

Gastro protective and antioxidant potential of *Euphorbia prostrata* against aspirin induced gastric ulcers in rabbits

Mahboob Ahmad¹, Bilal Aslam^{1*}, Faqir Muhammad¹, Mashkoor Mohsin² and Ahmad Raza¹

¹Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

²Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan

Abstract: Present study was planned to estimate the gastroprotective activity of *Euphorbia prostrata* plant against aspirin induced gastric ulcers in male adult albino rabbits. The ulcer was induced by oral administration of aspirin in all groups except normal control group. Gastric contents were used to estimate total acid output, gastric volume and gastric pH. Results showed that there was a significant decrease in gastric volume, total acid output, ulcer score and ulcer index in groups treated with extract of *E. prostrata* and it enhanced the pH of gastric mucosa. Blood samples were collected and serum was used for the estimation of total oxidant status (TOS), total antioxidant capacity (TAC), malondialdehyde (MDA) and catalase (CAT). Results suggested that *E. prostrata* extract significantly ($P<0.05$) enhanced the TAC and CAT activity comparable to synthetic antiulcer drug cimetidine while it caused a significant ($P<0.05$) reduction in TOS and MDA levels. Results of this study revealed that extract of *E. prostrata* at 10, 20 and 40mg/kg showed gastric protection of 33.79%, 53.15% and 70.66% respectively. Cimetidine was used as a synthetic antiulcer drug in the study, which showed 72.85% gastric protection. From the above mentioned results it was demonstrated that *E. prostrata* extract at dose rate of 40 mg/kg showed gastroprotective activity similar as cimetidine.

Keywords: CAT, *Euphorbia prostrata*, gastroprotective, MDA, TAC, TOS, ulcer.

INTRODUCTION

Peptic ulcer is a lesion in gastric mucosa that extends through the muscularis mucosa into sub mucosa or deeper (Kumar *et al.*, 2014). It is one of the important problems in developing countries (Gregory *et al.*, 2013). Gastric mucosa is the layer that covers the stomach protecting it from various agents such as acid, pepsin and different drugs (Onasanwo *et al.*, 2011). Gastric mucosal layer can be damaged when there is an imbalance between endogenous aggressive factors such as nonsteroidal anti-inflammatory drugs (NSAIDs), alcohol, smoking, stress, *Helicobacter pylori*, pepsin secretion (Choudhary *et al.*, 2014) bile acid, ischemia, leukotrienes, hydrochloric acid, hypoxia, reactive oxygen species and cytoprotective factors which include enzymatic and nonenzymatic antioxidants, mucous layer, bicarbonate barrier, prostaglandins, growth factors, mucosal blood flow and cellular regeneration (Awaad *et al.*, 2013).

Free radicals are species containing one or more unpaired electrons and are capable of independent existence. These are normally produced by aerobic metabolism in cells. Free radicals that are predominantly produced in cell are super oxide species (Hydroxyl and O_2). Hydrogen peroxide (H_2O_2) and peroxynitrite are not free radicals but they participate in redox state of cell. Collectively these species are known as reactive oxygen species (ROS). Main sources of ROS are oxidative metabolism in mitochondria, small molecules auto-oxidation and enzymatic reactions that involve mixed function oxidases

(Yadav *et al.*, 2013). Free radical formation in the cells has been studied for its damaging effects on cells and it has been proved that the production of free radicals which cause the oxidative stress is the reason for a number of diseases also for the peptic or gastric ulcers (Baigent *et al.*, 2009). *Helicobacter pylori* is a pathogen which is now known to be the most common and a major cause of gastric ulcer in human (Awaad *et al.*, 2013).

Aspirin is commonly used to treat pain, fever, inflammation, arthritis and cardiovascular thrombosis (Laine *et al.*, 2008). Long term use of Non-steroidal anti-inflammatory drugs (NSAIDs) leads to gastric bleeding, perforation, ulcerative lesions and gastric mucosal damage which increase mortality and morbidity (Wilson *et al.*, 2004). NSAIDs show systemic effects by inhibiting cyclooxygenase (COX). It is an enzyme which is involved in biosynthesis of prostaglandins. COX has two major forms COX-1 and COX-2. COX-1 is present in most tissues and protects the GIT by producing prostaglandins. COX-2 is formed on ulcer margins and improves the ulcer healing process by increasing cell proliferation and maintaining mucosal integrity. Prostaglandin which is responsible for the maintenance of gastric mucous is blocked by COX-1 inhibitors such as aspirin (Yin *et al.*, 2014). Prostaglandins play an important role in the integrity and defense of gastric wall since they control the blood flow of gastric mucosal layer, repair and production of epithelial cells, kidney functions and secretions of bicarbonate and mucus (Batista *et al.*, 2004).

