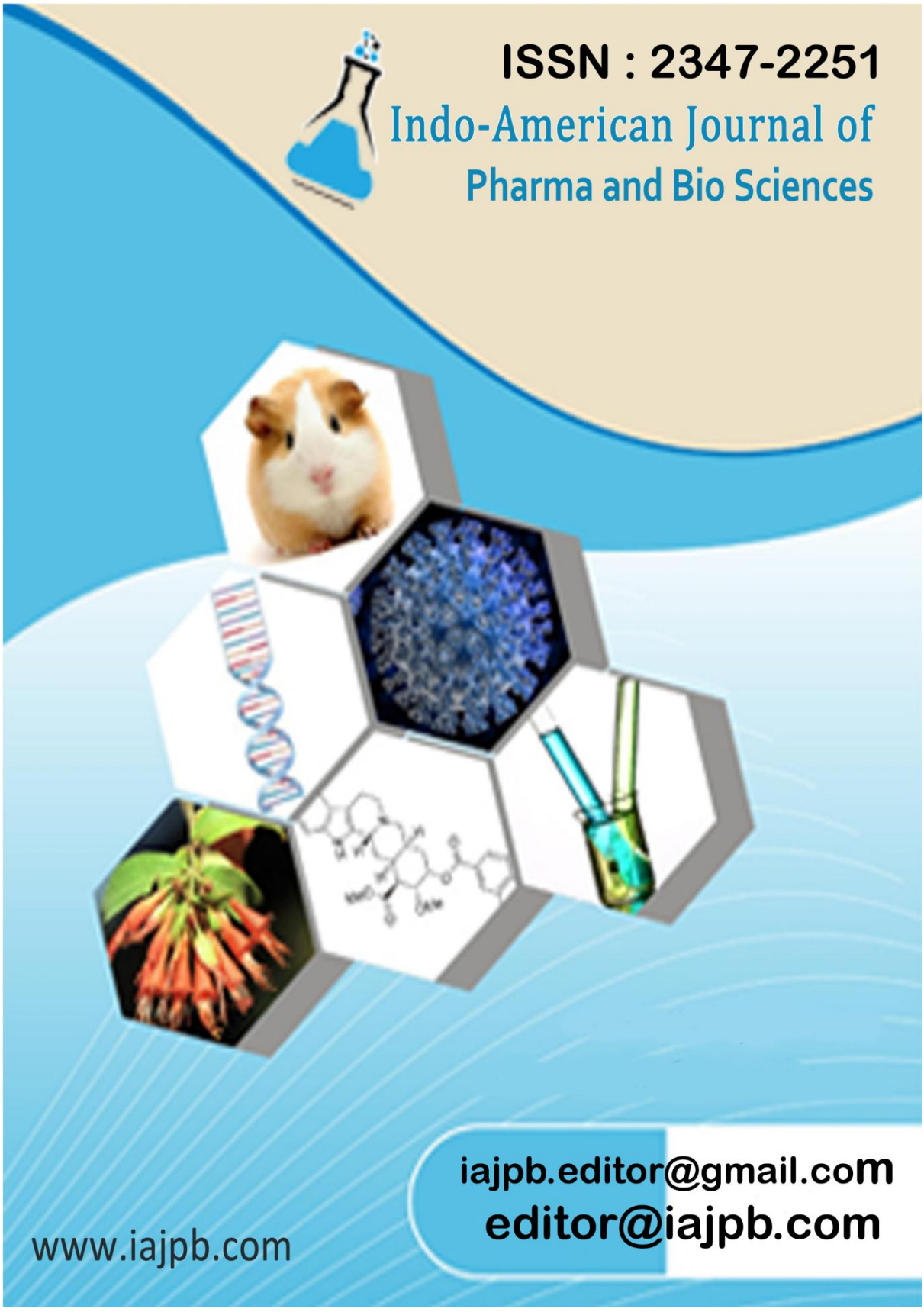
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Pharmacological and Phytochemical Screening of *Annona Squamosa L.* for Anti-Ulcer Activity

