal ulcer and together it is peptic ulcer. Gastric ulcers affects the gastric mucosa and are common in middle-aged and elderly men, especially on chronic usage of nonsteroidal anti-inflammatory drugs (NSAIDs), alcohol, or tobacco[(2)]. The prevalence of peptic ulcer increases with age. The lifetime prevalence is 11.22% with a peak prevalence of 28.8% in the 5th decade of life[(3)].

The common synthetic drugs used in the treatment of ulcer are H_2 receptor blockers, proton pump inhibitors, acid Neutralizers (Antacids) and drugs affecting the mucosal barrier. Even though a wide range of drugs are being used for the treatment of ulcer, many of these do not fulfill all the requirements and reported to have side effects like sedation, indigestion, headache, bowl upset, confusions, anti androgenic effect**.** These factors have prompted the researchers to investigate the pharmacological features of natural compounds with more effective, less side effects and less expensive for the treatment of peptic ulcer disease[(4)]. In this lead, the present work aims in identifying and evaluating a plant source that could be antiulcerogenic and cytoprotective. Hence ***Annona Squamosa*** **Linn.** belonging to the family *Annonaceae*, seen throughout India, has been selected and tested for its antiulcerogenic potential in HCl/ Ethanol induced ulcer models.

MATERIAL AND METHODS

Plant material

Fresh leaves of ***Annona Squamosa*** **Linn**. were collected from places in and around Tiruchirappalli, Tamilnadu, during the month of April, identified with the help of Flora of Presidency of Madras[(5)]and authenticated with the specimens deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's college, Trichy.

Preparation of aqueous extract:

The leaves of ***Annona Squamosa*** **Linn**. were shade dried and powdered coarsely. The powder was boiled in water (200g/1000ml water) and the decoction was reduced to one third in volume. Reduced decoction thus obtained was filtered and the filtrate was evaporated to dryness. The residue obtained was stored and used for pre clinical experiments. The percentage yield of the aqueous extract is 28g.

Experimental Animals

Wistar strain of albino rats weighing 100g-150g were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai. They were fed with standard rat chow pellet obtained form Sai Durga Food and Feeds, Bangalore, India and water *ad libitum*. Animals were maintained in a standard animal house in a controlled environment (temperature 25+2°C and 12hr dark/light cycle). The study was conducted after obtaining the necessary clearance from Institutional Animal Ethical Committee. CPCSEA approval no: 790/03/ac/CPCSEA.

Induction of Ulcer

Gastric ulcer was induced in group 2, 3, 4, & 5 animals. Animals were starved for 36 hours with access to drinking water *ad libitum*. Animals were given 1ml of 0.6M HCl / 80% ethanol solution (HCl/ethanol). One hour after induction the animals were sacrificed. The stomach tissue and blood were collected for further analysis [(6)].

Experimental Design

The rats were divided into five groups comprising of six rats each.

Group INormal control (Normal saline)

Group IIDisease control (1ml HCl – ethanol mixture on 11th day-Single dose).

Group IIIAnimals treated with **ALEAS** (aqueous leaf extract of *Annona squamosa* Linn.) (250mg/Kg body weight orally) for 10 days and 1ml HCl – ethanol mixture on 11th day (Single dose).

Group IVAnimals treated with **ALEAS** (aqueous leaf extract of *Annona squamosa* Linn.) (500mg/Kg body weight orally) for 10 days and 1ml HCl – ethanol mixture on 11th day (Single dose).

Group VAnimals treated with Ranitidine (2.5mg/ kg body weight orally) for 10 days and 1ml HCl – ethanol mixture on 11th day (Single dose).

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was collected and used for various biochemical estimations.

Determination of ulcer index in gastric tissues [7]

The stomach was removed, opened along the greater curvature and washed it slowly under the running tap water. Placed it on the glass slide and observed under microscope (10x) for ulcers. Mean ulcer score for each animal is expressed as ulcer index.

