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Research Article

EVALUATION OF ANTI-GASTRIC ULCER ACTIVITY OF THE FRUIT EXTRACTS OF TERMINALIA BELLERICA ROXB IN EXPERIMENTAL RODENTS

Shikha Mishra*[1], Ruchi Gupta [2], Arpit Shrivastava[1], Megha Shrivastava[1], Mahak Modi[1]
[1]*Adina Institute of Pharmaceutical Science, NH86A, Lahdara, Sagar, Madhya Pradesh, India*
[2]*Smriti College of Pharmaceutical Education, 4/1 Pipliya Kumar, MR-11, Dewas Naka, Indore, Madhya Pradesh, India*
**Corresponding author: pharmashikha026@gmail.com*

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ABSTRACT
Terminalia bellirica Roxb (*T. bellirica,* Combretaceae), known as Bahera or Beleric or bastard myrobalan is a large deciduous tree common on plains and lower hills in Southeast Asia, where it is also grown as an avenue tree. Glycoside, tannins, gallic acid, ellagic acid, ethyl galate, gallyl glucose, chebulanic acid are the main active phytoconstituents of medicinal importance. These phytoconstituents are responsible for many of the pharmacological roles. Different parts of the tree have various medicinal activities. In the present study, ethyl acetate and ethanolic extract of *T. bellirica* fruits, at the doses of 250 and 500mg/kg was evaluated for the anti-gastric ulcer activity using experimental gastric ulcer models induced by ethanol and aspirin. The phytochemical screening revealed the presence of triterpenoids, saponins, phenolic compounds, tannins, flavonoids, carbohydrate and glycosides. The parameters taken to assess anti-gastric ulcer activity were volume of gastric secretion, pH, free acidity, total acidity, ulcer index and histopathological studies of the stomach epithelium. The results indicate that the ethanolic and ethyl acetate extract of *T. bellirica* fruits produced a dose-dependent reduction of the volume of gastric acid secretion, pH, free acidity, total acidity and ulcer index with respect to standard (Omeprazole 20mg/kg) used in the present study. The findings therefore indicate that ethyl acetate and ethanol extract possess anti-gastric ulcer activities in experimental animals induced by alcohol and aspirin, which might be due to presence of phenolic compound and flavonoids whose presence, was confirmed by phytochemical analysis. Therefore, this study validates its anti-gastric ulcer use in Indian folk medicine. Further investigations on isolation of specific phytochemicals and elucidating mechanisms of action are needed.

Keywords: *Terminalia bellirica,* Anti-gastric ulcer, Phytochemical screening, Omeprazole, Ulcer index, Histopathology.

1. INTRODUCTION
Gastric ulcers are common and serious diseases, which have been a major cause of morbidity and mortality for more than a century [1]. The pathophysiology of gastric ulcer disease is based on an imbalance between aggressive and protective factors in the stomach [2, 3]. The gastric ulcer is a lesion characterized by necrosis, neutrophil infiltration, blood flow reduction, increased oxidative stress and inflammation. It is one of the common diseases of the human gastrointestinal system, affecting approximately 5-10% of the world population, and is difficult to cure completely, thus, relapse occurs often [4, 5]. The mechanism responsible for the development and relapse of a gastric ulcer is so complex that it has not been fully elucidated [6]. It is generally accepted that it results from an imbalance between exogenous damaging agents, such as reactive oxygen species, alcohol consumption, cigarette smoking, *Helicobacter pylori* and the frequent use of nonsteroidal anti-inflammatory drugs (NSAIDs) and the endogenous protective mechanisms of the stomach (the mucosal barrier, mucus secretion, efflux of bicarbonate, increasing antioxidant levels, cell regeneration, prostaglandins, and nitric oxide among others) [7, 8]. Despite the fact that many synthetic drugs are used in the clinic to manage gastric ulcer disease, including proton pump inhibitors, antacids and antihistaminic agents, these treatments are insufficient for complete ulcer healing, thus they are intimately linked to ulcer recurrence [9]. Additionally, the majority of treatments result in several adverse reactions, such as hypersensitivity, arrhythmia, hepatic injury, and