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Abstract:

This study investigates the anti-ulcer activity of the aqueous extract of *Annona squamosa* L. against Aspirin induced ulcer. Gastric ulcer disease is caused byan imbalance between mucosal defense factors and injurious factors, such as acid and pepsin. Ulcers caused by pylorus ligation result from increased gastric acid and pepsin accumulation, leading to auto digestion of gastric mucosa and breaking down the gastric mucosal barrier. NSAIDs are frequently associated with peptic ulcers.

The anti-ulcer effect is supported by decreased aggressive factors like gastric volume, decreased free and total acidity, and increased resistance factors like pH. The anti-ulcer agent may protect the mucosa fromacid effects by selectively increasing prostaglandins, which have a vital protective role. The mucosaldefense mechanism may be due to the epithelial cells of the gastric mucosa, which are impermeable to H+ ions and form a physical barrier.

The aqueous extract of *Annona squamosa linn* was evaluated using the aspirin induced ulcer model, showing dose-dependent inhibition percentages of 53.26% and 63.23% compared to the ulcer control. The standard drug Ranitidine (20mg/kg) showed a percentage inhibition of 71.48% when compared to the ulcer control.

The study also found that the extract can reduce reactive free radicals that might cause oxidative damage to tissue. It also reduced polymorphonucleur infiltration, TNF expression, and ROS formation in peptic mucosa. The anti-ulcerogenic activity of the extract may add to its beneficial effect against peptic ulcer disorder.

Keywords: PepticUlcer, Annona squamosa *linn,*Acid Secretion, Inflammation, Mucosa

Introduction:

Herbal Medicine

Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care. World Health Organization define herbal medicines as naturally occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices.[1]

Herbal medicines in systems have been used for a long time and are documented with their

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special theories and concepts, and accepted by the countries. For example, Ayurveda, Unani and Siddha. Modified herbal medicines have been modified in shape, or form including dose, dosage form, mode of administration, herbal medicinal ingredients, methods of preparation and medical indications. The WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. [2]

Peptic Ulcer

Peptic ulcers are acid-induced lesions of the digestive tract, typically located in the stomach or proximal duodenum. They are characterized by denuded mucosa with defects extending into the submucosa or muscularis propria. Total prevalence of peptic ulcer among the studied respondents was thus: 21.9% had peptic ulcer; 16.2% gastric ulcer and 5.6% duodenal ulcer. In 19.7% of the cases, the pain was severe, 92.4% reported that pain was precipitated by certain food. In addition to heartburn, 78.8% reported loss of appetite, 71.2% indigestion, 66.7% regurgitation, 59.1% nausea and vomiting and 42.4% with chest pain. Regarding the risk factors, coffee drinking came in first place (81.8%) followed by physical stress in 77.3%, spicy food in 57.6%, prolonged use of non-steroidal anti- inflammatory drugs (NSAIDs) in 33.3% and Helicobacter pylori (H. pylori)infection in 24.2%. A further 22.7% reported melena as a complication while only 10.6% reported hematemesis. [3]

"A peptic ulcer is a sore that forms when acidic digestive juices wear away the lining of the digestive system".

Plant Profile

Annona squamosa L. (Annonaceae), commonly known as the custard apple tree is a native of West Indies. But the cultivation is present throughout India, because of its edible nature. It is a fruit tree considered as a native of Central America also and hence has a wider cultivation throughout the regions of tropics. [4]

Taxonomical Classification

Kingdom	**Plantae**
Subkingdom	Angiosperms
Division	Magnoliophyta
Order	Magnoliales
Family	*Annonaceae*
Subfamily	Maloideae
Genus	Annona
Species	*A. squamosa L.*

