Kuwait J.Sci., Vol.49, No.(4),October.2022,pp(1-14)

Effect of *Trianthema triquetra* Rottl. ex Willed (Aizoaceae) on ethanol-induced gastric ulcers in experimental rats and assessment of various ulcer parameters

Hira Ijaz[1], Saiqa Ishtiaq[1,*], Faryal Rubab[1], Ans Munir[1], M. Sajid-ur-Rehman[1]

[1]*Punjab University College of Pharmacy, University of the Punjab, Lahore-54000, Punjab, Pakistan*

**Corresponding author: saiqa.pharmacy@pu.edu.pk*

Abstract

Trianthema triquetra Rottl. Ex. Willed (*T. triquetra*) is a medicinal plant that belongs to the family Aizoaceae. The plant has traditionally been used as fodder, a remedy for chronic ulcers, fever, and healing wounds**.** Therefore, the present study was intended to investigate the antiulcer ability of different fractions of *T. triquetra* to verify its folklore use in ulcer cure. Acute oral toxicity of all the fractions of *T. triquetra* was evaluated at 2 g/kg b.wt. The antiulcer potential of n-butanol (TTB), chloroform (TTC), ethyl acetate (TTEA), and aqueous (TTA) fraction of crude methanolic extract of *T. triquetra* was assessed by using ethanol-induced gastric ulcer model in rats. Omeprazole was used as the standard drug at a dose of 20 mg/kg b.wt. After 1 hour of administration of all the fractions of *T. triquetra*, at a dosage of 300 mg/kg b.wt. the gastric ulcer was induced in all animals by administering absolute ethanol (1mL/animal) orally except regular control group. After an hour, all the rats were sacrificed. Ulcer index, % age of ulcer inhibition, gastric pH, gastric volume, total acidity, gastric wall protein, gastric wall mucus, and histopathology of the stomach wall of rats were assessed. All fractions of *T. triquetra* showed a substantial decrease in ulcer index and improvement in percentage inhibition compared to the disease control group. In addition, there was a rise in gastric wall mucus content, total protein content, gastric pH, and a decrease in gastric volume and total acidity. Histopathological studies showed severe mucosal injury, leucocyte infiltration, and edema in the disease control group compared to omeprazole and plant fractions treated animal groups. The present work encourages the conventional use of *T. triquetra* to cure ulcers.

Keywords: Ethanol; gastric ulcer; NSAID's; omeprazole; *Trianthema triquetra.*

1. Introduction

Gastric ulcer (GU) and gastric hyperacidity are essential concerns for global health (Batista *et al*., 2013). These problems occur by a lack of balance between various mucosal defensive and harmful factors. The defensive factors include adequate mucous secretion and blood supply, intact mucous barrier, bicarbonate secretion, prostaglandins, phospholipids, antioxidants, and a sufficient nitric oxide level (NO). On the other hand, the harmful factors include gastric acids, ethanol, decreased blood flow to gastric mucosa, free radicals, misuse of NSAID's, and *Helicobacter pylori*. These factors contribute to gastric mucosal injury and ultimately lead to the development of gastric ulcers (Al-Rejai *et al*., 2012). The avoidance and cure of GU have become a global challenge. Antiulcer

medications which are commercially available have numerous side effects. The typical side effects of proton pump inhibitors (e.g., omeprazole) are diarrhea, nausea, stomach cramping, and constipation. H_2-receptor antagonists (e.g., cimetidine) can also have side effects such as galactorrhea and gynecomastia in females and males, respectively (Feldman & Burton, 1990). The shortfalls of current medicinal agents, their adverse effects, and their interactions with other drugs encourage the researchers to identify new therapeutic agents capable of scavenging reactive oxygen species (ROS) and providing protection against ulcer sores (Al-Rashdi *et al*., 2012). Nature presents a full reservoir of remedies to treat ailments of human beings. Medicinal plants or plant-derived products have been utilized for treatments and ailments of different diseases throughout the globe (Al-Naqeeb *et al*., 2003). Plants have medicinal value because of certain chemical compounds that have a specific physiological impact on the human body (Korcan *et al*., 2009). The therapeutic use of medicinal plants is increasing daily because of their lesser side effects and efficacy against antibiotic-resistant microorganisms (Samy *et al*., 1998). Genus "*Trianthema*" belongs to Aizoaceae, the ice plant family. This genus comprises 20 species, but only a few have been phytochemically reported (Geethalakshmi *et al*., 2010).

T. triquetra has been used traditionally as fodder for goats and cattle as a remedy for chronic ulcers, fever, and healing wounds (Wariss *et al*., 2014). Moreover, plant polyphenols have provided health benefits in several studies (Pandey *et al*., 2009). Preliminary phytochemical analysis of ethanolic root extract of *T. triquetra* has demonstrated the existence of multiple naturally occurring plant secondary metabolites such as saponins, tannins, alkaloids, flavonoids, phytosterols, and glycosides (Ghori & Humaira, 2016). The existence of various secondary metabolites can be responsible for the plant's gastroprotective impact. Hence, this study has been designed to confirm the traditional use of *T. triquetra* to cure GU disease.

2. Materials and methods

2.1 Plant material

The whole plant of *T. triquetra* was collected in July 2017 from Bahawalpur, Punjab, Pakistan. Expert plant taxonomist Dr. Zaheer-ud-din Khan at the Department of Botany, GC University, Lahore, Pakistan, authenticated the plant and issued voucher number GC.Herb.Bot.3445 (Salma & Saffan, 2003).

2.2 Drugs and chemicals

Omeprazole was kindly provided by Zafa Pharmaceuticals Laboratories Pvt. Ltd. Bovine Serum Albumin (BSA) was acquired from Bioshop (Canada). All other chemicals used in this study were of analytical grade and were obtained from Sigma-Aldrich (Germany).

