Original Article

Efficacy of Annona Squamosa (atafol) Leaf Aqueous Extract on Ethanol Induced Gastric Ulcer in Rats

Ahmed F[1], Shaha KC[2], Islam N[3], Mustafa N[4], Haque S[5], Ajmery S[6]

1. **Dr. Farzana Ahmed, Assistant Professor, Department of Pharmacology, Dhaka National Medical College*
2. *Dr. Kartick Chanda Shaha, Assistant Professor, Department of Pharmacology, Dhaka National Medical College*
3. *Dr. Naoshadul Islam, Senior Medical Officer, Department of Oncology, Delta Hospital Limited*
4. *Dr. Nafisa Mustafa, Assistant Professor, Department of Pharmacology, Dhaka National Medical College*
5. *Dr. Sania Haque, Assistant Professor, Department of Pharmacology, Aichi Medical College*
6. *Dr. Sunny Ajmery, Associate Professor, Department of Pharmacology, Jahurul Islam Medical College*

**** For Correspondence***

Abstract

Objective: *The aim of the present study was to assess the efficacy of Annona squamosa (atafol) leaf aqueous extract on ethanol induced gastric ulcer in Rats.*

Methods: *An Experimental study was conducted in the Department of Pharmacology of Dhaka Medical College, Dhaka from July 2015 to June 2016. A total number of 12 healthy Long Evan Norwegian rats of both sex weighting 150-200 grams were collected from the ICDDR, b Dhaka for this study. They were kept in animal house of department of Pharmacology, Dhaka Medical College. The leaf of Annona squamosa were collected from private plantation and identified and authenticated by National herbarium (DACB accession no. 42760), Mirpur, Dhaka. Experiment was comprised of 12 rats: Group-A and Group-B. Group-A served as disease control group and provided with distilled water (5 ml/kg body wt.). Group-B was provided with aqueous extract of Annona squamosa (500 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. Absolute ethanol caused marked gastric damage in negative control group which was prevented in aqueous extract of annona squamosa treated groups significantly. The protective effect was maximum with 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.*

Results: *Table 1 showed that the control group (Group A) of experiment had 33 lesions in the stomach. Total number of lesions in Group B were 22. The mean (± SD) number of lesion in Group A and Group B were respectively 5.5 ± 1.04 and 3.16 ±0.75. Table 2 showed that the mean lesion length (Mean ± SD) in Group A and Group B were 8.05 ± 2.54 mm and 2.38 ± 0.93 mm. Table 3 showed that the lesions breadth (Mean±SD) in Group – A and Group – B were 2.61±0.52 mm and 0.63 ± 0.21 mm. Table 4 showed that the mean lesion area (Mean ±SD) in Group A and Group B were 21.93 ± 9.97 mm2and 1.51 ± 0.74 mm2. Table 5 showed that the mean lesions index in Group A and Group B were 37.71 ±7.47 mm, and 6.16 ± 1.42mm.*

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.*

Keywords: *Annona squamosa, peptic ulcer, Ethanol.*

Introduction

Peptic ulcer is defined as disruption of the mucosal integrity of stomach and/or duodenum leading to a local defect or excavation due to active inflammation. The word 'peptic' refers to pepsin, a stomach enzyme that breakdown protein. Peptic ulcer located in stomach is called gastric ulcer[1]. It is the most common disease of upper GIT system. It affects 1 in 10 persons during lifetime. Peptic ulcers both affect men and women. 5 to 10% of people worldwide suffer from a peptic ulcer at least once in their lifetime. Duodenal ulcer is more common than gastric ulcer and usually occurs in people aged fewer than 50. Gastric ulcers are more common in people aged over 50 year[2]. There is, thus a need to search for natural alternatives having anti-ulcer properties. This has been the basis for the development of new anti-ulcer agent which includes herbal substances. 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]. Gastro protective activity of leaves of Annona squamosa was documented[4]. The crushed leaves are sniffed to overcome hysteria and fainting spells. Traditionally the leaves were applied to ulcer and wounds[5]. Annona squamosa is a woody, semi deciduous tree grown throughout Bangladesh in terrain with shallow and well drained soils. A bark decoction of this plant is used to prevent diarrhea, while the root is used in the treatment of dysentery. The fruits of Annona squamosa are hematinic, sedative and expectorant. The bark and leaves contain annonaine, an alkaloid which is found to possess many of this properties[6]. From the bark of Annona squamosa, a bioactive acetogenin with anti-cancer activity have been isolated (Hopp et al. 1998).flavonoids from leaves[7], aporphine alkaloids[8], glycoside and squamoline were isolated from this plant[9]. The present study will evaluate the effectiveness of Annona squamosa leaf aqueous extract in preventing formation of gastric ulcer experimentally by ethanol in rats.

