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EFFECT OF ETHANOLIC EXTRACT OF *EUGENIA JAMBOLANA* SEEDS ON GASTRIC ULCERATION AND SECRETION IN RATS

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Abstract : *Eugenia jambolana* (Jamun) fruit has been reported to give soothing effect on human digestive system. Present study includes the effect of ethanolic extract of seeds *of E. jambolana* (EJE) against gastric ulcers induced by 2 h cold restraint stress (CRS), aspirin (ASP, 200 mg/kg, 4 h), 95% ethanol (EtOH, 1 ml/200 g, 1 h) and 4 h pylorus ligation (PL) in rats. To ascertain the mechanism of action of EJE, its effect was studied on mucosal offensive acid-pepsin secretion, lipid peroxidation (LPO, free radical) and defensive mucin secretion, cell proliferation, glycoprotein and glutathione (GSH, an antioxidant). Acute and subacute toxicity studies were also conducted for the safety profile of *Eugenia jambolana.* EJE 200 mg/kg, when administered orally for 10 days in rats was found to reduce the ulcer index in all gastric ulcer models. It tended to decrease acid-pepsin secretion, enhanced mucin and mucosal glycoprotein and decreased cell shedding but had no effect on cell proliferation. It showed antioxidant properties indicated by decrease in LPO and increase in GSH levels in the gastric mucosa of rats. Acute toxicity study indicated LD_{50} to be more than 10 times (>2000 mg/kg) of the effective ulcer protective dose while subactue toxicity study (>1000 mg/kg) indicated no significant change in the general physiological and haematological parameters, liver and renal function tests. The result of the present study indicates that *E. jambolana* seed has gastro-protective properties mainly through promotion of mucosal defensive factors and antioxidant status and decreasing lipid peroxidation.

Key words : antioxidant *Eugenia jambolana* gastric ulceration mucosal offensive defensive factors toxicity study

INTRODUCTION

Worldwide interest in natural products as preventive and therapeutic agents has led to a greater appreciation of the rich heritage of traditional systems of medicine. Dietary and lifestyle modifications are the basis of Ayurvedic medicine, with herbal formulas rounding out therapeutic programs. Ayurvedic formulas contain many balancing herbs offering a high degree of safety and efficacy. Peptic ulcer is a major health

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hazard both in terms of morbidity and mortality. It occurs due to imbalance between offensive (acid-pepsin secretion. *H. pylori,* bile, increased free radicals and decreased antioxidants) versus impaired mucosal resistance (mucus, bicarbonate secretion, prostaglandins, blood flow and the process of restitution and regeneration after cellular injury). Various reports have shown that commonly used drugs for peptic ulcers such as H_2-blockers (ranitidine, famotidine etc.). M_1-blockers (pirenzepine, telenzepine etc), proton pump inhibitors (omeprazole , pantoprazole etc.) have danger of drug interaction, adverse effect and increased incidence of relapses during ulcer therapy (1).

Various herbal drugs notably *Musa sapientum Tectona grandis. Rhamnus procumbens, Rhammis triquerta* Wall. *Withania somnifera,* Shilajit, *Dhatura fastuosa, Fluggea microcarpa* and *Aegle marmelos, Zingiber officinale* and *Asparagus recemosus etc.* have been tried for their ulcer protective effects both experimentally (2) and clinically *(Musa sapientum)* (3) and their effects seem to be due to their predominant effect on mucosal defensive factors. Therefore, the search for an ideal antiulcer drug continues and has also been extended to herbal drugs for their better protection, easy availability, low cost and toxicity.

Various physical (cold restraint stress and pylorus ligation) and chemical (aspirin and ethanol) agents are used for induction of acute gastric ulcers. They induce ulcers by affecting the balance between offensive and defensive factors. NSAIDs can injure the gastro-duodenal mucosa by their topical and systemic effects causing direct tissue damage and through inhibition of prostaglandin synthesis. Ethanol leads to increase in gastric acid secretion and leukotriene C_4 synthesis and histamine release (4) Similarly PL leads to stasis of secretion leading to acid induced mucosal damage ‘ while, stress affects the gastric motility, vagal over activity (5) mast cell degranulation (6) and mucosal blood flow (7).