Gastric ulcer can be treated by neutralizing destructive factors and by stimulating the protection of gastric

*Corresponding author: e-mail: cba933@gmail.com

mucosa (Onasanwo *et al.*, 2013). The current management of gastric ulcer is mainly performed with proton pump inhibitors, H₂ receptor blockers and antimuscarinics. Prolonged use of these treatments produces adverse reactions like, hypersensitivity, arrhythmia, blood disorders, gynaecomastia, relapse, tolerance and impotence (Malfetheriner *et al.*, 2009). That is why, there is a strong need for new efficacious drugs for peptic ulcer therapy. The natural products for treating major diseases are easily available, economical and have been demonstrated to be clinically effective, less toxic and reduce the aggressive factors (Rahman and Parvin, 2014).

Euphorbia prostrata belongs to family Euphorbiaceae is a small prostrate annual herb present in many parts of the world including Pakistan. Two varieties found are green and red, having green leaves but rarely purplish red. Glucoside, galactoside, ellagic acid, β -sitosterol, luteolin, gallic acid, campesterol, luteolin-7-glucoside, stigmasterol, apigenin, apigenin-7-glucoside and kaempferol (Sharma *et al.*, 2012), terpenoids, flavonoids and tannins (Qaisar *et al.*, 2012) are the phytoconstituents of *E. prostrata*. Keeping in view the therapeutic value of its constituents, the gastroprotective activity of *E. prostrata* was evaluated in experimentally induced ulcerated male albino rabbits.

MATERIALS AND METHODS

Animals

A total of thirty six adult albino rabbits weighing 1.73 ± 0.26 Kg were purchased from the local market of Faisalabad and housed at experimental animal room, Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. The rabbits were kept in separate iron cages. They were kept in a room under controlled temperature ranging from $22 \pm 2^\circ\text{C}$ and relative humidity (65-70%). A twelve hour light dark cycle was provided artificially to the animals. Prior to the experimentation, the rabbits were acclimatized for one week and provided excess amount of standard feed and water.

Plant material

E. prostrata was collected from the premises of University of Agriculture, Faisalabad, Pakistan. The plant material was authenticated and compared to its standard in the herbarium maintained by Department of Botany, University of Agriculture Faisalabad. The samples were preserved in the pharmacology laboratory, department of Physiology and Pharmacology, University of Agriculture Faisalabad.

Drugs

Disprin® (Aspirin) and synthetic antiulcer drug cimetidine tablets (Cimit®, 15 mg) were purchased from Reckitt Benckiser Ltd Karachi, Pakistan and Ferozsons Laboratories Limited Nowshera, Pakistan respectively.

Extraction

Shade dried aerial parts of *E. prostrata* were subjected for extraction with methanol at room temperature occasionally shaking for 24 hrs. Extract was concentrated by Rotavapor-R20 at 37°C to obtain crude extract with the percent yield of 14.7. Five ml distilled water was used to dilute the *E. prostrata* extract before administration to the rabbits.

Experimental design

Animals were divided into six groups, including six animals in each group. These groups were numbered as 1, 2, 3, 4, 5 and 6. Group 1 served as the control group on normal diet, group 2 was given the aspirin (150 mg/kg) (Aslam *et al.*, 2013) and considered as the untreated control group, while group 3, named as treated control, received the cimetidine (standard treatment for ulcer) at the dose rate of 15mg/kg of body weight (Bukhari *et al.*, 2011) along with aspirin. Group 4, 5 and 6 were served with the three graded doses of plant extract as 10, 20, 40 mg/kg respectively along with aspirin. Animals were separately marked according to their groups.

Surgical procedures

After 14 days of treatment animals were fasted for 24 hours and were provided free access to water in this duration. After 14 days animals were sacrificed. Stomach was cut along the greater curvature and the contents were collected into tubes and centrifuged at 3000 rpm for 5 min. The supernatant was separated and was used for the estimation of various gastric parameters.

Blood samples were collected from 0-14 days. The samples were allowed to clot for 20 minutes at refrigeration temperature and then centrifuged for 5 min at 4000 rpm to separate serum. Serum was stored at -4°C till the estimation of different antioxidant parameters.

Procedure for antiulcer evaluation studies

Effect on gastric secretion

The pH and gastric volume of the supernatant was determined with the help of pH meter and by using graduated cylinder respectively. While acid output was determined by taking one ml of supernatant and diluting it to 10ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicated to the end point until the solution turned to orange color. Acid output was expressed by the following formula (Raju *et al.*, 2009).

$$\text{Acid output} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1}$$

Ulcer scores

The number of ulcers was noted and the severity was determined by the following scores (Kulkarni, 2002). Normal coloration (0.0), Red coloration (0.5), Spot ulcer (1.0), Hemorrhagic stress (1.5), Deep ulcers (2.0), Perforation (3.0).