hematopoietic changes, which limit their applications [10, 11]. Therefore, non-toxic, easily accessible and affordable antiulcer medications are urgently needed, thus, research is ongoing in this area [12]. Over recent years, abundant work has been accomplished to develop natural products to potentially provide rich sources of new agents with anti-ulcer activity. It is significant to clarify their prevention or management action against gastric ulcer. A few of plant extracts and plant-derived compounds have been found and proved to be safe, effective, relatively less expensive and globally competitive [13, 14]. *T. bellirica* is a large tree, up to 40 m height is found in deciduous forests throughout the greater part of India, but not in the arid regions, in upper Gangetic plains, Chota Nagpur, Bihar, Orissa, West Bengal, Konkan, Deccan and most of South India [15, 16]. *T. bellirica* contains different chemical constituents in different parts such as stem bark contains arjungenin and its glycosides, belleric acid, bellericosides. Fruits contain hexahydroxydiphenic acid, methyl ester, β-sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, chebulagic acid, mannitol, glucose, galactose and rhamnose [17]. *T. bellirica* bark is mildly diuretic and useful in anaemia and leucoderma. Fruits are anti-inflammatory, antihelmintic, expectorant, antipyretic, antiemetic, useful in asthma and bronchitis, dropsy, dyspepsia, cardiac disorders, skin diseases, leprosy, ulcer. Ripe fruits are used as astringent in combination with chebulic myrobalan (*T. chebula*) and *P. emblica* as the famous Triphala drug of Ayurveda are also useful in eye problems like cataract, glaucoma, progressive myopia, and conjunctivitis [18]. The bark of *T. bellirica* is used as an adulterant to the bark of *T. arjuna*. The deciduous tree *T. bellirica* found in Southeast Asia is extensively used in traditional Indian Ayurvedic medicine for the treatment of hypertension, rheumatism and diabetes [19]. This research was aimed at investigating the anti-gastric ulcer activity and acute oral toxicity from the ethyl acetate and ethanolic extract of *T. bellirica* fruits. This pharmacological activity will enhance the safe applications of *T. bellirica* as a natural pharmaceutical product.

2. MATERIAL AND METHODS

2.1. Plant material

The fruits of *T. bellerica* were purchased in the month of October from the local market of Sagar (M.P.). Fruits were authenticated by Dr. P. K. Tiwari (Bot./Her./ 1487) at the Department of Botany Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.) and preserved in the herbarium of the Department. After collecting of plant material, grinding was done using mechanical grinder was packed in air tight container and stored for phytochemical and biological studies.

2.2. Chemical reagents

Working standards of aspirin and omeprazole were obtained as gift samples from Cipla Pharmacauticals Limited, Mumbai. All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade.

2.3. Defatting of plant material

The shade dried powder of fruits of *T. bellerica* was extracted with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place completely was ensured by taking extract from siphon tube into china dish and evaporating it. Moreover, testing of residue by applying on skin caused no waxy touch.

2.4. Extraction by soxhlet extraction

The defatted marc of *T. bellerica* was exhaustively extracted with different solvent (petroleum ether, chloroform, ethyl acetate, ethanol and distilled water) by soxhlet extraction process. The extract was evaporated above their boiling points. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis [20].

2.5. Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures [21, 22]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

2.6. Animals

In the present investigation, the Wistar Albino rats of the either sex weighing between 150-200 gm (2-3 month old) were used for the anti-gastric ulcer activity. They were kept in the departmental animal house and maintained under standard environment condition (27±2°C and relative humidity 60±5%, light-dark cycle

of 12 hr). They were allowed free access to standard dry pellet diet and water *ad libitum*, under strict hygienic conditions. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. All animal procedure has been approved and prior permission from the Institutional Animals Ethics Committee of Dr. Hari Singh Gour University, Sagar was obtained as per the guideline.

2.7. Acute toxicity study

Preliminary experiments were carried out on rats (n=6). Various extract of fruits of *T. bellerica* were administered orally in different doses to find out the range of doses which cause zero and 100% mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) [23]. Animals were kept fasting providing only water, extract were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of different groups of rats (n=6) and the animals were kept under observation for mortality as well as any physical behaviour changes, any toxicity upto 24 hrs for evaluation of a possible anti-gastric ulcer activity.

