J. Dhaka National Med. Coll. Hos. 2018; 24 (01): 33-37

Original Article

Comparative gastroprotective effect of Annona squamosa (atafol) leaf aqueous extract with omeprazole on ethanol induced gastric ulcer in Rats

Farzana Ahmed[1], Naoshadul Islam[2], Nafisa Mustafa[3], Shusmita Saha[4], Md. Khorshed Alam[5]

[1]Lecturer, Dept. of Pharmacology, Dhaka National Medical College, [2]Medical Officer, Dept. of Oncology, Delta Hospital Limited, [3]Lecturer, Dept. of Pharmacology, Dhaka National Medical College, [4]Associate Professor, Dept. of Pharmacology, Dhaka National Medical College, [5]Professor, Dept. of Pharmacology, Dhaka National Medical College.

Abstract

Background: Annona squamosa is a fruit tree with long history of traditional use. Leaves of Annona squamosa have been reported to have gastroprotective effect.

Objectives: The present work was conducted to evaluate the gastroprotective effect of Annona squamosa leaf on ethanol induced gastric ulcer in comparison to omeprazole.

Study Design: To observe the gastroprotective effect of Annona squamosa leaf on ethanol induced gastric ulcer in rats an experimental study was done.

Place and period of study: The study was conducted during July 2015 to June 2016 in the Department of Pharmacology & Therapeutics, Dhaka Medical College, Dhaka.

Materials and Methods: A total number of 30 healthy Long Evan rats were used in this study. The experiment was divided into 2 parts: Experiment I and Experiment II. Experiment I was comprised of 12 rats: Group-A and Group-B having 6 rats in each group. Ulcer was produced by administration of absolute ethanol (1 mg/kg body wt.) in Group-B where Group-A served as control group and was provided with distilled water (5 ml/kg body wt.). All the rats were sacrificed after 60 minutes of ethanol administration, to confirm gastric ulcer by histopathology. Experiment II was comprised of 18 rats: Group-C, Group-D and Group-E having 6 rats in each group. Group-C served as disease control group and provided with distilled water (5 ml/kg body wt.). Group-D was provided with aqueous extract of Annona squamosa (500 mg/kg body wt.) and Group-E was provided with omeprazole suspension (10 mg/kg body wt.). After 10 days of treatment, animals were fasted for 24 hours. Then 1 ml of absolute ethanol (1 mg/kg body wt.) was administered and after 60 minutes all rats were sacrificed. Gross and microscopic examinations were performed to evaluate the results.

Results: The mean ± SD of Lesion Index in Group C, Group D and Group E were 37.71 ±7.47 mm, 6.16 ±1.42 mm and 3.33 ±0.64 mm respectively. Statistically highly significant of difference was observed between Group C and Group D (P value < 0.001), between Group C and Group E (P value <0.001) and between Group D and Group E (P value < 0.05). It was observed that Absolute ethanol caused marked gastric damage in negative control group which was prevented in omeprazole suspension and aqueous extract of Annona squamosa treated groups significantly. The protective effect was maximum with omeprazole followed by aqueous extract of Annona squamosa (500 mg/kg body wt.). Aqueous extract of Annona squamosa showed significant protection against ethanol induced gastric ulcer in rats as compared to omeprazole.

Conclusion: In conclusion this study establishes that Annona squamosa (Atafol) extract has gastro-protective ability following consumption of ethanol, in comparison to omeprazole.

Keywords: Annona squamosa; omeprazole; ethanol; gastric ulcer.

Introduction

Ulcer is defined as the erosion in the lining of the stomach or duodenum and is caused by the disruptions of the gastric mucosal defense and repair systems. Ulcer in the stomach is called gastric ulcer and in the duodenum is called duodenal ulcer and together peptic ulcer. In clinical practice, peptic ulcer is one of the most prevalent gastrointestinal disorders, commonly occurs in developed countries.[1] Upper gastrointestinal integrity is dependent upon the delicate balance between naturally occurring protective factor as mucous or prostaglandins and damaging force as

hydrochloric acid present in the digestive juices. An imbalance causes peptic ulcer formation and destruction of gastrointestinal tract mucosal lining. Ulcer may develop in the oesophagus, stomach, duodenum or other area of the alimentary canal. The ulcer irritates surrounding nerves and causes considerable amount of pain.[2]

The plant Annona squamosa (annonaceae) is commonly called as custard apple in English, sharifa in Hindi. The medicinal properties of Annona squamosa were reported as hypoglycemic, anti-diabetic, anti-tumor and anti-oxidant.[3,4] Hepatoprotective activity of leaves of Annona squamosa was documented.[5] The crushed leaves are sniffed to overcome hysteria and fainting spells. Traditionally the leaves were applied to ulcer and wounds.[6]

Phytochemical screening of the aqueous extract of Annona squamosa leaf revealed the presence of Alkaloids, Flavonoids, Saponins, Tannins.[7]

The possible mechanism of anti-ulcer benefit of aqueous extract of Annona squamosa leaf is due to its oxygen radicals scavenging property by inhibiting lipid peroxidation and by preventing loss of gastric mucous.[8]

This present study was done to evaluate the gastroprotective effect of Annona squamosa (atafol). leaf aqueous extract with omeprazole on ethanol induced gastric ulcer in Rats.