2.3 Preparation of plant extract and its fractions

The collected plant material was washed with distilled water, shade dried, and pulverized using a mechanical mill. The plant powder (500 g) was subjected to maceration using methanol (2.5L) as solvent. After seven days, the extract was filtered through Whatman filter paper to get the filtrate.

The filtrate was then concentrated in a rotary evaporator at 40 ºC and dried in the oven. The dried crude methanolic extract (71.88 g) was then subjected to fractionation and partitioned with chloroform (250 mL), ethyl acetate (250 mL), n-butanol (250 mL), and water (250 mL). The different fractions of *T. triquetra*, such as n-butanol (TTB), chloroform (TTC), ethyl acetate (TTEA), and water (TTA), were dried and stored in the refrigerator.

2.4 Experimental animals

Male and female albino rats weighing (190-210 g) were utilized in the experiment. Animals were housed at room temperature of 22±2 °C, 12 hours of light and dark intervals, and 45-55% relative humidity (Liu *et al.,* 2018). The animals were randomly divided into seven groups containing six rats in different cages and acclimatized for one week under standard environmental conditions with food and water *ad libitum*. Food was withdrawn 24 hours before the experiment; however, rats had free access to water for about 2 hours before the commencement of the experiment (Okonkon *et al*., 2009). The assay was conducted with the permission of the Bio-Ethical Committee from the Department of Zoology, University of the Punjab, Lahore, Pakistan (Ethic No. 1593).

2.5 Acute toxicity studies

Acute oral toxicity was conducted to determine the safe dose of different fractions of *T. triquetra*. Rats were randomly divided into 5 groups, and group 1 was orally administered with a vehicle (Distilled water, 5 mL/kg b.wt.). Group 2-5 was administered with plant fractions, TTC (2 g/kg b.wt.), TTB (2 g/kg b.wt.), TTEA (2 g/kg b.wt.), TTA (2 g/kg b.wt.) respectively. Rats were deprived of food for 24 hours before and 4 hours after. Animals were continuously observed for 4 hours after dosing for any behavioral or clinical abnormality. Mortality was recorded if any. The administered rats were followed up for 14 days. On the 15th day, blood samples were taken, and animals were sacrificed. The liver and kidney were taken for histopathological analysis (Goncalves *et al*., 2015).

2.6 Ethanol-induced acute gastric ulcer model

Group I (Normal group): Normal control group received distilled water (5 mL/kg b.wt.) orally (Al-harbi *et al*., 1994).

Group II (Negative control group): Negative or disease control group was orally given a vehicle (Distilled water, 5 mL/kg b.wt.).

Group III (Positive control group): The positive control group received an oral dose (20 mg/kg b.wt.) of omeprazole (Al-Wajeeh *et al*., 2016).

Group IV: TTC fraction was administered orally at 300 mg/kg b.wt.

Group V: TTB fraction was administered orally at a dose of 300 mg/kg b.wt.

Group VI: TTEA fraction was administered orally at a dose of 300 mg/kg b.wt.

Group VII: TTA fraction was administered orally at a dose of 300 mg/kg b.wt.

After 1 hour of the above treatment, absolute ethanol (1mL/animal) was orally administered to animals of all groups except the normal group (Madhuri *et al*., 2018). All animals were euthanized 1 hour after an overdose of xylazine and ketamine anesthetic drugs (Tayeby *et al*., 2017).

The stomach was isolated, and gastric contents were collected in centrifuge tubes. Then, the abdomen was opened with an incision along the broader curvature, washed with ice-cold normal saline, and placed on a soft white board for ulcer scoring (Abdulla *et al*., 2010).

2.6.1 Macroscopic and microscopic evaluation

The excised stomach tissues were observed under a magnifying glass and dissecting microscope to count lesions on the inner side of the stomachs (Dashputure & Naikwade, 2011).

2.6.2 Ulcer score

Ulcer Scores were assigned based on the severity of ulcers as follows:

No ulcer (0), Reddish mucosa (0.5), red spots (1), Hemorrhagic streak (1.5), Deep ulcer (2), and Perforations (3) (Khan *et al*., 2011).

2.6.3 Ulcer index (UI)

Ul was calculated using the following formula:

Ulcer index = (UN+US+UP) $\times 10^{-1}$

UN=Average number of ulcers per animal

US=Average of severity score

UP=Percentage of animals with ulcer

2.6.4 Percentage of ulcer inhibition (PI)

The percentage formula determined the extent of the protection from ulcers (Njar *et al*., 1995).

$$\%\ Protection = \frac{UI(disease\ control) - UI(\ treated)}{UI(disease\ control)}$$

2.6.5 Determination of gastric juice volume, pH, and total acidity

Drained the gastric contents in falcon tubes (10 mL) followed by centrifugation for 10 minutes at 3000 rpm. The volume and pH of the supernatant were measured (Nwinyi & Kwanashie, 2013). Transparent supernatant (1mL) was titrated against freshly prepared 0.1N Sodium hydroxide (NaOH). Phenolphthalein was used as an indicator, and the endpoint was colorless to light pink. Total acidity was measured using the equation below (Dahputure & Naikwade, 2011).

$$Acidity = \frac{volume\ of\ NaOH \times normality\ of\ NaOH}{0.1} \times 100$$

Results were expressed in terms of the clinical units (mEq/L)

2.6.6 Determination of gastric wall protein

The total Protein content of glandular tissue homogenate was calculated by the Lowry *et al.* method (Lowry *et al*., 1951).