Ulcer index calculations

Ulcer index was calculated by adding the total number of ulcers per stomach and total severity of ulcers per stomach. Ulcer index (UI) was calculated using the formula (Vogel, 2002).

$$UI = \frac{US + UN + UP}{10}$$

Where, US=Mean severity of ulcer score. UN=Average number of ulcers per animal. UP=Percentage of animals with ulcer incidence.

Percentage protection

The percentage protection was calculated for treated groups by using the following formula (Sharma *et al.*, 2011).

$$\text{Percentage (\%)} = \frac{[CUI - TUI]}{CUI} \times 100$$

Where, CUI = Ulcer index of control groups. TUI = Ulcer index of treated groups

Estimation of free radical generation

The gastric tissues and serum were analyzed for the estimation of free radicals by performing the following analysis tests for antioxidant and oxidant status.

Total antioxidant capacity (TAC)

TAC was determined with the help of spectrophotometer (Erel, 2004). Reading was taken at 670 or 660 nm on U.V spectrophotometer.

Total oxidant status (TOS)

TOS was measured with the help of spectrophotometer (Erel, 2005). The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micro molar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ Equiv. /L). Reading was taken at wave length of 560 nm on spectrophotometer.

Melondialdehyde (MDA)

MDA was determined according to the method developed by Ohkawa (1979). Absorption was taken at 532 wavelength of spectrophotometer.

Catalase (CAT)

CAT was determined by the commercially available kit (Goth, 1991). Reading was taken on spectrophotometer at wavelength of 405 nm.

STATISTICAL ANALYSIS

The values were expressed as mean $\pm$ SE. Statistical analysis was performed by one way analysis of variance (ANOVA) and statistical differences among different treatment groups were determined by Duncan's Multiple Range test at 5 % level of significance (Steel *et al.*, 1997).

RESULTS**Evaluation of gastro protective parameters**

The crude Extract of *E. prostrata* 10-40 mg/kg, given orally once daily for seven days showed dose dependant protective effect against gastric ulcer induced by aspirin. *E. prostrata* at the dose rate of 40 mg/kg significantly ($P < 0.05$) protected the animal and healed ulcer after seven days of treatment.

Ulcer index also showed the similar pattern of results as that of ulcer score (table 1). After seven days of the treatment ulcer index for group 1, 2, 3, 4, 5 and 6 was 1.12, 14.62, 3.97, 9.68, 6.85 and 4.29 respectively. Cimetidine (group 3) and highest plant dose (group 6) showed the significant results ($p < 0.05$). The percent curative ratio was much better with highest treated dose of *E. prostrata* as shown in table 1.

Total acid output of rabbits in units of mEq/L/100g body weight after seven days study is presented in table 1. The mean values for acid output were described that the aspirin increased the acidity in group 2 having mean value 96.63 ± 4.17 mEq/L/100g as compared to the normal group which has mean value for acidity 71.97 ± 2.18 mEq/L/100g, while the groups treated with test plant also showed the significant results at dose 2 and dose 3 (in group 5 and group 6 respectively). These were 65.86 ± 1.84 mEq/L/100g and 50.62 ± 1.77 mEq/L/100g respectively. Curative ratio, gastric pH and gastric volume shown in table 2.

Evaluation of antioxidant parameters

Results of the study showed that mean values of TAC for the control group was 1.53 ± 0.03 mmol/L. TAC decreased (0.80 ± 0.08 mmol/L) with ulcer production in stomach by the use of aspirin while it significantly ($p < 0.05$) increases up to normal values for groups 3 and 6 as 1.42 ± 0.04 and 1.38 ± 0.04 mmol/L respectively (table 3).

The mean values of TOS of normal control group was 3.54 ± 0.23 $\mu\text{mol/L}$. TOS increased (9.65 ± 0.23 $\mu\text{mol/L}$) with ulcer production in stomach by the use of aspirin while it significantly ($p < 0.05$) decrease up to normal values for groups 3 and 6 as 4.77 ± 0.18 and 4.99 ± 0.19 $\mu\text{mol/L}$ respectively when compared with untreated control (table 3).

Results of the current study also demonstrated that the mean values of MDA activity was increased (from 4.10 ± 0.25 to 9.45 ± 0.19 nmol/L) when aspirin was used alone for the production of ulcer in stomach. The mean values decrease significantly ($p < 0.05$) for group 3, 4, 5 and 6 as 4.74 ± 0.30 , 8.93 ± 0.27 , 7.11 ± 0.26 and 4.72 ± 0.21 respectively than untreated control (table 3).