2.8. Anti-ulcer activity

2.8.1. Aspirin-induced ulcer

Albino rats of either sex weighing between 150-200 gm were divided into seven groups, each group has six rats. The animals were fasted for 24 hours with free access to water. One Group represented the control group, which received 1% CMC orally. Second group received aspirin. Standard drug Omeprazole (20 mg/kg b. wt.) was administered to third group. Fourth and fifth groups received ethyl acetate extract of *T. bellerica* at 250 and 500 mg/kg respectively and sixth and seventh groups received ethanolic extract of *T. bellerica* 250 and 500 mg/kg respectively. In this model, gastric lesions were induced by aspirin (200mg/kg) administered to rats of group IV-VII after 1hr of respective drug treatment. The animals were anaesthetized after 4 hr of aspirin administration with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored [24, 25].

2.8.2. Ethanol induced ulcer

Ulcer was induced by oral administration of absolute ethanol (99.9%). All the animals were fasted for 24 hours with free access to water before administration of ethanol. The animals were randomly divided into seven groups, each consisting of six rats. One group represented the control group, which received only 1%CMC orally. Second group received absolute ethanol. Standard drug omeprazole (20 mg/kg) was administered to third group. Fourth and fifth groups received ethyl acetate of *T. bellerica* 250 and 500 mg/kg respectively and sixth and seventh groups received ethanolic extract of *T. bellerica* 250 and 500 mg/kg respectively. The gastric ulcers were induced in rats by administrating absolute ethanol (99.9%) at dose of 1ml/kg orally, after 1hr of ethyl acetate, ethanolic extract and omeprazole treatment. The animals were anaesthetized 1h latter with anaesthetic ether and stomach was incised along the greater curvature and ulceration will be scored [26, 27].

2.9. Estimation of parameters

2.9.1. Collection of gastric juice and pH

Gastric content collected from the each group of rats was centrifuged at 2000 rpm for 5 min. and the volume of gastric juice as well as pH of gastric juice was measured. The gastric juice was subjected to determination of other biochemical parameters.

2.9.2. Determination of total acidity

One ml of gastric juice was pipetted out in 50 ml of conical flask and diluted with 1 ml of distilled water. 2 to 3 drops of phenolphthalein indicator was added to flask and titrated with 0.01NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted.

2.9.3. Determination of free acidity

Free acidity was determined by using the Topfer's reagent as an indicator. 2 or 3 drops of Topfer's reagent was added to flask containing gastric juice and titrated with 0.01N Sodium hydroxide until all traces of red colour disappeared and the colour of the solution turned to yellowish orange. The volume of 0.01N NaOH consumed was noted. Acidity was calculated by using the formula [28].

Acidity = Vol. of NaOH × Normality of NaOH × 100 mEq/L/0.1

2.9.4. Histopathological examination

The stomachs were washed with saline solution and collected in small bottles containing 10% formalin solution and the ulcerated gastric tissues were examined under the microscope for histopathological changes such

as inflammation, infiltration, and erosion. Scoring was done as follows: 0 = Normal stomach, 0.5 = Red coloration, 1 = Spot ulcers, 1.5 = Haemorrhagic streaks, 2 = Ulcer > 3 mm but < 5 mm, 3 = ulcers > 5 mm [29]. Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined as follows [30]:

% Protective = Control mean ulcer index-Test mean ulcer index/ Control mean ulcer index ×100

2.10. Statistical analysis

All data were expressed as mean±SEM. The data were analyzed by ANOVA, Dunnett's test to determine differences between the groups. $p<0.05$ and $p<0.001$ were considered as statistically significant.

3. RESULT AND DISCUSSION

The crude extracts so obtained after soxhlet extraction process was concentrated on water bath by evaporating the solvents completely to obtain the actual yield of extract. The yield of extracts obtained from different samples using petroleum ether, chloroform, ethyl acetate, ethanol and distilled water as solvents are depicted in the Table 1. Phytochemical analysis of ethyl acetate and ethanolic extracts of fruits sample of *T. bellirica* showed the presence of triterpenoids, saponins, phenolic compounds, tannins, flavonoids, carbohydrate and glycosides (Table 2).