Biological assays

The total acidity in the gastric fluid was determined by using Sodium hydroxide-0.01N and Topfer's reagent-(Dimethyl-amino-azobenzene with phenolphthalein) **[31].** Determination of hemoglobin was carried out by using Drabkin's solution and Cyanmethaemoglobin as standard **[8]** and red blood cells (RBC) was enumerated by using Hayem's diluting fluid and hemocytometer**[8].** Activity of Alkaline phosphatase (ALP) was assessed by the method of King J, 1965**[9].** The activity of enzymatic antioxidants superoxide dismutase (SOD) was determined by the method of Misra HP and Fridovich I, 1972**[10]** and glutathione peroxidase (GPx) was determined by the method of. Rotruck *et al.*, 1973**[11].** Non-enzymatic antioxidants lipid peroxides (LPO) was assessed by the method of Ohkawa , 1970**[12]** and reduced glutathione (GSH) by Morton *et al.*, 1979 method**[13]**.

Statistical analysis

All the results were expressed as mean ± S.E.M. The data were statistically analyzed by one – way analysis of variance (ANOVA). P values <0.05 were considered as significant.

RESULT AND DISCUSSION

Antiulcerogenic and cytoprotective effect of ALEAS

The effects of aqueous leaf extract *Annona squamosa* Linn. on gastric lesion induced in albino rats are shown in **Table 1**. Oral administration of HCl/ethanol solution to control group produced characteristic mucosal lesion as evidenced by the number of lesions in stomach. Pretreatment with **ALEAS** (group III and IV) caused significant (P< 0.05) protective effect against HCl/ethanol induced gastric lesion **(Table 1)**. The results were comparable to standard drug. The plant source provides increased mucosal resistance and regulate mucosal repair.

Antacid effect of ALEAS

Group - II animals showed profound increase in total acid output **(Table 1)**. The pH of gastric content was also found to be more acidic compared to normal control. Oral administration of test drug **ALEA** for 10 days provoked a marked decrease (P< 0.05) in total gastric acid together with an increase in pH values (Group III and IV). This may be related to an antacid effect or cytoprotective effect of *Annona squamosa* Linn. on HCl/ ethanol induced gastric irritability.

Effect of ALEAS on RBC and Hemoglobin levels

Effect of **ALEAS** at varying dose on hemoglobin level and RBC count were depicted in **Table- 1**. Ulcer is a condition where there is profound hemorrhage due to the lesion in the gastric mucosa. This was evidenced from the decreased level of Hb and RBC count obtained for group-II animals **(Table 1)**. Pretreatment with plant extract inhibited ethanol induced ulcer, congestion, hemorrhage and necrosis in gastric mucosa. Hence there was significant raise in the level of Hemoglobin and RBC count (P< 0.05). The results obtained were in par with reference drug ranitidine (group-V).

Inter- group comparison

a*P < 0.05.statistically significant when compared Group II with Group I,

b*P < 0.05.statistically significant when compared Group III, IV & V with Group II,

Effect of ALEAS on Alkaline phosphatase (ALP), Non-Enzymatic and Enzymatic Antioxidants

Alcohol ingestion caused damages to gastric mucosa and results in the release of marker enzyme (ALP) into the blood, hence there was a marked increase in the ALP activity in group II animals **(Table 2)**. Aqueous extract of *Annona squamosa* Linn. exhibited marked gastroprotection in HCl/ethanol induced ulcer model. The extract significantly (P<0.05) reduced the ALP activity when compared to untreated alcohol administrated control (group-II). The reference drug ranitidine also showed similar effect **(Table 2)**. The effect of **ALEAS** may be due to its better quality of mucosal reconstruction and regeneration of gastric tissues.

Inter- group comparison:

a*P < 0.05.statistically significant when compared Group II with Group I,

b*P < 0.05.statistically significant when compared Group III, IV & V with Group II,

The present study indicated that **ALEAS** showed ulcer protective effect against HCl/Ethanol induced gastric ulcer in rats. Group II animals which has not been pretreated with plant extract had increased level of LPO, decreased level of GSH and altered the activity of SOD and GPx. **(Table 2).**