Table 1: Mean± SE values of ulcer score, ulcer index and curative ratio after 14 days of treatment with per-oral drugs and *E. prostrata* extract in rabbits

Group	Dose	Ulcer Score	Ulcer index	Curative ratio %
1 (Control)	Routine feed	0.17±0.11	1.12±0.10	-
2 (Disprin®)	150 mg/kg	2.25±0.25*	14.62±0.33	-
3 (Cimetidine + Disprin®)	10 mg/kg	0.92±0.20	3.97±0.12	72.85%
4 (<i>E. prostrata</i> + Disprin®)	10 mg/kg	2.17±0.17*	9.68±0.27	33.79%
5 (<i>E. prostrata</i> + Disprin®)	20 mg/kg	1.50±0.22*	6.85±0.35	53.15%
6 (<i>E. prostrata</i> + Disprin®)	40 mg/kg	0.92±0.15	4.29±0.29	70.66%

Table 2: Mean± SE values of Acid output, pH and gastric volume after the 14 days of treatment with per-oral drugs and *E. prostrata* extract in rabbits

Group	Dose	Acid output (mEq/L/100g)	pH	Gastric Volume (ml/100g)
1 (Control)	Routine feed	71.97±2.18	2.48±0.12	16.43±0.37
2 (Disprin®)	150 mg/kg	96.63±4.17*	1.62±0.13*	20.77±0.27*
3 (Cimetidine + Disprin®)	10 mg/kg	49.37±3.61	4.07±0.08*	11.37±0.27
4 (<i>E. prostrata</i> + Disprin®)	10 mg/kg	82.83±3.72*	2.08±0.12	14.78±0.40*
5 (<i>E. prostrata</i> + Disprin®)	20 mg/kg	65.86±1.84*	3.13±0.06*	13.47±0.30*
6 (<i>E. prostrata</i> + Disprin®)	40 mg/kg	50.62±1.77	3.84±0.11*	11.52±0.29

Table 3: Mean± SE values of TOS, TAC, MDA and CAT after the 14 days of treatment with per-oral drugs and *E. prostrata* extract in rabbits

Groups	Dose	TOS (μmol/L)	TAC (mmol/L)	MDA (nmol/L)	CAT (KU/L)
1 (Control)	Routine diet	3.54±0.23	1.53±0.03	4.10±0.25	7.78±0.21
2 (Disprin®)	150 mg/kg	9.65±0.23*	0.80±0.08*	9.45±0.19*	3.71±0.19*
3 (Cimetidine + Disprin®)	10 mg/kg	4.77±0.18*	1.42±0.04*	4.74 ±0.30*	7.62±0.22*
4 (<i>E. prostrata</i> + Disprin®)	10 mg/kg	8.49±0.21*	0.94±0.06*	8.93±0.27*	4.09±0.12*
5 (<i>E. prostrata</i> + Disprin®)	20 mg/kg	6.66±0.34*	1.17±0.09*	7.11±0.26*	5.76±0.19*
6 (<i>E. prostrata</i> + Disprin®)	40 mg/kg	4.99±0.19*	1.38±0.04*	4.72±0.21*	7.67±0.18*

Values are mean±SE; (n=6) *p<0.05 when compared with control

The mean values of CAT activity are expressed in KU/L. The CAT activity decreased up to 3.71±0.19 KU/L when aspirin was used alone for the production of ulcer in stomach. This value is significantly (p<0.05) decreased from normal value i.e. 7.78±0.21 KU/L. And it significantly seemed to be increased value when treated with cimetidine (7.62±0.22 KU/L) and it also has normal range of values for CAT activity when plant was used at highest dose as 7.67±0.18 KU/L (table 3).

DISCUSSION

Peptic ulcer is the lesion that perforates the mucosal layer of stomach (Batista *et al.*, 2004). Pathogenesis of gastric ulcer includes discrepancy between offensive and protective factors (Mirje and Zaman, 2014). Ulcerations takes place due to disturbance in normal balance produced by either increased aggression or reduced mucosal resistance. Integrity of gastric mucosa is retained by homeostatic equilibrium between destructive and protective factors (Junaidi *et al.*, 2013).