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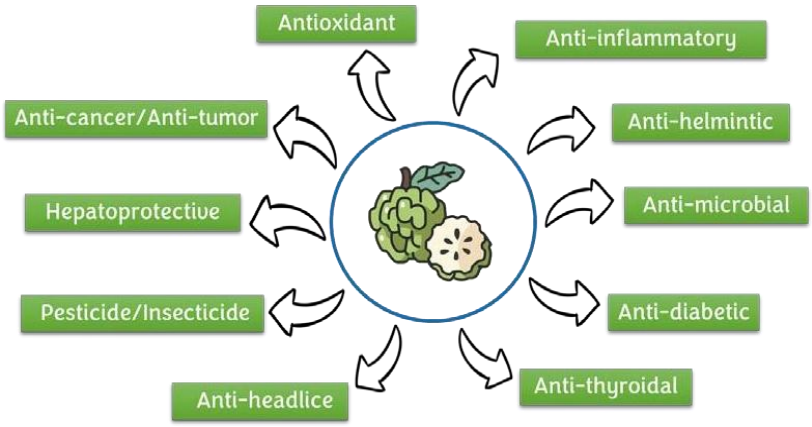
Pharmacological Properties of *Annona Squamosa*: [5]



Materials and Methods:

The plant material was collected, authenticated and then the leaves were washed to remove the dust Particles and allowed to air dry in a shade for complete drying. Then the dried leaves without moisture were powdered in a mixer grinder.

Preparation of extract

The coarse powder was packed tightly in the soxhlet apparatus and extracted with ethanol for 72 hours with occasional shacking maintained at 60ºc throught out the extraction process. The extract was concentrated to of its original volume by evaporation. The resulting aqueous extract of the *Annnona Squamosa (L.)* was subjected to phytochemical study. [6]

Phytochemical analysis

The aqueous extract of *Luffa acutangula (L.) Roxb.* were subjected to qualitative phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, and free aminoacids and triterpenoids. [7]

Test for carbohydrate

Small quantity of extract was dissolved in 5ml of water and filtered.

Molisch test

The filtrate was treated with a few drops of α-napthol (20% in ethyl alcohol). Then 1 ml of concentrated H_2SO_4 was added along the sidesof inclined test tube and observed for formation of violet coloured ring at the interface.

Test for glycosides and anthroquinones

Borntrager's test

A small amount of ethanolic extract was hydrolysed withhydrochloric acid for few hours on water bath and the hydrosylate was extracted with benzene. The benzene layer was treated with dilute ammonia solution and observed for the formation of reddish pink colour.

Legal test

The extract was dissolved in pyridine and made alkaline with few drops of 10% NaOH and freshly prepared sodium nitroprusside was added and observed for formation of blue colour.

Test for flavonoids

Ammonia test

Filter paper strips were dipped in the dilute solution of the extract, ammoniated and observed for colour change from white to yellow

Test for Proteins and Amino acids

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Small amount of extract was dissolved in distilled water and filtered

Biuret's test
To the ammoniated alkaline filtrate add 2-3 drops of 0.002% copper sulphate and observed for appearance of red or violet colour.

Millon's test

To 2 ml of filtrate 5-6 drops of millons reagent (1 g of mercury + 9 ml of fuming nitric acid solution) was added and observed for red precipitates.

Ninhydrin test
To the filtrate lead acetate solution was added to

precipitate tannins and filtered. The filtrate was spotted on paper chromatogram and sprayed with ninhydrin reagent and heated at 110ºC for five minutes and observed for red or violet colour.

Xanthoprotein test
To the filtrate a few drops concentrated nitric acid was added by the side of test tube and observed for appearance of yellow colour.

Test for sterols and tri terpenes
The extract was refluxed with alcoholic potassium hydroxide until the completion of saponification. Then the mixture was diluted with distilled water and extracted with diethyl ether. The ethereal extract was evaporated and the unsaponifiable matter was subjected to the following tests.

Salkowski's reaction
To the ether soluble residue 2 ml of concentrated sulphuric acid was added and observed for the formation of yellow ring at the junction which turns red after one minute.