Materials & Methods

An Experimental study was conducted in the Department of Pharmacology of Dhaka Medical College, Dhaka from July 2015 to June 2016. A total number of 12 healthy Long Evan Norwegian rats of both sex weighting 150-200 grams were collected from the ICDDR, b Dhaka for this study. They were kept in animal house of department of Pharmacology, Dhaka Medical College. The leaf of Annona squamosa were collected from private plantation and identified and authenticated by National herbarium (DACB accession no. 42760), Mirpur, Dhaka. Experiment was comprised of 12 rats: Group-A and Group-B. Group-A served as disease control group and provided with distilled water (5 ml/kg body wt.). Group-B was provided with aqueous extract of Annona squamosa (500 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. Absolute ethanol caused marked gastric damage in negative control group which was prevented in aqueous extract of annona squamosa treated groups significantly. The protective effect was maximum with 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.

Results

Table I showed that the control group (Group A) of experiment had 33 lesions in the stomach. Total number of lesions in Group B were 22. The mean (± SD) number of lesion in Group A and Group B were respectively 5.5 ± 1.04 and 3.16 ±0.75.

Table I: Showing the effect of pretreatment of aqueous extract of A. squamosa on total number of lesions and mean number of lesions in ethanol treated rats in each group

Groups	**Total number of Rats**	**Number of lesions**	**lesions in each group**
Group A	6	33	5.5 ±1.04
Group B	6	19	3.16 ±0.75

Table II showed that the mean lesion length (Mean ± SD) in Group A and Group B were 8.05 ± 2.54 mm and 2.38 ± 0.93 mm.

Table II: showing the effect of pretreatment of aqueous extract of A. squamosa on mean lesions length in ethanol treated rats in each group

Groups	Number of Rats	Mean lesion length (±SD)in mm
Group A	6	8.05 ± 2.54 mm
Group B	6	2.38 ± 0.93 mm

Table III showed that the lesions breadth (Mean±SD) in Group – A Group – B were 2.61±0.52 mm and 0.63 ± 0.21 mm.

Table III: Showing the effect of pretreatment of aqueous extract of A. squamosa on lesions breadth in ethanol treated rats in each group

Groups	Number of Rats	Mean lesion breadth (±SD)in mm
Group A	6	2.61±0.52 mm
Group B	6	0.63 ± 0.21 mm.

Table IV showed that the mean lesion area (Mean ±SD) in Group A and Group B were 21.93 ± 9.97 mm2and 1.51 ± 0.74 mm2.

Table IV: Showing the effect of pretreatment of aqueous extracts of A. squamosa on mean lesions area in ethanol treated rats in each group

Groups	Number of Rats	Mean Lesion area (±SD in mm^2)
Group A	6	21.93 ± 9.97 mm^2
Group B	6	1.51 ± 0.74 mm^2

Table V showed that the mean lesions index in Group A and Group B were 37.71 ±7.47 mm, and 6.16 ± 1.42mm.

Table V: Showing the effect of pretreatment of aqueous extract of A. squamosa on mean lesion index in ethanol treated rats in each group

Groups	Number of Rats	Mean Lesion index
Group A	6	37.71 ±7.47 mm
Group B	6	6.16 ± 1.42mm.