Jamun is a very common, large evergreen tree of Indian subcontinent. The scientific name of jamun is *Eugenia jambolana* (EJ) or *Syzygium cumini Linn* and it belongs to the myrtaceae family. Other common names for jamun are Java plum, black plum, jambul, Indian blackberry, doowet, faux pistachier etc. It grows naturally in tropical as well as in subtropical zones. The juice is carminative, diuretic and gives a soothing effect on human digestive system (8). The juice of ripe fruit is used for preparing sauces as well as beverages. It is also dried with salt and preserved as a digestive powder or *churan*. The bark, flowers and seeds have been used in diabetes for their hypoglycemic activity (9). Fruits and leaves juices were advocated for dysentry and gingivitis (bleeding gums) (8). *E. jambolana* seeds were further reported to have hypoglycemia (10), anti-inflammatory (11), neuropsycho-pharmacological (12), anti- bacterial (13), anti-HIV (14) and anti-diarrhoeal (15) effects.

MATERIALS AND METHODS

Animals : Charles Foster strain albino mice (25–35 g) and rats (150–200 g) of either sex were obtained from the Central Animal House of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. The

animals were provided with standard rodent pellet diet (Pashu Aahar, Varanasi) and the food was withdrawn 18–24 h before experiment though water was allowed *ad libitum*. All experiments in rats were carried out in accordance with the recommendation of the guidelines for care and use of laboratory animals approved by the Institutional Animal Ethics Committee (NIH publication no. 82–23, revised 1985).

Drug collection and extraction: Fruits of *Eugenia jambolana* (Hyderabad colony, Banaras Hindu University and authenticated by Dr. V. K. Joshi, Head, Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University, Varanasi) were collected in the months of June/July. The seed pulp was taken out, dried under shade and powdered. The ethanolic extract of EJ (EJE) was obtained by soaking the dried seed powder in adequate amounts of ethanol for 7 days and the extract so obtained was filtered. The procedure was again repeated twice using adequate amount of ethanol at an interval of 3 days. The extract was again filtered and mixed with the previous lot. It was then vacuum dried and stored in a refrigerator until further use. The yield was 12.3% of the dried powdered seeds. Ulcer protective anti secretory drug ranitidine (RAN. 2.5 mg/kg) (16) was selected as a standard drug.

Drug treatment: An initial dose-response study was undertaken with 100, 200 and 400 mg/kg of EJE suspended in 1% carboxymethyl cellulose (CMC) when administered orally daily for 10 days to find out the optimal ulcer protective dose against 2 h cold restraint stress (CRS)-induced gastric ulcers in rats. A dose of 200 mg/kg of EJE was then selected on the basis of optimal ulcer protective dose for further studies. The experimental groups received EJE (200 mg/kg) or ranitidine (RAN, 2.5 mg/kg) suspended in 1% CMC' once daily, orally for 10 days, the last dose being given 1 h prior to the experiment to 18 h fasted rats while, the control animals received 1% CMC. The concentration of test drugs was prepared in such a manner that each animal received drug suspension as 1 ml/100 g of body weight.

Antiulcer study: Gastric ulcers in rats were produced by 2 h cold restraint stress (17), 4 h pylorus ligation (18) aspirin (18) and ethanol (19) following the methods reported earlier (16). Briefly, CRS-gastric ulcer (GU) was induced in 18 h fasted rats by strapping them for 2 h at 4–6°C. 4 h pylorus ligation (PL) GU was induced by ligating the pyloric end of the stomach without causing any damage to the blood supply under pentobarbtone (35 mg/kg i.p.) anesthesia while, aspirin (ASP)-induced GU was produced by administering ASP in dose of 200 mg/kg (20 mg/ml). The animals in both PL- and ASP- induced GU were sacrificed after 4 h. The ulcer index in the above groups was calculated by adding the total number of ulcers per stomach and the total severity of ulcers as +1 per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer in pluses (+) after histological confirmation (20). Ethanol (EtOH)-induced GU was produced by administering EtOH orally in the dose of 1 ml/200 g. The animals were sacrificed after 1 h of EtOH administration. Ulcer index was scored based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm^2/rat) (19).