Table 1: Percentage yield of extract of *T. bellirica*

Solvents	(%) Yield
Petroleum ether	6.2
Chloroform	1.3
Ethyl acetate	3.9
Ethanol	5.8
Aqueous	3.1

Table 2: Phytochemical screening of extracts of *T. bellirica*

S. NO.	Test	Pet. ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
1	**Carbohydrate**					
	Molisch	-ve	-ve	-ve	+ve	+ve
	Fehling's	-ve	-ve	-ve	+ve	+ve
2	**Protein and amino acid**					
	Ninhydrin	-ve	-ve	-ve	-ve	+ve
	Xanthoproteic	-ve	-ve	-ve	-ve	+ve
3	**Steroids**					
	Salkowski	+ve	-ve	-ve	-ve	-ve
	Libermann's Burchard	+ve	-ve	-ve	-ve	-ve
4	**Triterpenoids**					
	Salkowski	-ve	-ve	+ve	+ve	-ve
	Libermann's Burchard	-ve	-ve	+ve	+ve	-ve
5	**Saponins**					
	Foam	-ve	-ve	+ve	+ve	+ve
6	**Phenols**					
	Ferric chloride	-ve	-ve	+ve	+ve	+ve
7	**Tannins**					
	Lead acetate	-ve	-ve	+ve	+ve	+ve
8	**Flavonoid**					
	Alkaline test	-ve	-ve	+ve	+ve	+ve
8	**Glycosides**					
	Borntrager's test	-ve	-ve	-ve	+ve	+ve
	Legal's test	-ve	-ve	-ve	+ve	+ve
9	**Alkaloids**					
	Wagner's	-ve	+ve	-ve	-ve	-ve
	Dragendorff's	-ve	+ve	-ve	-ve	-ve
	Mayer's	-ve	+ve	-ve	-ve	-ve

In aspirin and ethanol induced model, the total acid, output of the gastric juice, free acidity, pH and accumulation of gastric secretary volume were increased. Ethyl acetate extract showed significant % protection 45.32% and 59.38% with the dose of 250 and 500 mg/kg respectively as compared to control in aspirin induced model. In ethanol induced model, % protection of ethyl acetate extract was found to be 46.55% and 60.33% with the dose of 250 and 500 mg/kg, respectively, as compared to control. Ulcer protection of ethanolic extract could be seen only at doses 500 mg/kg in both types of models. Percentage protection of ethanolic extract at 500mg/kg for aspirin and ethanol induced were 48.43% and 53.44%, respectively (Table 3 & 4).

Table 3: Effect of ethyl acetate and ethanolic extract of *T. bellerica* on pH, gastric volume, total acidity and free acidity in aspirin induced ulcers in rats

Treatment Groups	Treatment and Dose	Mean ulcer index ± SEM	% Protection	pH	Gastric Volume (ml/100g)	Total acidity (mEq/L/100g)	Free acidity (mEq/L/ 100g)
Group I	Control (1%CMC)	5.33 ±0.091	-	2.25 ± 0.028	1.94 ± 0.15	46.51 ± 0.98	27.78 ± 0.53
Group II	Aspirin (200mg/kg)	7.00 ±0.632	-	1.6 ±0.085**	2.50 ± 0.09**	68.16 ±0. 40**	41.98 ± 0.50**
Group III	Standard 20mg/kg	1.16 ±0.210**	78.13%	3.86 ± 0.17**	1.21 ± 0.06**	29.62 ± 0.41**	14.41 ± 0.76
Group IV	EA extract (250mg)	2.91 ± 0.490*	45.32%	2.71 ± 0.07*	1.56 ± 0.05*	42.31 ± 0.83**	23.30 ± 0.60**
Group V	EA extract (500 mg)	2.16 ±0.683**	59.38%	3.53 ± 0.10**	1.30 ± 0.07**	36.13 ± 0.56**	18.42 ± 0.32**
Group VI	EHOH extract (250 mg)	3.5 ± 0.50	34.37%	2.43 ± 0.12	1.70 ± 0.04	45.50 ± 0.20	25.88 ± 0.25
Group VII	EHOH extract (500 mg)	2.75 ± 0.512*	48.43%	2.67 ± 0.10*	1.58 ± 0.09*	43.93 ± 0.45*	23.55 ± 0.32**