Materials and method

This experimental study was conducted in the department of Pharmacology & Therapeutics, Dhaka Medical College, Dhaka, Bangladesh in collaboration with the Department of Pathology, Dhaka Medical College, Dhaka, Bangladesh from July 2015 to June 2016.

Materials:

1. Aqueous extract of Annona squamosa. 2. Omeprazole powder 3. Distilled water. 4. Absolute ethanol. 5. Normal rat food.

Animals:

A total number of 30 healthy Long Evan Norwegian rats of both sex weighting 150-200 grams were collected from the icddr,b Dhaka for this study.

Living condition of animals: They were kept in animal house of department of Pharmacology, Dhaka Medical College. Rats of different batches of different groups were kept in different metallic cages & they were allowed to feed on standard laboratory diet and to drink ad libitum. The rats were acclimatized for eleven days, at normal temperature and humidity.

Plant material:

The leaf of Annona squamosa were collected from private plantation and identified and authenticated by National herbarium (DACB accession no. 42760), Mirpur, Dhaka.

Preparation of aqueous extract of Annona squamosa leaf:

Preparation of aqueous extract was performed in the Department of Chemistry of Dhaka University, Dhaka. The leaves of Annona squamosa Linn were dried and powdered coarsely. The powder was dissolved in distilled water (200gm/1000ml water) and residue was filtered by using mesh and filter funnel. The filtrate was extracted by rotatory evaporator. 15 gm extract was found. Stock solution of Annona squamosa aqueous extract was prepared by dissolving 500mg of extract into required amount of distilled water to get a solution of 100 mg/ml. Mixtures were left to mix homogenously at room temperature 27°c overnight.

Experiment Design:

The experiment was divided into two parts:

1. Experiment I

2. Experiment II

Experiment I:

This part of experiment was comprised of 12 rats which were divided into 2 groups each having 6 rats. Groups were labeled as Group-A and Group-B. This part of the experiment was carried out to demonstrate the effect of vehicle (distilled water) and ethanol extract to induce gastric ulcer in experimental rats.

Group A: This group was served as a control group and received normal diet and 1 ml of distilled water (5 ml/kg body wt.) for 10 days orally by gastric tube. On 11th day, all rats were sacrificed and prepared for dissection.

Group B: Gastric damage experimental group was received normal diet and 1 ml distilled water (5 ml/kg body wt.) for 10 days and after 24 hour fasting 1 ml of absolute ethanol orally by gastric tube. After 24 hour fasting, on 11th day, 1 ml absolute ethanol was orally administered to all groups by gastric tube. After 60 minutes of ethanol administration, on 11th day all rats were sacrificed and prepared for dissection.

Experiment II:

This part of experiment was comprised of 18 rats which were divided into 3 groups. Each having 6 rats. 3 Groups were labeled as Group C, Group D & Group E. This part of the experiment was carried out to demonstrate the effect of pretreatment with aqueous extract of Annona squamosa and omeprazole on ethanol induced gastric ulcer in experimental rats.

Group C: This group was served as control group and provided with normal diet & distilled water (5ml/kg body wt.) orally by gastric tube for 10 days.

Group D: This group was served with aqueous extract of Annona squamosa (500 mg/kg body wt.) orally by gastric tube for 10 days.

Group E: This group was served with omeprazole suspension (10 mg/kg body wt.) orally by gastric tube for 10 days.

After 24 hour fasting, on 11th day, 1 ml absolute ethanol was orally administered to all groups by gastric tube. After 60 minutes of ethanol administration, all rats were sacrificed on 11th day and prepared for dissection.

Morphological parameters:

1. Number of lesions per rat in each group. 2. Individual lesion length in millimeter for each group. 3. Mean ulcer index (sum of length of the all lesions in each stomach) in millimeter for each group.

Ulcer Index

Stomach was cut along the greater curvature ,washed and placed on a card board and Ulcer Index was counted from glandular portion. Each lesion was measured along the greatest length and evaluated singly according to their dimensions and severity.

The maximum length is measured and mean ulcer index per rat in each group was calculated and used in lesion index for each group.The ulcer index or lesion index (UI) was determined as the sum of length of all gastric lesion in mm for each stomach.