The goal of this study was to estimate the effectiveness of *E. prostrata* extract in preventing the formation of gastric ulcer experimentally by aspirin-induced gastric damage in albino rabbits. Ulcer parameters such as gastric pH,

gastric volume, total acid output, ulcer score, ulcer index and percentage protection were measured to determine the antiulcer activity of *E. prostrata* extract. Biochemical analysis were also carried out to determine the efficacy of *E. prostrata* extract against aspirin induced gastric ulcerative damage.

pH of gastric secretions was measured in the current study. Administration of aspirin significantly reduced the pH of gastric mucosa as described in previous studies (Wang *et al.*, 2008; Aslam *et al.*, 2013). Administration of cimetidine and *E. prostrata* extract with aspirin significantly enhanced the pH of gastric secretions. Extract of *E. prostrata* at dose of 40 mg/kg significantly enhanced the pH and it produced similar results as synthetic antiulcer drug cimetidine (table 1). Aspirin causes the gastric damage by making the stomach pH more acidic which increases the acidity in the gastric mucosa by enhancing the concentration of hydrogen ions. These results are similar as described in previous studies (Sami *et al.*, 2013; Yadav *et al.*, 2013; Choudhary *et al.*, 2014).

Results of above studies showed that gastric volume and total acid output were significantly increased in aspirin treated rabbits. Aspirin increases the acid secretions in

gastric mucosa due to its acidic nature which enhances the volume of gastric secretions. Administration of cimetidine and *E. prostrata* along with aspirin significantly reduces the gastric volume and total acid output. *E. prostrata* extract at dose of 40 mg/kg significantly reduced gastric volume and total acid output and its results were statistically similar with synthetic antiulcer drug cimetidine (table 1). The above mentioned results are in accordance with literature studies on aspirin induced gastric ulcer (Choudhary et al., 2013; El-Meligy et al., 2013; Nwangwu et al., 2013).

It is observed that enhanced stimulation of cholinergic neurotransmitters may potentiate the acid secretions from parietal cells which cause severe gastric damage (Garrick et al., 1986). A study has reported the anticholinergic activity of *E. prostrata* which may be responsible for the reduction in gastric secretions (Chaudhry et al., 2001). Histamine is an inflammatory mediator which causes dilation of blood vessels. It also acts on T-cells and releases interleukins (Cannon et al., 2006). Studies with the standardized extract of *E. prostrata*, when administered orally showed an inhibition of histamine-induced edema can suppress histamine Ellagic acid and flavonoids present in *E. prostrata* exhibit gastroprotective, antisecretory and cytoprotective activities in mammals as they reduce histamine secretions by inhibiting histidine decarboxylase (Gupta, 2012; Awaad et al., 2013).

In this study ulcer scores and ulcer index were measured. Results demonstrated that ulcer scores and ulcer index were significantly increased in animals treated with aspirin. Groups treated with cimetidine (groups 3) and *E. prostrata* (groups 6) significantly reduced the ulcer scores and ulcer index when compared with control group (aspirin treated group). These results of ulcer scores and ulcer index are in accordance with previous studies (Vidya et al., 2011; Choudhary et al., 2014; Mirje and Zaman, 2014).

Curative ratio is very important parameter in ulcer study which determines the %age protection of treatments. In current study curative ratio was measured by using the values of ulcer index for all groups. Results suggested that synthetic antiulcer drug cimetidine showed 69.02% protection against aspirin induced gastric ulcer. *E. prostrata* extract at 10, 20 and 40 mg/kg showed 27.84%, 44.66% and 69.02% respectively. *E. prostrata* extract at 40 mg/kg % protection almost near to synthetic antiulcer drug cimetidine. These results of curative ratio are also in accordance with previous research studies (Vidya et al., 2011; Mirje and Zaman, 2014; Aslam et al., 2015).

Aspirin is nonsteroidal antiinflammatory drug that promotes the production of reactive oxygen species. It has been suggested that gastric lesions generated by NSAIDs

include the inhibition of electron transport chain and separation of oxidative phosphorylation causing partial oxygen reduction. ROS can cause oxidative stress by damaging DNA, cellular lipids and proteins (Droge, 2002). Nuclear factor kappa B plays an important role in generation of damage to mucosal cells during oxidative stress induced by NSAIDs. Transcription factor (NF- κ B) is regulated by redox status intracellularly and causes the expression of cyclooxygenase-2 and inducible nitric oxide synthase during NSAIDs use in mucosal cells (Laube et al., 2013). Tannins, phenolic acid and flavonoids (luteolin-7-glucoside, apigenin-7-glucoside) are the active constituents of *E. prostrata*. In murine macrophages pro-inflammatory cytokine production, tyrosine phosphorylation and gene expression mediated by nuclear factor kappa B are inhibited by luteolin. COX-2 and iNOS (inducible nitric oxide synthase) are significantly inhibited by apigenin. For prevention of inflammation and carcinogenesis, such type of variations in iNOS and COX-2 by apigenin are essential (Bakhshi et al., 2008).