Gastric secretion study : The gastric juice was collected 4 h after PL and centrifuged for 5 minutes at 2000 rpm. The volume of the supernatant was expressed as ml/100 g body weight. Total acid output was determined by titrating with 0.01*N* NaOH, using phenolpthalein as indicator and is expressed as µEq/ml concentration or µEq/4 h as output. Peptic activity was determined using hemoglobin as substrate and has been expressed as µmol of tyrosine/ml as concentration or µmol of tyrosine/4 h as output (21). Dissolved mucosubstances were estimated in 90% alcoholic precipitate of the gastric juice. The precipitate, thus obtained was either dissolved in 1 ml of 0.1 *N* NaOH or 1 ml of 0.01 *N* H_2SO_4. The former was used for the estimation of protein (22), total hexoses, hexosamine and fucose, while the latter was used for the estimation of sialic acid (23). The results are expressed in µg/ml. The ratio of total carbohydrate (TC) (Sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity. DNA content was estimated and expressed as µg/ml gastric juice/100 g weight of rat (24).

Estimation of mucosal glycoprotein: Samples of gastric mucosal scraping were homogenized in distilled water and glycoprotein was estimated in 90% alcoholic precipitate of the gastric mucosal homogenate treated with 90% ethanol. The precipitate so obtained was subjected for the estimation of carbohydrates and proteins following the methods as described above for gastric juice contents.

Cell proliferation: Mucosal scraping was homogenized in 2.5 ml of ice cold 0.6N perchloric acid (PCA). DNA (25) and protein (22) were estimated. The concentration of DNA was expressed as µgDNA/mg protein which is a reliable index of cell proliferation as reported earlier (25).

Free radical and antioxidant: Gastric mucosal lipid peroxidation (LPO) and glutathione (GSH) were estimated in the gastric mucosal homogenate in ice cold normal saline. LPO product malondialdehyde (MDA) and GSH were estimated following the methods of Ohkawa *et al* (26) and Sedlak and Lindsay (27) respectively and were expressed as nmoles/g wet tissue.

Toxicity studies : Acute toxicity study was conducted in equal number of both the sexes of mice (n=10) using the following doses of 100, 200, 500, 1000 and 2000 mg/kg of EJE in 1% CMC. The animals were observed for 72 h to note any changes in behavioural pattern including level of consciousness, gait, food and water intake and mortality. Subacute toxicity study was carried out in equal number of both male and female rats (n=8). EJE was given in dose of 1000 mg/kg, once daily for 4 weeks. Food and water intake, body weight changes and mortality were also observed in this group at an interval of one week up to 4 weeks of treatment with EJE in the above dose. At the end of 4 weeks, the animals were sacrificed by decapitation, blood was collected for hematological studies, liver and renal function tests using commercial kits. Organs were collected, weighed and preserved for histopathological studies.

Statistical analysis : All data were presented as mean ± SE. Statistical significance was calculated from one-way analysis of variance (ANOVA) followed by

Dunnett's multiple comparison tests. Unpaired 't' test was applied for the subacute toxicity study. The differences were considered to be significant when $P<0.05$.

RESULTS

A preliminary study using 100, 200, 400 mg/kg against CRS-induced gastric ulcers showed a dose-dependent decrease in ulcer index (control ulcer index- 31.2 ± 2.7; 18.9% to 48.4% ulcer protection). EJE 200 mg/kg, showing optimal ulcer protective effect was selected for further study. EJE (200 mg/kg) when tested against 4 h PL-, aspirin- and ethanol-induced gastric ulceration in rats showed significant ulcer protection (34.1% to 58.3% protection) and was comparable with standard anti-ulcer drug, ranitidine (Table I).