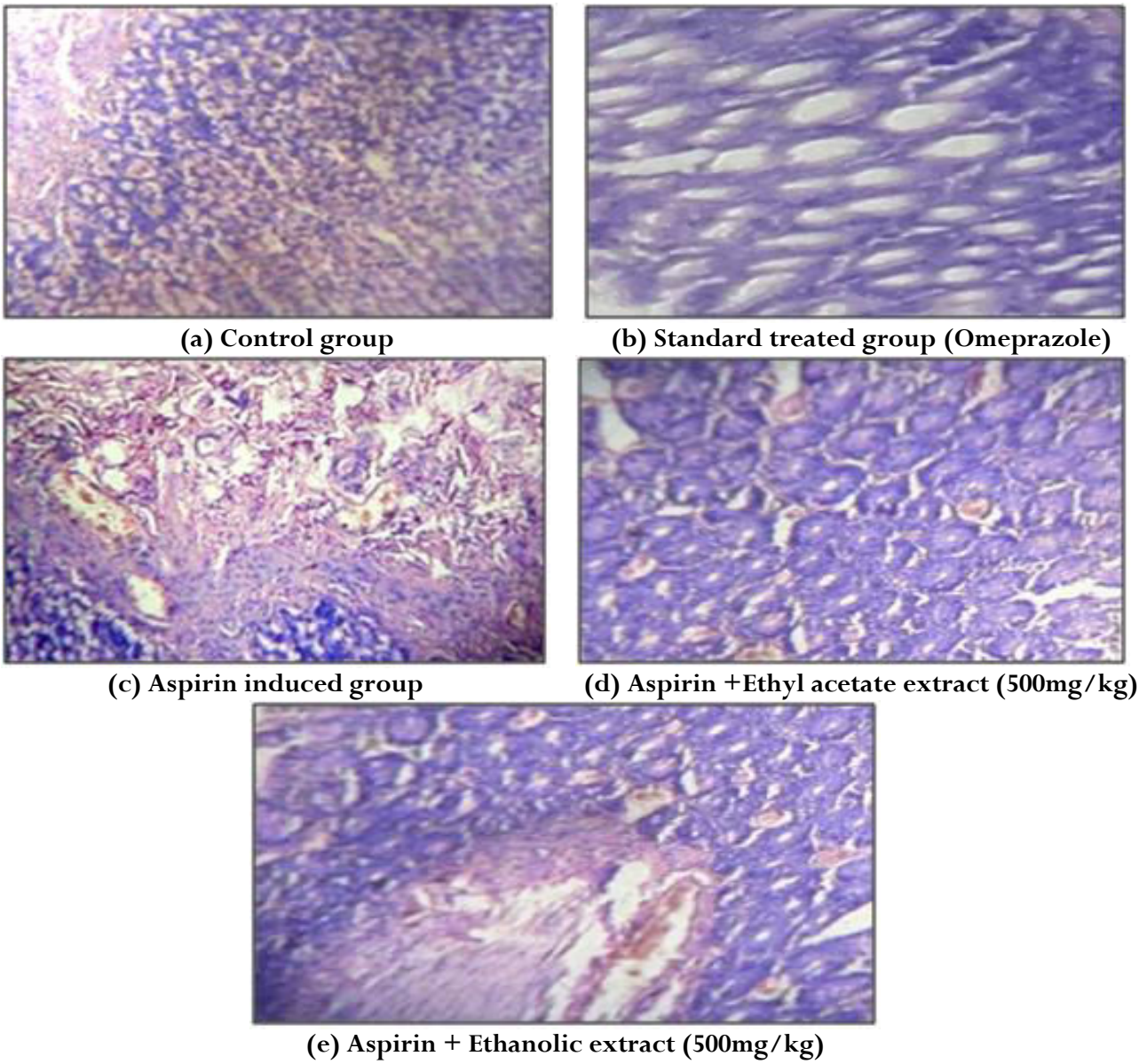
*EA=ethyl acetate extract, EHOH= ethanolic extract, All values are expressed as mean ± S.E.M; n=6 animals in each group. Statistical at *P<0.05 and **P<0.01 compared to control group*