Malondialdehyde levels show membranous lipid peroxidation. Its level was extensively elevated, after aspirin induction, which is associated to cell injury (Yin et al., 2014). To know whether the protecting effect of *E. prostrata* is intermediated by its antioxidant activity, MDA was calculated. The study showed increased levels of MDA and decreased level of mucus contents of gastric mucosa in the ulcer control group. The decreased level of MDA in the rabbits pre-treated with *E. prostrate* extract and positive control group exhibited cytoprotective action in the aspirin induced gastric ulcer. The higher concentration of extract was able to reduce more oxidative stress process in rabbits. Our results showed that oxidative stress induced by aspirin was reduced by *E. Prostrata* extract.

CAT enzymes are the protective antioxidants and are basic defense for reactive oxygen species (Jainu and Devi, 2006). CAT is mostly present in per oxisomes and converts H_2O_2 into nonreactive oxygen species and water (Gonzalez-Rey and Bebianno, 2012). Results of this study suggested that aspirin significantly reduced the CAT levels. However administration of cimetidine and *E. prostrata* extract along with aspirin significantly enhanced CAT activity. From results it was obvious that antioxidant potential of *E. prostrata* at 40 mg/kg was statistically as effective as of synthetic antiulcer drug cimetidine. The above mentioned results are in accordance with previous research studies (Aslam et al., 2013; Yadav et al., 2013; El-Meligy et al., 2013; Yin et al., 2014).

Results of the study suggested that aspirin significantly enhanced the total oxidant status and significantly reduced the total antioxidant capacity. However

administration of cimetidine and *E. prostrata* extract along with aspirin significantly reduced the total oxidant status while it significantly enhanced total antioxidant capacity. From results it was obvious that antioxidant potential of *E. prostrata* at 40 mg/kg was statistically as effective as of synthetic antiulcer drug cimetidine (table 3). These results show that *E. prostrata* extract has the ability to elevate antioxidant enzymes potential with good efficiency in albino rabbits. These above mentioned results are in accordance with previous research studies (Aslam *et al.*, 2013; Yadav *et al.*, 2013; El-Meligy *et al.*, 2013; Begum *et al.*, 2014; Yin *et al.*, 2014). Serum oxidative stress was markedly increased in untreated toxic group (received aspirin) as compared to normal healthy subjects. The possible reason for this increase is due to increased reactive oxygen species production leading to elevation of oxidant level in ulcerative rabbits.

Further studies are recommended on *E. prostrata*, for evaluating the pharmacological actions against AIDs, tumor, heart disease, aging, ischemic stroke, prostate cancer cells and antiestrogen-resistant breast cancer. More detailed studies on *E. prostrata* using different doses and covering longer periods of observation are needed before reaching a clear cut conclusion. Although the exact chemical compounds responsible for the gastro protective effect of *E. prostrata* extract still remain speculative yet experimental evidence obtained in the present study can justify the inclusion of *E. prostrata* in the management of gastric ulcer in traditional medicine.

CONCLUSION

E. prostrata extract significantly enhanced the TAC and CAT activity comparable to synthetic antiulcer drug cimetidine while it caused a significant reduction in TOS and MDA levels. Results of the study revealed that extract of *E. prostrata* at 10, 20 and 40 mg/kg showed gastric protection of 33.79%, 53.15% and 70.66% respectively. Cimetidine was used as a synthetic antiulcer drug in the study, which showed 72.85% gastric protection. From the above mentioned results it was demonstrated that *E. prostrata* extract at dose rate of 40 mg/kg showed gastroprotective activity similar as cimetidine.