2.6.7 Determination of gastric mucus content

Glandular segments from the stomachs were excised and weighed. These portions were soaked in 0.1% alcian blue dye (in 0.16 M sucrose buffered with 0.05 M sodium acetate adjusted to a pH=5). Excess dye was drained with 0.25 M sucrose solution by rinsing for 15 and 45 minutes. The dye complex with gastric wall mucus was extracted with 10 mL of 0.5 M magnesium chloride for 2 hours with consecutive shaking for 1 minute after 30 minutes intervals. 4 mL of this solution was shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3000 rpm, and the absorbance of the aqueous layer was measured at 580 nm (Al-Batran *et al.,* 2013).

2.6.8 Histopathological evaluation

Samples of the gastric wall of rats of all groups were placed in a 10 % formalin solution. They were subsequently processed and firmly inserted into paraffin. Different portions of the stomach were sliced through microtome at a thickness of 5 µm. Staining has been achieved by eosin and hematoxylin. The slides were examined and photographed under a microscope (Al-Wajeeh *et al*., 2017).

3. Statistical analysis

The results were statistically evaluated using one way analysis of variance (ANOVA) followed by Dunnet's test. All results have been presented as Mean ± SEM.

4. Results

Results of the current studies indicate that the Ulcer Index (UI) (Table 1**)** in the disease control group was (12.48), which is an indication of an ulcer. In contrast, the UI of the omeprazole-treated animal group was significantly reduced (5.13) and in the animal groups treated with fractions of *T. triquetra* as compared to the disease control group, which is a clear indication of antiulcer activity of *T. triquetra*. The UI of TTB, TTEA, TTC and TTA fractions are (7.22, 9.23, 10.95, and 11.28), respectively. The mean PI (Table 1**)** of the omeprazole treated group is 58.88%, whereas the mean PI of TTB, TTEA, TTC, and TTA is 42.20%, 26.04 %, 12.28%, and 9.61%, respectively. TTB has shown a maximum PI that is comparable with the standard drug omeprazole. It was observed that all four fractions of *T. triquetra* had raised the stomach pH and reduced gastric content volume (Table 2**)** compared to the disease control animal group. The gastric volume in the normal control group is 1.45±0.13 mL, and its pH is 3.6±0.06. The gastric volume in the disease control group is 10.88±0.33 mL, and the pH is 2.53±0.04. In the omeprazole-treated animal group, gastric volume and raised pH were reduced with $p<0.001$. Similar results have been observed in test samples of *T. triquetra*, in which TTB has shown a maximum decrease in gastric volume of 3.98±0.11 mL and increased pH of 5.62±0.04 among all the fractions of *T. triquetra*. Whereas TTEA has a gastric volume of 5.23±0.09 mL and pH is 5.1±0.06, TTC with 5.17±0.05 mL and pH

of 5.02±0.05 and gastric volume of TTA was 5.92±0.06 mL with pH 4.53±0.06. The total gastric acidity (Table 2**)** of disease control and omeprazole-treated groups was 94.5±3.28 mEq/L and 33 mEq/L, respectively. Similarly, all the fractions of *T. triquetra* reduced the stomach acidity compared to the disease control group. Values of gastric acidity of TTB, TTEA, TTC, and TTA were 41.67±4.94 mEq/L, 50±6.55 mEq/L, 51.5±7.94 mEq/L, and 56.5±7.63 mEq/L respectively and are mentioned in **(**Table 2**)**. Total protein content in the disease control group was 281.83±15.30 µg/mL, whereas a significant increase in protein content was observed in omeprazole treated animal group, e.g., 450.25±54.48 µg/mL ($p<0.01$). At the same time, a substantial increase in gastric protein content (Table 3**)** in TTB was observed with 441.17±35.94 µg/mL ($p<0.01$) and TTEA with 431.58±28.81 µg/mL ($p<0.05$) has been observed. But in the case of TTC and TTA treated animal groups, a non-significant increase in protein content has been observed, e.g., 344.667±45.13 µg/mL and 341.42±5.31 µg/mL, respectively. The gastric wall mucus content (Table 3) in the normal group was 189.584 ±5.50 µg/g of glandular tissue ($p<0.05$), and in the disease control group, 114.92±12.64 µg/g of glandular tissue was determined. A non-significant increase in gastric mucus content has been observed in all *T. Triquetra* fractions treated animal groups. TTB, TTEA, TTC, and TTA have gastric mucus content values as follows; 172.67±30.91 µg/g of glandular tissue, 141.92±25.02 µg/g of glandular tissue, 136 ±9.44 µg/g of glandular tissue and 130.417±14.65 µg/g of glandular tissue respectively. In an acute oral toxicity study, oral administration of the plant's fractions (2g/kg b.wt.) treated rats showed no mortality or signs of toxicity during 14 days study period. Results of biochemical parameters (Bilirubin, SGPT, SGOT & ALT) of liver function and (Urea and Creatinine) of kidney function used in acute oral toxicity studies showed non-significant changes between the normal control and plant fractions treated rats (Table 4)**.** Moreover, the vital organs of plant fractions treated rats, such as kidneys and liver, revealed no altered histology and signs of toxicity (Figure 3)**.** Sheets of connective tissue divide the liver into small units called lobules. A lobule is hexagonal, with portal triads and a central vein. Normal histological structure of the central vein and surrounding hepatocytes was present. No fatty changes, necrosis, or necrobiosis were seen. Average-sized kidney and cellularity of glomeruli, normal tubules, endothelial lined vessel, and interstitium were present. No basement membrane thickening, deposits, necrosis, thrombosis, edema, and inflammatory changes were seen.