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. 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 Mahmoud et al. (2005)[10,11]. 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. Liu et al. (2002) in a study showed that acute per oral administration of absolute ethanol (5.0 ml/kg) to fasted rats produced extensive necrosis of gastric mucosa and pretreatment with per oral administration of propolis ethanol extract (PEE) could effectively and dose dependently prevent such necrosis[12]. 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[10,11]. Mahmood 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. Effects of aqueous extract of Annona squamosa were demonstrated both in normal, untreated and ethanol induced gastric damage in rats[11]. Mean number of lesions, mean lesion length, mean lesion breadth, mean lesion area, lesion index was more in group A and less

in group B. By calculating the inhibition percentage of gastric lesion, all the treatment was compared to ulcer control to measure the percentage that each treatment inhibited gastric lesion. All treatments showed statistical significance of difference when compared to ulcer control. In our study mean lesion area (Mean ±SD) in Group A and Group B were 21.93 ± 9.97 mm2and 1.51 ± 0.74 mm2. In our study mean lesions index in Group A and Group B were 37.71 ±7.47 mm, and 6.16 ± 1.42mm. This gastro-protective effect is statistical significant when compared to control. Palanisamy et al., (2012) in a study showed that ulcer index which was produced by control group and 50 and 100 mg Annona squamosa extract was 18.71± 0.10, 8.74± 0.08 and 6.47± 0.08 respectively[13]. That showed significant decrease ($p<0.001$) in ulcer index. Results are similar to our study.

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. Valle, J.D. 'Peptic ulcer diseases and related disorders'. Harrison's principles of internal medicine. 2005;16: 1746-62.

2. Gupta, S.K., Singhvi, J.I. "Herbal and hepatoprotective drugs acting on PU & Liver Disease". International journal of pharmacy & technology. 2011;3 (1):824-53.

3. Shirwaikar, A., Rajendran, K. and Kumar, C.D. 'In vitro antioxidant studies of Annona squamosa Linn. Leaves'. Indian journal of experimental biology. 2004;42(8):803-7.

4. Saleem, T.M., Christina, A.M., Chidambaranathan, N., Ravi, V. and Gauthaman, K. 'Hepatoprotective activity of Annona squamosa Linn.on experimental animal model'. International Journal of Applied Research in Natural Products. 2008;1(3):1-7.

5. Pandey, N. and Brave, D. 'Phytochemical and Pharmacological Review on Annona squamosa Linn'. Int J Biomed Pharmaceut Sci. 2011;2(4):1404-12.

6. Vohora, S.B., Kumar, I. and Naqvi, S.A.H. 'Phytochemical, Pharmacological, Antibacterial and anti-ovulatory studies on Annona squamosa'. Plantamedica. 1975;28(05):97-100.

7. Seetharaman, T.R. 'Flavonoids from the leaves of Annona squamosa and Polyalthialongifolia'. Fitoterapia.1986;57(3):198-199.

8. Bhakuni, D.S., Tewari, S. and Dhar, M.M. 'Aporphine alkaloids of Annonasquamosal'. Photochemistry. 1972;11(5):1819-1822.

9. Forgacs, P., Desconclois, J.F., Provost, J., Tiberghien, R. and Touche, A. 'Unnouvelheterosidenitréextraitd'Annonasquamosal'.Phytochemistry.1980;19(6):1251-52.

10. Mequanente, S., Makonnen, E., Asfaw, D.A. 'Gastro protective effect of aqueous extract of trigonellafoenum-gracum and linumussitatissimum seed extracts in mice'.Pharmacologyonline. 2006;2:324-34.

11. Mahmood, A.A., Sidik, K. and Salmah, I. 'Anti—ulcer and Gastro Protective Effects of Honey in Combination with T rigonellafoenumgmecum Seeds Extract on Experimental Gastric Ulcer in Rats'. International journal of molecular medicine and Advance sciences.2005;1 (3):225-9.

12. Liu, C.F., Lin, C.C., Lin, M.H., Lin, Y.S. and Lin, S.C. 'Cytoprotection by propolis ethanol extract of acute absolute ethanol-induced gastric mucosal lesions'. The American journal of Chinese medicine. 2002;30:245-54.

13. Palanisamy, A, Rajesh, V., Perumal, P., Vamsi,B. 'Antiulcer activity of ethanolic extract of Annona squamosa leaves'.2012;4(1):162-7.