REFERENCES

- Aslam B, Majeed W, Javed I, Muhammad F, Khaliq T, Khan JA, Ali A and Sindhu ZD (2013). Gastroprotective effect of *Berberis vulgaris* (zereshk) seeds against gastric ulcer induced by aspirin in male adult albino mice. *Indo Amer. J. Pharmace. Res.*, **3**: 4518-4527.
- Aslam B, Awan T, Javed I, Khaliq T, Khan JA and Raza A (2015). Gastroprotective and antioxidant potential of *Glycyrrhiza glabra* on experimentally induced gastric ulcers in albino mice. *Int. J. Curr. Microbiol. App. Sci.*, **4**(2): 451-460.
- Awaad AS, Meligy RME and Soliman GA (2013). Natural products in treatment of ulcerative colitis and peptic ulcer. *J. Saudi Chem. Soc.*, **17**: 101-124.
- Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, Buring J, Hennekens C, Kearney P, Meade T, Patrono C, Roncaglioni MC and Zanchetti A (2009). Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomized trials. *Lancet.*, **373**: 1849-1860.
- Bakhshi GD, Langade DG and Desai VS (2008). Prospective, open label study of *Euphorbia prostrata* extract 100 mg in the treatment of bleeding haemorrhoids. *Bomb. Hosp. J.*, **50**: 577-583.
- Batista LM, de Almeida AB, de Pietro Magri L, Toma W, Calvo TR, Vilegas W, Souza and Brito AR (2004). Gastric antiulcer activity of *Syngonanthus arthrotrichus silveira*. *Biol. Pharm. Bull.*, **27**: 328-332.
- Begum R, Aslam B, Javed I, Khaliq T, Muhammad F and Raza A (2014). Gastro protective and antioxidant effect of *Euphorbia prostrata* against indomethacin induced gastric ulcers in healthy adult male albino rabbits. *Int. Res. J. Pharm.*, **5**(11): 846-850.
- Bukhari MH, Khalil J, Qamar S, Qamar Z, Zahid M, Ansari N and Bakhshi IM (2011). Comparative Gastroprotective Effects of Natural Honey, *Nigella sativa* and Cimetidine against Acetylsalicylic Acid Induced Gastric Ulcer in Albino Rats. *J. Coll. Physicians. Surg. Pak.*, **21**: 151-156.
- Cannon JG (2006). Cytokines and Eicosanoids. In: *Moderation Nutrition in Health and Disease*. 10th Ed. Philadelphia Lippincott Williams and Wilkins, pp.655-669.
- Chaudhry BA, Janbaz KH, Uzair M and Ejaz AS (2001). Biological studies of *Conyza* and *Euphorbia* species. *J. Res. Sci.*, **12**: 85-88.
- Choudhary M, Kumar V and Singh S (2014). Gastric antisecretory and cytoprotective effects of hydro alcoholic extracts of *Plumeria alba* Linn leaves in rats. *J. Integr. Med.*, **12**: 42-51.
- Choudhary MK, Bodakhe SH and Gupta SK (2013). Assessment of the antiulcer potential of *Moringa oleifera* root-bark extract in rats. *J. Acupunct. Meridian. Stud.*, **6**: 214-220.
- Droge W (2002). Free radicals in the physiological control of cell function. *Physio. Rev.*, **82**: 47-95.
- El-Meligy RM, waad ASA, Zain ME and Alafeefy AM (2013). Evaluation of selected desert plants as anti-ulcerogenic natural products. *Aust. J. Bas. Appl. Sci.*, **7**: 431-436.
- Erel O (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.*, **37**: 277-285.
- Erel O (2005). A new automated colorimetric method for measuring total oxidant status. *Clin. Biochem.*, **38**: 1103-1111.