Table 4: Effect of ethyl acetate and ethanolic extract of *T. bellerica* on pH, gastric volume, total acidity and free acidity in ethanol induced ulcers in rats

Groups	Treatment and Dose	Mean ulcer index ± SEM	% Protection	pH	Gastric Volume (1ml/100g)	Total acidity (mEq/L/100g)	Free acidity (mEq/L/ 100g)
Group I	Control (1%CMC)	4.83 ± 0.799	-	2.28 ± 0.04	1.98 ± 0.02	47.13 ± 0.50	26.76 ± 0.49
Group II	Ethanol (1ml/kg)	6.33 ± 0.368	-	1.81 ± 0.05*	2.61 ± 0.07**	69.54 ± 0.48**	42.49 ± 0.52**
Group III	Standard (20mg/kg)	0.91 ±0.200**	81.03%	3.98 ±0.21**	1.36 ± 0.08	30.17 ± 0.29**	15.99 ± 0.22**
Group IV	EA extract (250mg)	2.58 ± 0.401*	46.55%	2.77 ± 0.05*	1.73 ± 0.04*	41.73 ± 0.22**	24.17 ± 0.45**
Group V	EA extract (500 mg)	1.91 ±0.335**	60.33%	3.68 ±0.10**	1.58 ± 0.03**	38.02 ± 0.26**	17.79 ± 0.27**
Group VI	EHOH extract (250 mg)	3.16 ± 0.153	34.47%	2.66 ± 0.16	1.78 ± 0.06	45.85 ± 0.17	25.99 ± 0.20
Group VII	EHOH extract (500 mg)	2.25 ± 0.557**	53.44%	2.80 ± 0.07*	1.76 ± 0.04*	44.07 ± 0.62**	23.82 ± 0.53**

*EA=ethyl acetate extract, EHOH= ethanolic extract, All values are expressed as mean ± S.E.M; n=6 animals in each group. Statistical at *P<0.05 and **P<0.01 compared to control group*

The prepared slides were viewed under light microscope. The observed stomach epithelium was severely eroded with perforation in case of control group of animals. The numbers of ulcer were more in control group as compared to the treated group. The numbers of ulcer and severity of ulceration was less in treated group with ethyl acetate extract of *T. bellerica* as compared to ethanolic extract of *T. bellerica* (Fig. 1 & 2). NSAID's like aspirin causes gastric mucosal damage by decreasing cytoprotective prostaglandin levels through inhibition of prostaglandin synthesis [31], increasing acid secretion and block diffusion of H^+ [32]. Ethanol challenge induces gastric injury due to production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane [33]. Ethanol also increased cell permeability and gastric blood flow, which contributes to the development of the haemorrhage and necrotic aspects of tissue injury [28]. Administration of extract of *T. bellerica* resulted in a significantly reduced pH, gastric volume, total acidity, free acidity and ulcer index in dose dependent manner, compared to control. The ethyl acetate and ethanolic extract exhibited the anti-gastric ulcer activity which might be due to presence phenolic acids and flavonoids confirmed by phytochemical analysis.



(a) Control group **(b) Standard treated group (Omeprazole)**

(c) Aspirin induced group **(d) Aspirin +Ethyl acetate extract (500mg/kg)**

(e) Aspirin + Ethanolic extract (500mg/kg)

Fig. 1: Histopathological examination of stomach epithelium of rats with aspirin-induced gastric ulcers in the control, omeprazole and *T. bellerica* fruits ethyl acetate and ethanol extract treated groups