Table 1. Effect of *T.triquetra* fractions on gastric ulcer in experimental rats

Sr.	Groups	Ulcer number	Ulcer score	Incidence of ulcer (%)	Ulcer index (UI)	% Inhibition (PI)
1	Normal	0***	0***	0	0	-
2	Diseased	20.67±2.76	4.17±0.97	100	12.4833	0
3	Omeprazole	0.83±0.40***	0.5±0.26***	50	5.13333	58.8785
4	TTB	4±1.48***	1.5±0.48**	66.66	7.216	42.1949
5	TTEA	7±1.97***	2±0.68*	83.33	9.233	26.0374
6	TTC	8±0.52***	1.5±0.13**	100	10.95	12.283
7	TTA	10.67±0.84***	2.167±0.25*	100	11.2833	9.61282

All values are written as Mean±SEM. Significant at $p<0.05$*, $p<0.01$**, $p<0.001$***, ns=non-significant with respect to disease control group

Table 2. Effect of *T.triquetra* fractions on gastric juice parameters

Sr.	Groups	Gastric volume (mL)	Gastric pH	Total acidity (mEq/L)
1	Normal	1.45±0.13***	3.6±0.06***	25±2.28***
2	Diseased	10.88±0.33	2.53±0.04	94.5±3.28
3	Omeprazole	2.417±0.09***	6.083±0.04***	33±2.577**
4	TTB	3.983±0.11***	5.617±0.04***	41.67±4.94***
5	TTEA	5.233±0.09***	5.1±0.06***	50±6.55***
6	TTC	5.167±0.05***	5.017±0.05***	51.5±7.94***
7	TTA	5.917±0.06***	4.533±0.06***	56.5±7.63***

All values are written as Mean±SEM. Significant at p<0.05*, p<0.01**, p<0.001***, ns=non-significant with respect to disease control group

Table 3. Effect of *T.triquetra* fractions on gastric wall protein and mucus content

Sr.	Groups	Protein content(µg/mL)	Mucus content (µg of alcian blue/g wet tissue)
1	Normal	474.67±17.80**	189.58±5.50*
2	Diseased	281.83±15.30	114.92±12.64
3	Omeprazole	450.25±54.48**	177.58±14.62^{ns}
4	TTB	441.17±35.94**	172.67±30.91^{ns}
5	TTEA	431.58±28.81*	141.92±25.02^{ns}
6	TTC	344.67±45.13^{ns}	136±9.44^{ns}
7	TTA	341.92±5.21^{ns}	130.417±14.65^{ns}

All values are written as Mean ± SEM. Significant at p<0.05*, p<0.01**, p<0.001***, ns= non - significant with respect to disease control

Table 4. Effect of *T. triquetra* fractions on biochemical parameters in the acute oral toxicity study

		Biochemical Parameters					
Sr	**Groups**	**Urea (mg/dl)**	**Creatinine (mg/dl)**	**Bilirubin (mg/dl)**	**SGPT (U/L)**	**SGOT (U/L)**	**ALP (U/L)**
1	Normal	19.5±1.41	0.78±0.06	0.65±0.05	100.83±5.54	111.33±8.05	159±7.33
2	TTB	20.5±1.26^{ns}	0.79±0.05^{ns}	0.65±0.06^{ns}	102.5±6.29^{ns}	113±5.42^{ns}	165.5±9.5^{ns}
3	TTEA	21±0.97^{ns}	0.75±0.05^{ns}	0.64±0.07^{ns}	108.33±9.46^{ns}	116.33±8.59^{ns}	163±10.05^{ns}
4	TTC	19.67±1.23^{ns}	0.80±0.05^{ns}	0.61±0.05^{ns}	110.83±8.60^{ns}	114.67±7.80^{ns}	161.83±7.94^{ns}
5	TTA	18.67±1.02^{ns}	0.75±0.04^{ns}	0.63±0.06^{ns}	105±8.47^{ns}	119.67±4.05^{ns}	158±10.54^{ns}

All values are written as Mean ± SEM. Significant at p<0.05*, p<0.01**, p<0.001***, ns= non - significant with respect to normal control group