- Garrick T, Buack S and Bass P (1986). Gastric motility is a major factor in cold restraint induced lesion formation in rats. *Am. J. Physiol.*, **250**: 191-199.
- Gonzalez-Rey M and Bebianno MJ (2012). Does non-steroidal anti-inflammatory (NSAID) ibuprofen induce antioxidant stress and endocrine disruption in mussel *Mytilus galloprovincialis*? *Environ. Toxicol. Pharmacol.*, **33**: 361-371.
- Goth L (1991). A simple method for determination of serum catalase and reversion of reference range. *Clinica. Chimica. Acta.*, **2**: 143-150.
- Gregory M, Divya B, Mary RA, Viji MM, Kalaichelvan VK and Palanivel V (2013). Anti-ulcer activity of *Ficus religiosa* leaf ethanolic extract. *Asian. Pac. J. Trop. Biomed.*, **3**: 554-556.
- Gupta J, Kumar D and Gupta A (2012). Evaluation of gastric anti-ulcer activity of methanolic extract of *Cayratia trifolia* in experimental animals. *Asian. Pac. J. Trop. Dis.*, **2**: 99-102.
- Jainu M and Devi CS (2006). Gastroprotective action of *Cissus quadrangularis* extract against NSAID induced gastric ulcer: role of pro inflammatory cytokines and oxidative damage. *Chem. Biol. Interact.*, **161**: 262-270.
- Junaidi M, Anjum H, Afreen M, Geetha K and Pamu S (2013). A review on antiulcer drugs of natural origin. *Intern. J. Pharm. Res. Rev.*, **1**: 380-390.
- Kumar MS, Ghosh A, Kumar YA, Dileep K and Kumar SA (2014). *Kamadugha rasa* an effective ayurvedic formulation for peptic ulcer: A review. *Global J. Res. Medic. Plants Indig. Med.*, **3**: 24-32.
- Laine L, Takeuchi K and Tarnawski A (2008). Gastric mucosal defense and cytoprotection: Bench to bedside. *Gastroenterology*, **135**: 41-60.
- Laube M, Knies T and Pietzsch J (2013). Radio labeled COX-2 inhibitors for non-invasive visualization of COX-2 expression and activity- A critical update. *Molecules*, **18**: 6311-6355.
- Malfertheiner P, Chan FK and McColl KE (2009). Peptic ulcer disease. *Lancet*, **374**: 1449-1461.
- Mirje MM and Zaman SU (2014). Evaluation of the antiulcer activity of *Ocimum sanctum* (tulsi) in indomethacin-induced gastric ulcers in albino rats. *Intern. J. Life Sci. Biotech. Pharm. Res.*, **3**: 274-279.
- Nwangwu S, Ajeigbe K, Omotoso D, Josiah S, Olayanju A and Bolaji A (2013). Acid buffering effects of aqueous leaf extract of *Ocimum gratissimum* L. in the rabbit stomach. *Turk. J. Gastroenterol.*, **24**: 204-210.
- Ohkawa H, Ohishi N and Yag K (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analy. Biochem.*, **95**: 351-358.
- Onasanwo SA, Emikpe BO, Ajah AA, Elufioye TO. (2013). Antiulcer and ulcer healing potentials of *Musa sapientum* peel extract in laboratory rodents. *Pharmacognosy. Res.*, **5**: 173-178.
- Onasanwo SA, Singh N, Olaleye SB and Palit G (2011). Anti-ulcerogenic and proton pump (H⁺, K⁺ ATPase) inhibitory activity of Kolaviron from *Garcinia kola* Heckel in rodents. *Indian. J. Exp. Biol.*, **49**: 461-468.
- Qaisar M, Gilani SN, Farooq S, Rauf A, Naz R, Shaista and Perveez S (2012). Preliminary comparative phytochemical screening of *Euphorbia* species. *American-Eurasian J. Agric. Envir. Sci.*, **12**: 1056-1060.
- Rahman S and Parvin R (2014). Therapeutic potential of *Aegle marmelos* (L) an overview. *Asian Pac. J. Trop. Dis.*, **4**: 71-77.
- Raju D, Ilango K, Chitra VI and Ashish K (2009). Evaluation of antiulcer activity of methanolic extract of *Zerimalia chebula* fruits in experimental rats. *J. Pharmace. Sci. Res.*, **1**: 101-107.
- Sami G. Alsabri, Nouri B. Rmeli, Abdulmottaleb A. Zetrini, Salah B. Mohamed, Marwa I. Meshri, Khaled M. Aburas, Salah M. Bensaber, Ibrahim A. Mrema, Akram A. Mosbah, Khowla A. Allahresh, Anton Hermann and Abdul Gbaj (2013). Phytochemical, antioxidant, anti-microbial, anti-inflammatory and anti-ulcer properties of *Helianthemum lippii*. *J. Pharmacog. Phytochem.*, **2**: 86-97.
- Sharma SK, Singh J and Singh S (2012). Pharmacognostical and phytochemical investigation of *Euphorbia prostrata* Ait. *Intern. J. Pharmace. Sci. Res.*, **3**: 1043-1048.
- Sharma SK, Singh S and Singh J (2011). Anthelmintic effect of *Euphorbia prostrata* Ait. extracts. *Indian J. Pharm.*, **43**: 743-744.
- Steel RGD, Torrie JH and Dickey DA (1997). Principles and procedures of statistics. McGraw Hill, New York, USA.
- Vidya S, Ramesh A, Alekhya N and Lohitha I (2011). Antiulcer activity of *Eugenia jambolana* leaves against ethanol induced ulcer in albino rats. *Intern. J. Pharm. Res. Develop.*, **3**: 106-112.
- Vogel HG (2002). Drug Discovery and Evaluation, Pharmacological Assay. 2nd Ed. Springer, Germany. pp.759-767.
- Wang H, Zhou CL, Lei H, Zhang SD, Zheng J and Wei Q (2008). Kaempferol: A new immunosuppressant of calcineurin. *IUBMB Life.*, **60**: 549-554.
- Wilson I, Langstrom G, Wahlqvist P, Walan A, Wiklund I and Naesdal I (2004). Management of gastroduodenal ulcers and gastrointestinal symptoms associated with NSAID therapy: A summary of four comparative trials with omeprazole, ranitidine, misoprostol and placebo. *Curr. Therap. Res.*, **62**: 835-850.
- Yadav AK, Khatoon R, Chandana VR and Chandana A (2013). Antiulcer activity of *Momordica dioica* fruits, extract. *Wor. J. Pharm. Pharmace. Sci.*, **2**: 1921-1944.
- Yin H, Pan X, Song Z, Wang S, Yang L and Sun G (2014). Protective effect of wheat peptides against indomethacin-induced oxidative stress in IEC-6 cells. *Nutrients*, **6**: 564-574.