Pharmacological Screening:

Animals
Wistar albino rats weighing 150- 200 gm were used for this study. The animals were obtained from animal house, Vedic Institute of Pharmaceutical Education and Research Sagar (M.P). On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2ºc and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow.[8]

Animal approval

The study was conducted after obtaining from Committee for the Purpose of Control and Supervision Experiments on Animals (CCSEA) and Institutional Animal Ethics Committee (IAEC), proposal number (Vedic/CCSEA/2025/06).

Preparation of Dosage Form:

The suspension of extract was prepared by Triturating the accurately weighed quantity of the extract with 1% Tween 80 in a glass mortar, with gradual addition of distilled water, to make up the required volume and to prepare the clear solution. Ranitidine was diluted with 1 % Tween 80. [9]

Administration of Doses:

The test substances are administered in a single dose by gavage using a stomach tube. Animals should be fasted prior to dosing with the rat, food but not water should be with held overnight, following the period of fasting, the animals should be weighed and the test substances are administered. After the substances had been administered, Food with held for a further 3-4 hours in rats. Where the dose is administered in fractions over a period. [10]

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Evaluation of Antiulcer activity:

Aspirin-induced ulcer model:

Non steroidal anti-inflammatory drugs (NSAIDs) like aspirin, cause gastric mucosal damage by decreasing the prostaglandin levels through inhibition of PG synthesis. In aspirin-induced ulcer model, Wistar albino rats were divided according to groups of three animals each. Animals were fasted 36 h before administration of aspirin.

Groupings of animals:

Group I Normal Saline 2ml/kg

Group II: 20mg/kg Ranitidine

Group III: 175mg/kg Aqueous extract of *Annona squamosa L*

Group IV: 250mg/kg Aqueous extract of *Annona squamosa L.*

All treatments were administered 1 h before administration of aspirin. Ulcers were induced by the administration of aspirin orally at the dose of 200 mg/kg. Animals were sacrificed by cervical dislocation after 6 h of administration of aspirin, and ulcer scoring was done counting the gastric lesion after opening the stomach along the greater curvature. [11]

Determination of Free acidity and Total acidity:

After dissection of the stomach, the contents of the stomach were drained into A graduated centrifuge tube. The gastric contents were then centrifuged at 1000 rpm for 10 mins. 1 ml of the supernatant liquid was then pipette out and diluted to10ml with distilled water. A volume of 2ml diluted gastric juice was titrated with 0.1 N NaOH run from a micro burette using 3-4 drops of Topfer‟s reagent as indicator until Canary yellow color was observed. Volume of NaOH required was noted.

This corresponds to Free Acidity .Further 2-3 drops of phenolphthalein were added and titrated with NaOH until pink color was restored which corresponded to Total Acidity. Free Acidity and Total Acidity is expressed in terms of 0.1 N HCl per 100 gm of gastric contents. This is the same as mEq/lit. To obtain this figure multiply the Burette reading obtained from titration by 10. [12]

Determination of Ulcer Severity and Ulcer Index

The Ulcer Index was calculated and the Ulcer Severity graded as mentioned below: -

Ulcer Severity

0 –No ulcer,

1 –Superficial ulcer

2 –Deep ulcer

3 – Perforation.

Ulcer Index

Ulcer Index was calculated using following formula: Ulcer index=10/ X

Where,

$$X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

5.10 Histopathological Evaluation:

The gastric tissue samples were fixed in neutral buffered formalin solution for duration of 24 hrs. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity of aqueous extract of ***Annona squamosa leaves***. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined

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microscopically for Patho morphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes.[13]

squamosa was found to be dark brown in colour and the consistency was found to be sticky. The extracts were subjected to phytochemical screening for the presence of type of phyto-constituents. [14]

Results:

Preliminary Phytochemical Evaluation:

The colour of the Aqueous extract of ***Annona***

Phytoconstituents	Aqueous Extracts
Carbohydrates	+
Glycosides	-
Alkaloids	+
Phytosteroids	-
Flavonoids	++
Proteinandaminoacids	-
Saponin	-
Phenols&tannins	+
AnthaquinoneGlycosides	-
Triterpenoids	+

(-) Represents Absence; (+) Represents Presence.