In 4 h PL rats, EJE tended to decrease acid and pepsin secretion and showed a significant increase in total hexoses and TC: P ratio which is a reliable index of mucin secretion. Further, EJE showed a tendency to decrease cell shedding as evidenced by a decrease in µg DNA/mg protein (Table II). EJE tended to increase the gastric mucosal glycoproteins in terms of individual carbohydrates on TC : P ratio compared to the control group. There was little or no change in cell proliferation either with EJE or RAN compared with control group (Table III).

CRS caused an increase in LPO level by 55.8% while EJE reversed the increase in LPO level induced by CRS near to control value (Table IV). Glutathione (GSH) level was significantly decreased in ethanol-treated control rats. EJE pretreatment caused a reversal in GSH level in rats treated with ethanol near to control value (Table IV).

The acute toxicity study in mice after oral administration of various doses of EJE (100, 200, 500, 1000 and 2000 mg/kg) indicated no behaviourial changes or

TABLE I: Effects of EJE and RAN on gastric ulceration induced by physical (Cold restraint stress and 4 h pyloric ligation) and chemical agents (aspirin and ethanol) in rats.

Oral treatment (mg/kg, od×10 days)	*CRS (4–6°C, 2 h)*		*Pylorus ligation (4 h)*		*Aspirin (200 mg/kg, po, 4 h)*		*Ethanol 1 ml/200 gm, po, 1 h)*	
	Ulcer index	*Percentage protection*	*Ulcer index*	*Percentage protection*	*Ulcer index*	*Percentage protection*	*Ulcer index*	*Percentage protection*
Control (1% CMC)	31.2±2.7	–	10.8±1.3	–	13.3±2.1	–	17.9±1.8	–
EJE 200	18.0±3.2*	34.1	4.5±1.7*	58.3	4.7±1.7*	64.7	9.3±1.8*	48.1
RAN 2.5	16.5±2.4*	48.1	1.8±0.7*	83.3	4.9±1.3*	54.9	9.4±1.7*	47.5
One way ANOVA								
F	8.298		13.434		8.454		7.964	
df	17		17		17		17	
P	<0.05		<0.001		<0.05		<0.001	

Results are mean±SE of 6 rats in each group.
One-way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group. The difference was considered to be significant when *P<0.05 when compared with control group.

TABLE II: Effects of EJE and RAN on gastric secretion parameters in 4 h pylorus ligated rats.

Juice parameters	*NR*	*EJE*	*RAN*
Volume (ml/100 g)	1.74±0.17	1.63±0.18	1.11±0.06*
Acid			
Concentration (µEq/ml)	81.0±7.5	76.3±3.2	57.2±3.6*
Output (µEq/4 h)	135.8±13.5	125.5±17.7	80.4±5.5*
Pepsin			
Concentration (µmol/ml)	258.6±24.9	191.7±28.4	171.7±14.0*
Output (µmol/4 h)	465.3±67.7	289.0±31.4	242.6±24.5*
Mucoproteins (µg/ml)			
Total hexoses	277.0±15.9	360.4±21.8*	284.3±15.5
Hexosamine	151.9±15.4	176.1±23.2	166.4±16.1
Fucose	82.6±4.4	84.4±3.4	75.0±4.7
Sialic Acid	27.4±3.8	26.9±1.5	24.7±2.1
Total carbohydrate (TC)	538.4±30.1	647.8±41.7	550.8±25.2
Protein (P)	603.8±44.5	526.4±37.8	625.2±26.8
TC : P	0.93±0.06	1.25±0.05*	0.88±0.03
Cell shedding			
DNA (µg/ml)	129.0±17.6	82.3±8.3	94.5±8.7

Results are mean±SE of 12 rats in NR group and 8 rats in EJE and RAN group.
One-way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group. The difference was considered to be significant when *P<0.05 when compared with control group.