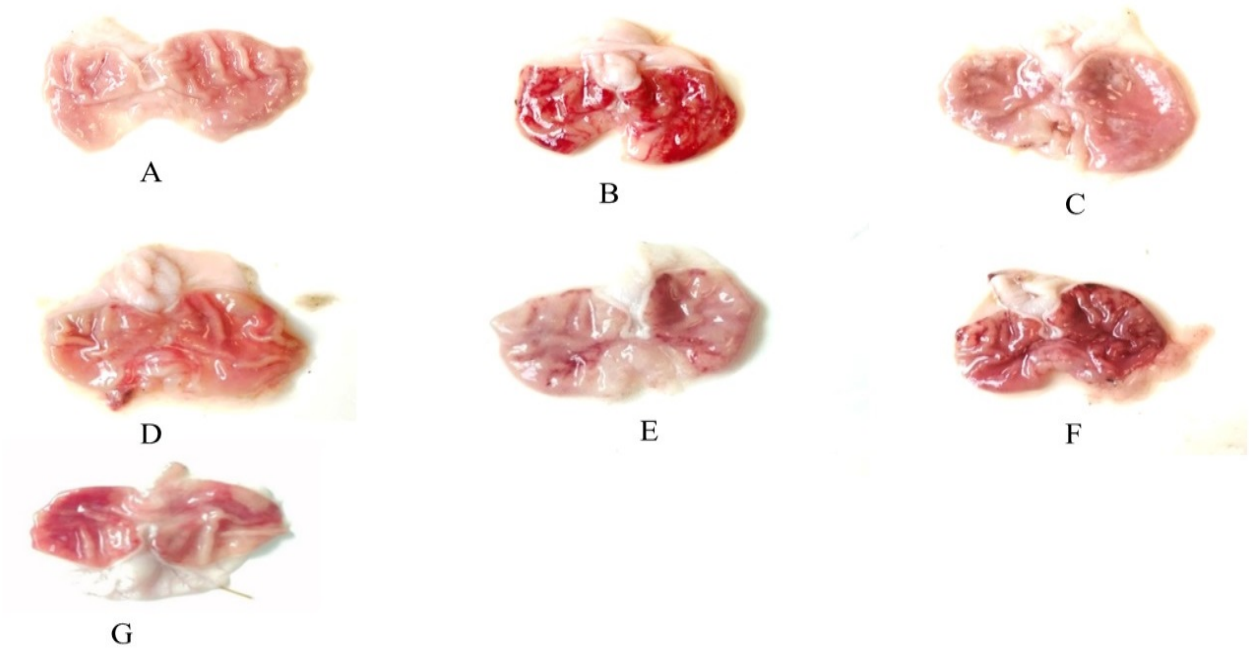

Fig. 1. Effect of *T.triquetra* fractions on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric ulcer model in rats**.** (A) Normal control group, (B) Disease control group, (C) Omeprazole/Standard control group, and (D-G) were given *T.triquetra* fractions such as TTB, TTEA, TTC, and TTA, respectively

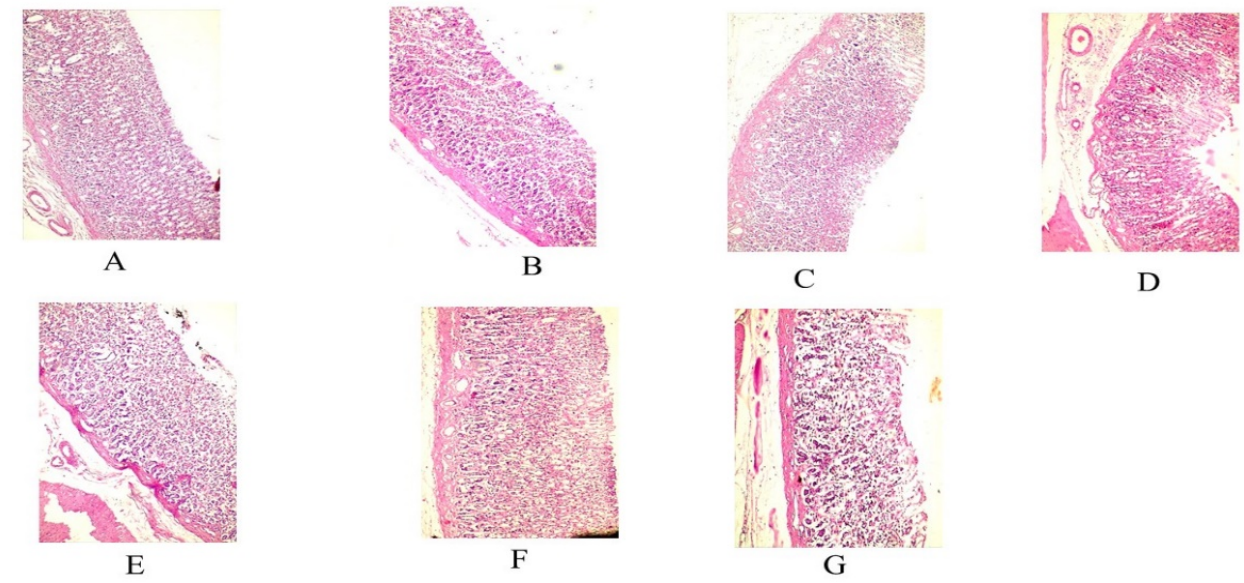

Fig. 2. Effect of *T.triquetra* fractions on the histology of stomach of rats in ethanol-induced gastric ulcer model. (A) Normal control group, (B) Disease control group, (C) Omeprazole/Standard control group, and (D-G) were given *T.triquetra* fractions such as TTB, TTEA, TTC, and TTA, respectively