Acute Toxicity Study:
Acute Toxicity study was performed on rats. The extracts were administered Orally at a dose of 1500 mg/kg body weight. They were then observed for signs of Toxicity, continuously for 2 hours, and for mortality up to 24 hours after oral administration. [15]

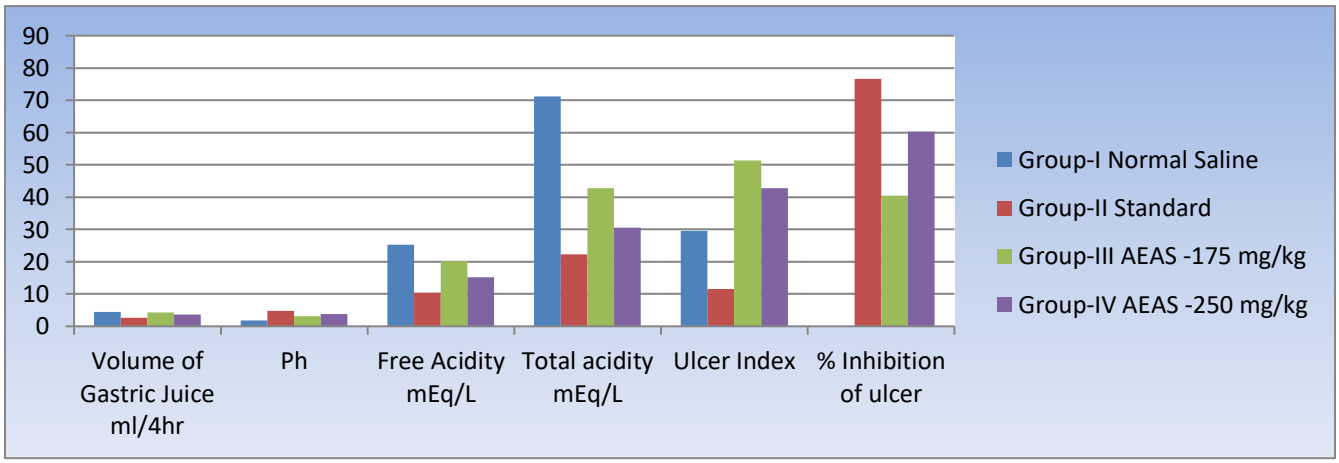
Screening of Anti Ulcer Activity:

Aspirin induced Antiulcer activity:

Groups	Dose	Volume of gastric juice ml/4hr	Ph	Free acidity mEq/L	Total acidity mEq/L	Ulcer index	% Inhibition of ulcer
Group –I Normal saline	2 mg/kg	4.48 ± 0.117	1.74 ±0.2	5.34 ±0.08	71.16 ±0.20	29.6 ±1.5	------

Group –II Standard (Ranitidine)	20 mg/kg	2.68 ± 0.18**	4.86 ±0.1*	10.42±0.02**	22.24 ±0.1*	11.6 ±0.8***	76.61***
Group –III Aqueous extract of *Annona Squamosa L.*	175 mg/kg	3.98 ± 0.098***	3.61±0.14***	20.18±0.05**	51.41±0.38***	20.88 ±1.5*	60.31*
Group –IV Aqueous extract of *Annona Squamosa L.*	250 mg/kg	3.18 ± 0.075***	3.96 ±0.1*	14.21 ±0.04*	38.71 ±0.38**	15.77 ±1.2**	68.25**

Values were mean ± SEM, (n=3), *P<0.05, **P<0.01 Vs control. Data were analyzedby using One-way ANOVA followed by Dunnett's test



Graph: Graphical presentation of the results of anti-ulcer activity against aspirin-induced gastric ulcer model.