Statistical Analysis:

All relevant information for each rat was recorded in a predesigned data collection sheet. Collected data was tabulated and statistical analysis was done by appropriate significant test (Unpaired student's 't' test). Statistical significance was considered at $p<0.05$, $p<0.01$ and $p<0.001$.

Results

Table I Effect of distilled water (Group A) and absolute ethanol (Group B)on mean number of lesions, mean lesions length and Mean lesion index on Group A and Group B.

Parameters	Group A	Group B
Mean number of lesions (±SD)	0	4.83±0.75
Mean lesion length (±SD)	0	6.52±3.80
Mean lesion index (±SD)	0	31.2±9.61

Statistically highly significant of difference were observed between the groups (P value < 0.001)

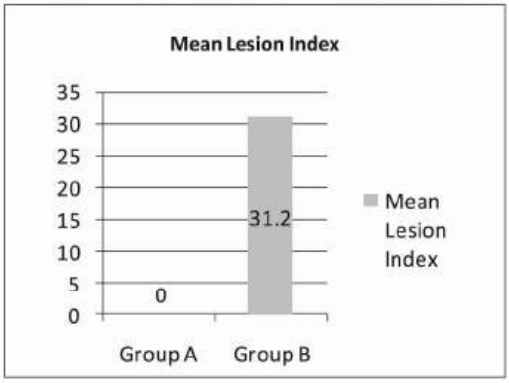

Figure 1 showing the effect of Distilled water and Ethanol on Mean Lesion Index respectively in Group A and GroupB.

Table II Effect of absolute ethanol (group C), aqueous extract of Annona squamosa 500mg (Group D) and Omeprazole suspension (Group E) on mean number of lesions, mean lesions length and Mean lesion index on Group A and Group B.

Paeters	Group C	Group D	Group E
Mean number of lesions (±SD)	5.5 ±1.04	3.16±0.75	2.83 ± 0.98
Mean lesion length (±SD)	8.05 ± 2.54	2.38 ±0.93	1.91 ±0.70
Mean lesion index (±SD)	37.71 ± 7.47	6.16 ±1.42	3.33 ± 0.64

Statistically highly significant of difference were observed between the groups (P value < 0.001)

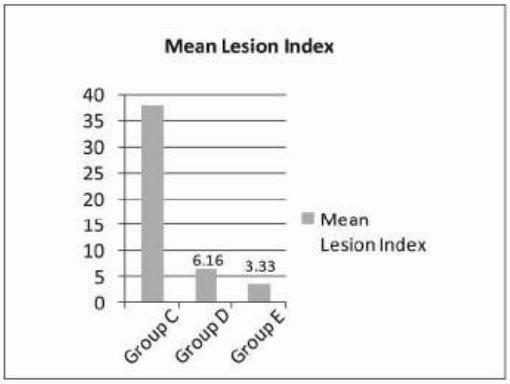

Figure 2 showing the effect of Ethanol , Aqueous extract of Annona squamosa leaf 500 mg and Omeprazole suspension 10 mg on Mean Lesion Index respectively in GroupC, GroupD, Group E.

Comparing Group-D with Group-C (control) There was a significant reduction in number of ulcers, ulcer length, ulcer index seen in Group-D which was pre-treated with, Aqueous extract of Annona squamosa leaf. Results were highly significant ($p<0.001$). Thus, aqueous extract of Annona squamosa leaf showed to have a significant gastro-protective effect in rats.

Comparing Group-D with Group-E

Reduction of number of ulcers, ulcer length and ulcer index was not significant between the groups ($p>0.05$). Thus, Annona squamosa leaf showed to have equal efficacy as omeprazole in gastro-protection in experimental rats.

Discussion

The present study was carried out to evaluate the gastro protective effect of aqueous extract of Annona squamosa leaf (Atafol) on ethanol induced gastric lesion in rats. For this purpose, effect of aqueous extract of Annona squamosa were demonstrated both in normal, untreated and ethanol induced gastric damage in rats.

In the present study, Absolute ethanol was used as agent to induce stomach ulcer in rats. The dose and routes of administration was selected according to Mequanente et al. (2006) and Mahmood et al. (2005).[9,10] Ethanol induced gastric ulcers are commonly used for evaluation of anti-ulcer activity. Absolute ethanol penetrates the gastric mucosa very quickly, which explains why 30 minutes was sufficient for developing gastric lesions in rats. Mequanente et al. (2006) and Mahmood et al. (2005) also in their study showed that acute per oral administration of absolute ethanol (5.0 ml/kg) to fasted rats produced extensive necrosis to gastric mucosa.[9,10] Mahmood and kuppusamy et al. (2005) in another study showed that acute per oral administration of absolute ethanol (5.0 ml/kg) to fasted rats produced extensive hemorrhagic lesions of gastric mucosa and pretreatment with per oral administration of honey alone or honey in combination with O. bacilicum oil extract or honey in combination with cimetidine orally 30 minutes before administration of absolute ethanol could effectively and dose dependently prevent the formation of such lesion.[10]