Generation of lipid peroxide radicals and related substance occurs due to gastric mucosal membrane damage, which was evidenced from group II untreated ulcerated animals. Lipid peroxidation was inhibited significantly (P<0.05) on prior treatment with plant extract for 10 days (Group III and IV). The inhibition was found to be dose dependent **(Table 2)**. GSH is a major non-protein thiol in living organisms that play a vital role in coordinating the body's antioxidant defense mechanism **[14]**. Excessive peroxidation that occurs in disease conditions causes increased GSH consumption **[15]**. The similar effect was observed in Group II HCL/Ethanol induced gastric lesion. Treatment with **ALEAS** resulted in increased (P<0.05) activity of total tissue GSH compared to the untreated ulcerated rats. The antioxidant activity of the herbal extract would have protected the gastric lining from excessive peroxidation.

Activity of SOD, one of the antioxidant enzymes were also decreased in ulcerated, untreated rats. Aqueous extract of *Annona squamosa* Linn. due to its antioxidant property, prevented HCL/Ethanol induced lesion. This was evidenced by the increased activity of SOD in group-III and IV animals **(Table 2).** Activities of GPX was also altered in diseased rats (group-II). Oral administrations of test drug were significantly (P<0.05) prevented the oxidative damage caused by HCL/Ethanol mixture on gastric mucosa. Hence resumption in the activity of GPX was seen as depicted in **table 2**. From the antioxidant studies it was revealed that the plant extracts counteract oxidative damage caused by HCl/Ethanol toxicity. **ALEAS** thus prevented the ulcer formation and enhances ulcer healing.

Table 1: **Effect of ALEAS on Ulcer index, Total acidity, pH, Hemoglobin level and RBC count**

Groups	Ulcer Index	Total Acidity	pH	Hemoglobin (g%)	RBC
I	1.2 ± 0.34	118.5±33.24	5.4 ± 0.08	16.0 ± 3.41	5.85 ± 0.92
II	29.4 ± 4.21a*	140.1±32.56a*	3.8 ± 0.06a*	12.4 ± 5.62a*	3.42 ± 1.31a*
III	11.5 ± 3.82b*	123.4±23.52b*	4.1± 0.08b*	14.7± 4.92b*	3.93 ± 2.01b*
IV	3.4 ± 1.91b*	116.5±34.51b*	5.2± 0.10b*	16.2 ± 5.31b*	6.01 ± 1.52b*
V	3.5 ± 1.04 b*	118.9±43.42 b*	6.1 ± 0.07 b*	15.6 ± 2.61 b*	5.64 ± 0.91 b*

Values are mean ±S.E.M (n = 6)

Table 2: **Effect of ALEAS on Alkaline phosphatase (ALP), Non-Enzymatic and Enzymatic Antioxidants**

Groups	ALP	LPO	GSH	SOD	GPx
I	98.4± 18.21	33.6 ± 3.42	92.7 ± 10.41	56.7 ± 5.45	43.7 ± 4.62
II	121.5± 29.43a*	67.9 ± 8.45a*	54.0 ± 7.21a*	23.7 ± 3.04a*	24.5 ± 3.21a*
III	109.6± 20.31b*	51.4 ± 7.06b*	72.9 ± 6.56b*	36.6 ± 4.79b*	30.6 ± 4.42b*
IV	97.6± 12.45b*	36.1* ± 6.52b*	89.1± 7.31b*	51.4 ± 6.64b*	44.7 ± 5.31b*
V	95.5± 11.22b*	38.4* ± 7.04b*	88.7 ± 9.79b*	49.6 ± 4.51b*	42.7 ± 3.61 b*

Values are mean ± S.E.M (n = 6)

CONCLUSION

The obtained data reveals the antiulcerogenic effect of aqueous leaf extract of *Annona squamosa* Linn. by markedly inhibiting acid secretion and the occurrence of lesion in stomach. The possible mechanism of **ALEAS** antiulcer benefit may be due to its oxygen radicals scavenging property by inhibiting lipid peroxidation and by preventing loss of gastric mucus. The precise mechanism of action of *Annona squamosa* Linn. in protecting rats against HCl/ethanol induced gastric lesion is unknown. Further in-depth studies are needed to be carried out with isolated compounds and to identify the mechanism of action of the active principle responsible for the gastroprotective effect of the test drug.

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