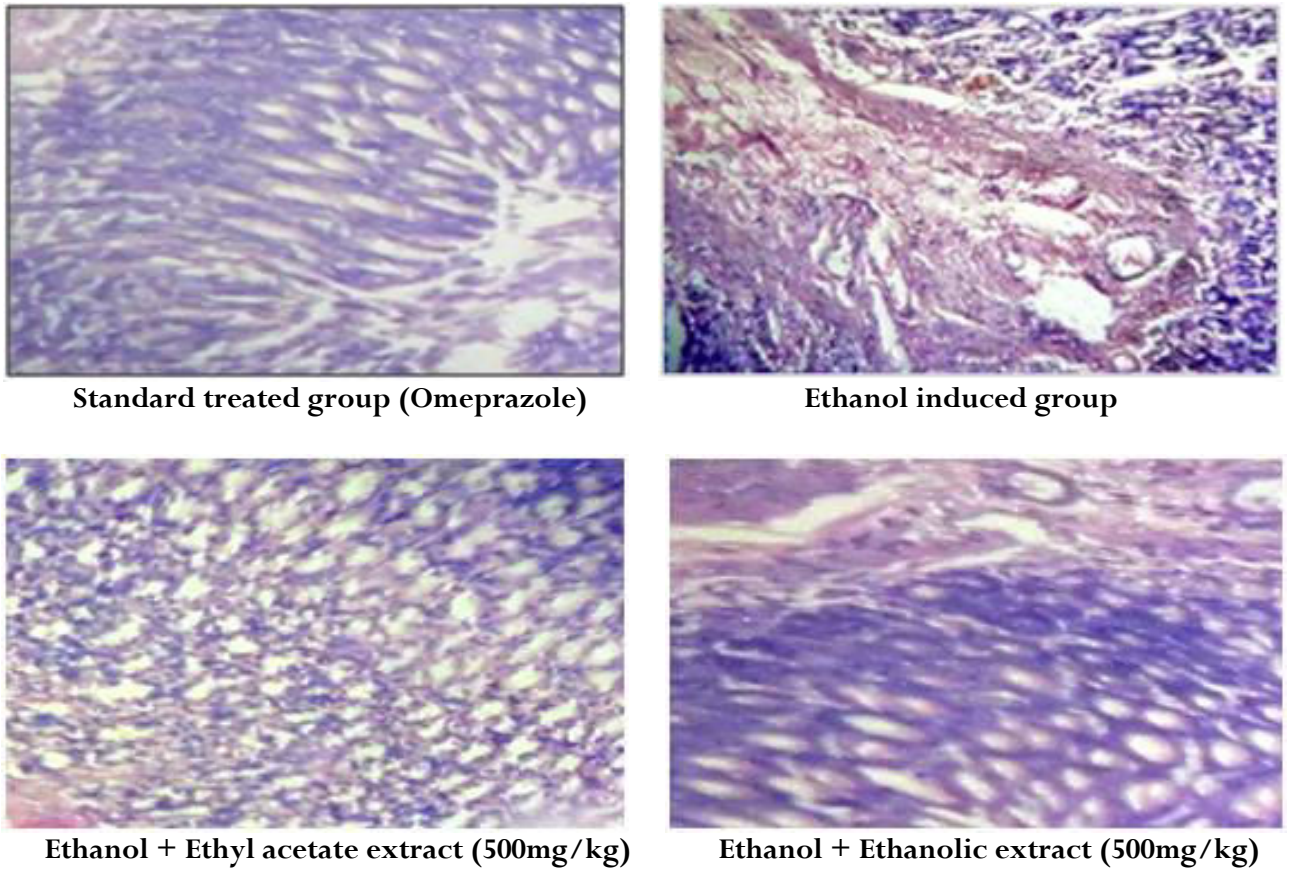


Fig. 2: Histopathological examination of stomach epithelium of rats with alcohol-induced gastric ulcers in the control, omeprazole and *T. bellerica* fruits ethyl acetate and ethanol extract treated groups

4. CONCLUSION

From the results of this study, it is clear that the ethyl acetate and ethanolic extract from *T. bellerica* has significant anti-gastro ulcer activity in aspirin and ethanol induced models and the absence of oral acute toxicity at the doses employed. Its action might involve due to richness of phenolic and flavonoids compound of *T. bellerica*. This interesting observation indicates that the ethyl acetate and ethanolic extract from *T. bellerica* fruits can be a potential source for the treatment of gastric ulcers. The finding of this experimental study could lead to further isolation of specific phytochemicals and elucidating mechanisms of action

Conflict of interest

None declared

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