The control group (Group A) had no lesion in the stomach. In group B the total number of lesions was 29, So in Group B administration of absolute ethanol produces a highly significant ($p<.001$) change in gastric damage parameters. Present study findings reconfirm the previously demonstrated studies that absolute ethanol produces gastric damage. All the rats are pretreated for 10 days according to Jothi et al (2012) study.[8]

In the current study, omeprazole suspension than aqueous extract of Annona squamosa 500 mg/kg body significantly reduced the mean number of gastric ulcer, mean length of lesion, mean breadth of lesion, mean area of lesion, ulcer index, caused by ethanol. The total number of lesion and area of lesion seems to be more prominent in aqueous extract of Annona squamosa in 500 mg/kg body wt. dose than omeprazole provided group. Jothi et al study similiarly showed that, pretreatment with aqueous extract of Annona squamosa caused significant protective effect against ethanol induced gastric lesion. Mean number of lesions, mean lesion length, mean lesion breadth, mean lesion area, lesion index and was maximum in group C and minimum in group E and more in group D than group C. It showed that aqueous extract of Annona squamosa and omeprazole has gastroprotective effect. It was similar to Palanisamy et al. (2012) study.[11]

All treatments showed statistical significance of difference when compared to ulcer control. Rats pre-treated with aqueous extract of Annona squamosa followed by rats pre-treated with omeprazole. Larsson et al. (1983) in a study stated that omeprazole was found to interfere directly with the gastric proton pump when acting in vivo in rats. Present study findings correlates well with previous study.[12]

Conclusion

The observation and result of this study provide a rationale for use of Annona squamosa in the development of a new drug, much needed to reduce or prevent the severity of peptic ulcer. In conclusion this study establishes that Annona squamosa (Atafol) extract has gastro-protective ability following consumption of ethanol. Further experiments are however required to better understand the gastro-protective mechanism of aqueous extract of Annona squamosa (Atafol) following ethanol induced gastric lesion

References

1. Marslin G, Vithalrao KP, Franklin G, Kalaichelavan V. Anti-Ulcer (Ulcer-Preventive) Activity of Ficus arnottiana Miq. (Moraceae) Leaf Methanolic Extract. Am J of Pharmacol and Toxicol 2009; 4 (3): 89-93.
2. Jamal A, Siddiqui A,Tajuddin, Jafri MA. A review on gastric ulcer remedies used in Unani system of medicine. Nat Prod Rad 2006; 5(2) : 153-59.
3. Shirwaikar A, Rajendran K, Kumar CD. In vitro antioxidant studies of Annona squamosa Linn. leaves. Indian J Exp Biol 2004;42: 803-7.
4. Kaleem M, Asif M, Ahmed QU, Bano B. Antidiabetic

and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats. Singapore Med J Aug 2006; 47(8):670-5.

5. Saleem TM, Christina AM, Chidambaranathan N, Ravi V, Gauthaman K. Hepatoprotective activity of Annona squamosa Linn. on experimental animal model. International Journal of Applied Research in Natural Products. Sep 2008; 15;1(3):1-7.
6. Pandey N, and Barve D. Antioxidant Activity of Ethanolic Extract of Annona squamosa Linn Bark. Int. J. Res. Pharm. Biomed. Sci.,2011; 2(4): 1692-1697.
7. Saleem TS, Pradeep Kumar R, Priyanka N. Antiulcerogenic effect of aqueous extract of Annona squamosa Linn. Int. J. Res. Phytochem. Pharmacol 2012 ; 2(3): 157-159.
8. Jothi G, Radhika J, Palani M, Ganesh kumar K. Protective effect of Annona squamosa Linn. Leaf extract on HCL-ethanol induced gastric ulcer in Albino rats. Int J Pharm Pharm Sci 2012; 4: 83-85.
9. Mequanente S, Makonnen E, Debella A. Gastric acid Antisecretory and acid neutralization effect of Aqueous Triogonella feonum-gracum and Linum ussitatissimum Seed extracts on experimental models. Pharmacology online. 2006;3:192-200.
10. Mahmood AA, Sidik K, Salmah I. Antiulcer and gastro protective effects of honey in combination with trigonella foenum graecum seeds extraction experimental gastric ulcer in rats. International journal of molecular Medicine and Advance sciences. 2005; 1(3): 225-229.
11. Palanisamy A, Rajesh V,Perumal P. Antiulcer activity of ethanolic extract of Annona Squamosa leaves. IJPRD, 2011; vol 4(1) : 162-167.
12. Larsson H, Carlsson E, Junggren U, Olbe L, Inhibition of gastric acid secretion by omeprazole in dog and rat. Gastroenterology. 1983;85: 900-907.