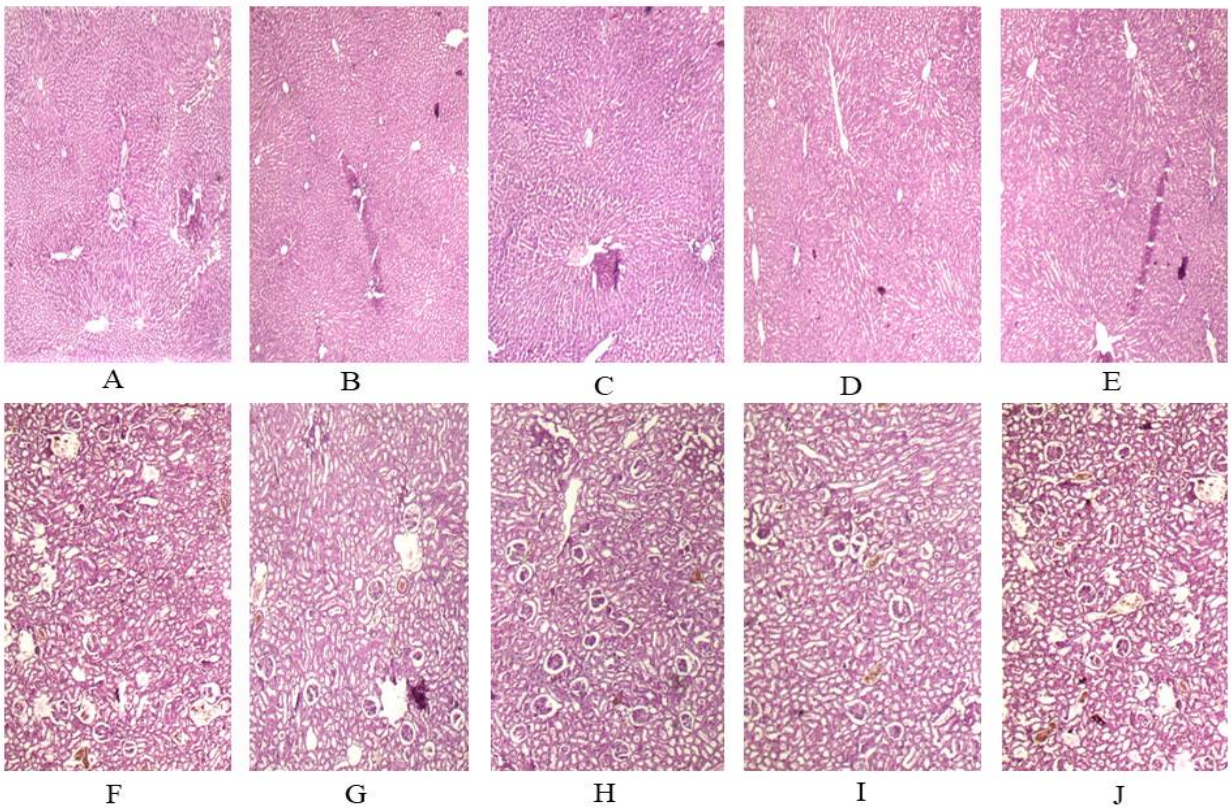

Fig. 3. Histopathology of the liver (A, B, C, D, E) and Kidney (F, G, H, I, J) of normal control and *T. triquetra* fractions treated rats in acute toxicity study for 14 days. A and F: normal control; B and G: TTC 2g/kg b.wt; C and H: TTB 2g/kg b.wt; D and I: TTEA 2g/kg b.wt; E and J: TTA 2g/kg b.wt

5. Discussion

An acute oral toxicity study was carried out on different fractions of *T. triquetra* at 2 g/kg b.wt. After 15 days of administration of plant fractions, all animals were found healthy and alive. No significant differences in kidney and liver function biochemical parameters between normal, and plant fractions treated groups. No histological changes in the liver and kidney of the treated rats were observed. So it may be concluded that all the fractions of *T. triquetra* have a lethal dose LD_{50} above 2 g/kg b.wt. Thus the present dose regimen (300 mg/kg b.wt.) was chosen for the current study. *T. triquetra* has traditionally been used in treating chronic ulcers (Ketuly *et al*., 2011). Recent research has been conducted to evaluate the antiulcer potential of different fractions of *T. triquetra* using an ethanol-induced ulcer model in rats. Ethanol damages the gastric mucosa and results in gastric hemorrhage and tissue necrosis. Alcohol penetrates speedily into the mucus secretion lining of the stomach. It induces damage to the plasma membrane leading to cell death due to the increased accessibility of sodium and water into the intracellular membrane (Gupta *et al*., 2012). These effects are due to the lipid peroxidation and generation of free radicals before cell injury and death (Sannomiya *et al*., 2005). Omeprazole is used to cure disorders related to gastric acidity and protects against mucosal damage. Omeprazole specifically binds and inactivates

the enzyme H^+, K^+- ATPase (Ode & Asuzu, 2011). It is a proven fact that stomach acid secretions have an essential role in GU (Dokmeci *et al.,* 2005). All fractions of *T. triquetra* induced a substantial increase in pH of the stomach and decreased the volume of gastric content and gastric acidity compared to the disease control group indicating an antisecretory mechanism that can be a result of blockade of the H^+, K^+- ATPase enzyme (Bhajoni *et al*., 2016). Moreover, UI and PI are other parameters used for determining gastro-protective effects. All the fractions of *T. triquetra* have shown a significant decline in the UI and improved PI compared to the disease control group. This indicates the gastroprotective effects of *T. triquetra*. The stomach histology of rats of the normal control group has shown no histological changes. No gastric lesions and intact gastric mucosa were observed. Normal gastric glands were seen with rounded nuclei. In the disease control group, a deep ulcer was observed. Severe disruption of the surface epithelium with hyperplastic gastric glands was seen. Histological evaluation of the standard drug omeprazole treated animal group revealed mild disruption of the gastric mucosa. The histological studies of the TTB-treated animal group were similar to the common drug omeprazole-treated animal group. Histological evaluation demonstrated that in the TTB-treated animal group's case, optimum gastric mucosa safety was observed with moderate mucosal epithelium disruption with normal lamina propria. The histological studies of other groups had shown less destruction of gastric mucosa relative to the disease control group. No severe ulcer has been found, and shallow, superficial erosions accompanied by mild leucocyte infiltration and edema in the lamina propria were seen (Figure 2). Total protein content was also evaluated; a decline in the total protein content is a valuable tool for identifying cellular dysfunction. In our research, however, plant fraction treated groups caused an increase in protein synthesis, which was considered self-mechanism leading to a regeneration process (Sharma & Shukla, 2011). Treatment of rats with *T. triquetra* fractions has improved the quantity of mucus in the gastric mucosa suggesting that the plant has antiulcer potential. Although the exact mechanism is unknown, the gastroprotective effect might be due to the protection of the mucus in the stomach wall. Mucus consists of the mucin-type glycoproteins that can be detected by alcian blue dye (Wong *et al*., 2013). The gastroprotective effect may be due to the development of defensive complexes between *T. triquetra* fractions and mucus, serving as an obstacle to ethanol-induced damaging factors in the stomach. Past experiments have shown that the ethanolic root extract of *T. triquetra* holds ROS scavenging activity which might be responsible for its gastroprotective action (Chitra & Nithyanandhi, 2007).