In the aspirin induced ulcer model, oral administration of AEAS at two distinct dosages (175 mg/kg and 250 mg/kg) resulted in a substantial reduction in ulcer index, stomach volume, free acidity, and total acidity when compared to the control group. Oral administration of aqueous extract of *Annona Squamosa* at doses of 175 and 250 mg/kg exhibited dose dependent inhibition percentage of 60.31 and 68.25 respectively compared to the ulcer control, provingth anti-ulcer activity. The standard drug ranitidine (20mg/kg) has shown 76.61 percentage inhibition of ulcer when compared with ulcer control. *Aspirin*is acyclooxygenase inhibitor which suppresses gastro duodenal bicarbonate secretion reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow in animals. [16]

Histopathological of stomach:

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The histopathological images of stomach of Control group, Standard drug group, aqueous extract extract of *Annona squamosa* can be illustrated as follow:

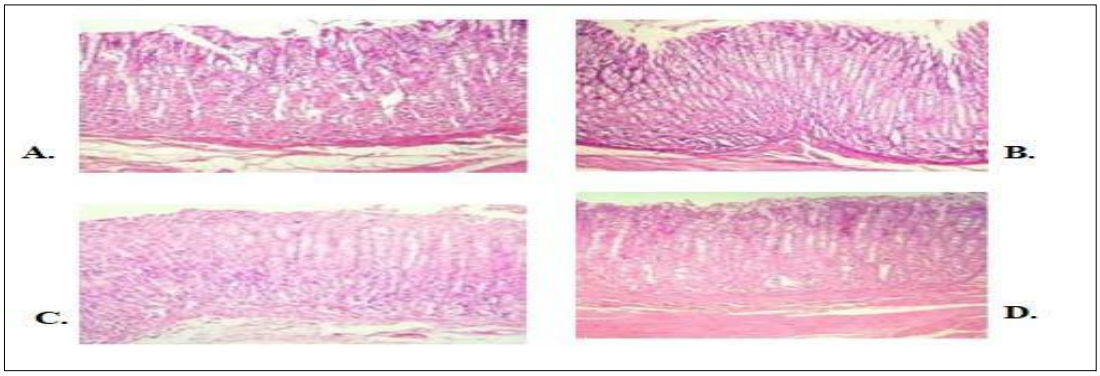

Fig.15: Histopathological study of stomach

The Histopathology Examination reports demonstrate a prominent damage, loss of mucus and chief cells as well as marked infiltration of the leucocytes to the stomach surface of the rats in group-I treated only with aspirin. In Group-II, the standard group showed no damage to the gastric mucosa. The histopathological sections of group- III, treated with aqueous extract of *A. squamosa* shown a reduction in the ulcer focus and a hyperplastic gastric mucosa with regenerating mucosal epithelium. The section of IV- group rats shown mucosal erosion and ulceration to the stomach surface illustrating a less protection to the mucosa and the gastric epithelium. [17]

Conclusion:

In our study, the AEAS significantly reduces the ulcer formation in models by forming a mucosal protecting layer on the epithelial surface of stomach which reduces gastric juice volume, free acidity, total acidity, total acid output, ulcer score and ulcer index when compared to control group of animals. This implicates that the AEAS suppress gastric acid secretion in stomach by aggressive factors. The results of the present investigation clearly demonstrate that *Annona Squamosa* possesses significant anti-ulcer activity against aspirin-induced gastric ulcers. The gastro protective effect may be attributed to the presence of phytoconstituents such as flavonoids and phenolic compounds, which exert antioxidant, cytoprotective, and anti-secretory effects.

The ability of *Annona Squamosa* extract to reduce gastric lesions and improve mucosal defense suggests its potential as a natural anti-ulcer agent. These findings support the traditional use of the plant in gastrointestinal disorders.

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