6. Conclusion

The current study's findings underpin the conventional usage of *T. triquetra* as an antiulcerogenic agent. *T. triquetra* plant can be a novel drug candidate for the cure of ulcers. The TTB fraction of *T. triquetra* has shown the most prominent antiulcer activity among all the other fractions of the plant. Previous studies reported that the plant contains alkaloids, flavonoids, phytosterols, tannins, glycosides, and saponins. The presence of antioxidants like phenols, flavonoids, and tannins may also contribute to ulcer cure. Further experiments are advised to establish the mode of action and active compounds responsible for the antiulcer potential.

References

Abdulla, M.A., Al-Bayaty, F.H., Younis, L.T. & Abu Hassan, M.I. (2010). Antiulcer activity of *Centella Asiatica* leaf extract against ethanol-induced gastric mucosal injury in rats. Journal of Medicinal Plants Research, **4**(13): 1253-1259.

Al-Batran, R., Al-Bayaty, F., Al-Obaid, M.M.J., Abdualkader, A.M., Hadi, H.A., Ali, H.M. & Abdulla, M.A. (2013). In vivo antioxidant and antiulcer activity of *Parkia spicosa* ethanolic leaf extract against an ethanol-induced gastric ulcer in rats. *PLoS. One*. **8**(5): e64751.

Al-harbi, M.M., Qureshi, S., Raza, M., Ahmed, M.M., Afzal. & Shah, A.H. (1994). Evaluation of *Caralluma tuberculata* pretreatment for the protection of rat gastric mucosa against toxic damage. Toxicology and applied pharmacology, **128**(1), 1-8.

Al-Naqeeb M.A., Thomson, M., Al-Qattan, K., Kamel, F., Mustafa, T. & Ali, M. (2003). Biochemical and histopathological toxicity of an aqueous extract of ginger in female rats. Kuwait Journal of Science and Engineering, **30**(2): 35-48.

Al-Rashdi, A.S., Salman, S.M., Alkiyami, S.S., Abdulla, M.A., Hadi, A.H.A., Abdelwahab, S.I., Taha, M.M., Hussaini, J. & Asykin, N. (2012). Mechanisms of gastroprotective effects of ethanolic leaf extract of *Jasmium sambac* against HCl/Ethanol-induced gastric mucosal injury in Rats. Evidence-Based Complementary & Alternative Medicines, **2012**: 1-15.

Al-Rejaie, S.S., Abuohashish, H.M, Ahmed, M.M., Aleisa, A.M. & Alkhamees, O. (2012). Possible biochemical effects following inhibition of ethanol-induced gastric mucosa damage by *Gymnema Sylvestre* in male Wistar albino rats. Pharmaceutical Biology, **50**(12): 1542-1550.

Al-Wajeeh, N.S., Hajerezaie, M., Noor, S.M., Halabi, M.F., Al-Henhena, N., Azizan, A.H.S. & Ali, H.M. (2016). The gastroprotective effects of *Cibotium barometz* hair on ethanol-induced gastric ulcer in Sprague-Dawley rats. BMC Veterinary Research, **13(**1): 1-12.

Al-Wajeeh, N.S., Hajrezaie, M., Al-Henhena, N., Kamran, S., Bagheri., E., Zahedifard, M., Saremi, K., Noor, S.M., Ali, H.M. & Abdulla, M.A. (2017). The antiulcer effect of *Cibotium barometz* leaves in rats with experimentally induced acute gastric ulcer. Drug Design, Development, and Therapy, **11**: 995-1009.

Batista, L.M., Almeida, A.N.B., Lima, G.R.M., Falcao, H.S., Ferreira, A.L., Magri, L.P., Coelho, R.G., Calvo, T.R., Vileges, W. & Brito,A.R.M.S. (2013). Gastroprotective effect of the ethanolic extract and fractions obtained from *Syngoanthus bisulcatus.* Rul. Records of Natural Products, **7**: 35-44.

Bhajoni, P.S., Meshram, G.G. & Lahkar, M. (2016). Evaluation of the antiulcer activity of the leaves of *Azadirachta indica*. An experimental study. Integrative Medicines International, **3**:10-16.

Chitra, M. & Nithyanandhi, K. (2007). Radical scavenging activity of *Trianthema triquetra* in male albino rats intoxicated with CCl_4. Journal of Environmental Biology, **28**(2): 283-285.

Dashputra, N.L. & Naikwade, N.S. (2011). Evaluation of Antiulcer activity of methanolic extract of *Abutilon Indicum* Linn. Leaves in experimental rats. International Journal of Pharmaceutical Sciences and Drug Research, **3**(2): 97-100.

Dokmeci, D., Akpolat, M., Aydogdu, N., Dogany, L. & Turan, F.N. (2005). L-carnitine inhibits ethanol-induced gastric mucosal injury in rats. Pharmacology Reports, **57**(4): 481-488.

Feldman, M. & Burton, M.E. (1990). H_2-receptor antagonists: Standard therapy for acid-peptic diseases. New England Journal of Medicine, **323**: 1672-1680.

Geetalakshami, R., Sarada, D.V.L. & Ramasamy, K. (2010). *Trianthema decandra* L: a review on its phytochemical and pharmacological profile. International Journal of Engineering Science and Technology, **2**(5): 976-979.

Ghori, S.S. & Humaira, T. (2016). Evaluation of ethanolic extract of roots of *Trianthema triquetra* for antiulcer activity in albino rats. World Journal of Pharmaceutical and Life Sciences, **2**(5): 385-392.

Goncalves, N., Lino Junior, R.d.S., Rodrigues, C.R., Rodrigues, A.R., & Cunha, L.C.d. (2015). Acute oral toxicity of *Celtis iguanaea* (Jacq.) Sargent leaf extract (Ulmaceae) in rats and mice. Revista Brasileira de Plantas Medicinais, **17**(4), 1118-1124.

Gupta, J., Kumar, D. & Gupta, A. (2012). *Cayratia trifolia* in experimental animals. Asian Pacific Journal of Tropical Diseases, **2**(2): 99-102.

Ketuly, K.A., Abdulla, M.A., Hadi, H.A., Mariod, A.A. & Abdel-Wahab, S.I. **(2011).** Antiulcer activity of the 9 alpha-Bromo analogues of Beclomethasone dipropionate against ethanol-induced gastric mucosal injury in rats. Journal of Medicinal Plant Research. **5**(4): 514-520.

Khan, M.S.A., Hussain, S.A., Jais, A.M.M., Zakaria, Z.A. & Khan, M. (2011). Antiulcer activity of *Ficus religiosa* stems bark ethanolic etract in rats. Journal of Medicinal Plant Research, **5**(3): 354-359.

Korcan, S. E., Cigerci, I.H., Dilek, M., Kargioglu, M., Cenkci, S. & Konuk, M. (2009). Antimicrobial activity of endemic species, Thermopsis turcica, Turkey. Kuwait Journal of Science and Engineering, **35**(1A): 101-112.

Liu, W., Yang, M., Chen, X., Li, L., Zhou, A., Chen, S., You, P. & Liu, Y. (2018). Mechanism of the antiulcer effect of an active ingredient group of modified Xiao Chaihu decoction. Evidence-Based Complementary and Alternative Medicines, **2018:** 1-10.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein estimation by Lowry's method. Journal of Biological Chemistry. **193**: 265.

Madhuri, Y., Narendra Babu, A., Kumar, E. & Yanadaiah, P. (2018). Antiulcer activity of *Hibiscus sabdariffa* on albino rats. International Journal of Pharmaceutics, **11**(3): 13-26.

Njar, V., Adesanwo, J. & Raji, Y. (1995). Methyl Angolenate: Antiulcer agent from the stem bark of *Entandrophragma angolense*. Planta Medica, **61**: 91-91.

Nwinyi, F.C. & Kwanashie, H.O. (2013). Comparative effects of *Sorghym bicolor* leaf base extract on tissue isolated from some body systems of experimental animals. Research Journal of Medicinal Plants, **7**(41): 3041-3051.

Ode, O.J. & Asuzu, O.V. (2011). In rats, investigate *Cassia singueana* leaf extract for antiulcer effects using ethanol-induced gastric ulcer models. International Journal of Plant, Animal and Environmental Sciences, **1**(1): 1-7.

Okokon, J., Antia, B. & Umoh, E. (2009). The antiulcerogenic activity of ethanolic leaf extract of *Lasianthera Africana*. African Journal of Traditional, Complementary and Alternative Medicines, **6**(2): 150-154.

Pandey, K.B., Mishra, N. & Rizvi, S.I. (2009). Myricetin can mitigate altered redox status in type 2 diabetic erythrocytes. Kuwait Journal of Science and Engineering, **36**(2A): 115-124.

Salma, H.M.H. & Saffan, S.E.S. (2003). Triterpenoids and flavonoids from the air-dried aerial parts of Plantago amplexicaulis. Kuwait Journal of Science and Engineering, **30**(2): 109-118.

S**amy, R.P., Ignacimuthu, S. & Sen, A. (1998).** Screening of 34 Indian medicinal plants for antibacterial properties. Journal of Ethnopharmacology, **62**(2): 173-181.

Sannomiya, M., Fonseca, V.B., Silva, M.A., Rocha, L.R.M., Santos, L.C., Hiruma- Lima, C.A., Brito, A.R.M.S. & Vilegas, W. (2005). Flavonoids and antiulcerogenic activity from *Byrosonima crassa* leave extracts. Journal of Ethnopharmacology, **97**(1): 1-6.

Sharma, N. & Shukla, S. (2011). Hepatoprotective potential of aqueous extract of *Butea monosperma* against CCl_4 induced damage in rats. Experimental and Toxicologic Pathology, 63(6-7): 671-676.

Tayeby, F., Salman, A.A.A., Kamran, S., Khaing, S.L., Salehen, N.B. & Mohan, G.M.A.D. (2017). Ulcer prevention effect of 3, 4, 5, Trihydroxy-NO-[(2-Methyl-1H-Indol-3yl) Methylidene] Benzohydrazine in HCl/Ethanol-induced gastric mucosal damage in rats. International Journal of Medical Sciences, **14**(13): 1317-1326.

Wariss, H.M., Ahmad, S., Anjum, S. & Alam, K. (2014). Ethnobotanical studies of dicotyledonous plants of Lal Suhanra National Park, Bahawalpur, Pakistan. International Journal of Science and Research, **3**(6): 2452-2460.

Wong, J.Y., Abdulla, M.A., Raman, J., Phan, C.W., Kuppusamy, U.R., Golbabapour, S. & Sabaratnam, V. (**2013)**. Gastroprotective effects of Lion's Mane mushroom *Hericiumerinaceus* (Bull.: Fr.) Pers. (Aphyllophoromycetideae) extract against an ethanol-induced ulcer in rats. Evidence-Based Complementary and Alternative Medicines, **2013**: 1-9.

Submitted: 20/09/2020
Revised: 10/10/2021
Accepted: 30/10/2021
DOI: 10.48129/kjs.10605