TABLE III: Effects of EJE and RAN on gastric mucosal glycoprotein and cell proliferation in 4 h pylorus ligated rats.

Mucosal parameters	*NR*	*EJE*	*RAN*
Glycoproteins (µg/ml)			
Total hexoses	2297±103	2471±164	2266±120
Hexosamine	1518±84	1636±114	1500±101
Fucose	204±18	271±20*	186±19
Sialic Acid	104±7	128±11	102±9
Total carbohydrate (TC)	4117±161	4506±142	4053±134
Protein (P)	5547±327	5681±333	5766±138
TC : P	0.74±0.03	0.81±0.05	0.71±0.02
Cell shedding			
Proteins (µg)	6025±446	5917±361	6527±263
DNA (µg)	651±26	673±21	673±25
µgDNA/mg protein	108±4	114±13.7	104±3.5

Results are mean±SE of 12 rats in NR group and 8 rats in EJE and RAN group.
One-way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group. The difference was considered to be significant when *P<0.05 when compared with control group.

TABLE IV: Effects of EJE and RAN on rat gastric mucosal lipid peroxidation (LPO) and glutathione (GSH) levels.

Treatment		*Lipid peroxidation*	*Treatment*	*Glutathione*
		LPO (nmol/g wet tissue)		GSH (µmol/g wet tissue)
Control (1% CMC)		189.5±17.4	Control (1% CMC)	367±15
CRS (1% CMC)		295.3±16.0*	EtOH (1% CMC)	295±13*
EJE + CRS		201.3±19.3[a]	EJE + EtOH	393±27[a]
One-way ANOVA	F	10.839		6.647
	df	17		17
	P	<0.01		<0.05

Results are mean±SE of 6 rats in each group.
One-way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group. The difference was considered to be significant when *P<0.05 compared to respective control group, [a]P<0.05 compared to CRS or EtOH group.

mortality up to 72 h after treatment. Subacute toxicity studies with EJE (1 g/kg) in rats (4 weeks treatment) indicated no significant change in general physiological parameters (body weight, food and water intake), organ weight (liver, kidney, adrenals and testis), haematological parameters (hemoglobin and WBC count), liver function (total bilirubin, SCOT, SGPT, alkaline phosphatase, total protein and albumin) and renal function (blood urea and creatinine) tests. Histological studies of liver, kidney, testis and ovaries did not indicate any gross or microscopic changes.

DISCUSSION

Peptic ulcer is defined as disruption of the mucosal integrity of the stomach and/ or duodenum leading to a local defect or excavation due to active inflammation. Despite the constant attack on the gastroduodenal mucosa by a host of noxious agents (acid, pepsin, bile acids, pancreatic enzymes, drugs, and bacteria), integrity is maintained by an intricate system that provides mucosal defense and repair. This intricate biologic system consist of mucus-bicarbonate layer, surface epithelial cells and a rich submucosal micro-circulatory bed which provides bicarbonate ions to neutralize the acid generated by parietal cell secretion of hydrochloric acid. Moreover, this microcirculatory bed provides an adequate supply of micronutrients and oxygen while removing toxic metabolic by products.

The present study indicated that ethanolic extract *of Eugenia jambolana* (EJE) showed ulcer protective effects against cold restraint stress-, pylorus ligation-, ethanol- and aspirin-induced gastric ulcers in rats. In all these conditions, mucosal erosions or ulceration arise when the caustic effects of aggressive factors (acid, pepsin, bile) and diminished mucosal blood How overwhelm the defensive factors of the gastrointestinal mucosa (mucus and bicarbonate secretion, prostaglandins, blood flow and the process of restitution and regeneration after cellular injury).

As mentioned earlier stress plays an important role in the pathogenesis of ulcers

by playing role in number of factors like increase in gastric motility, vagal over activity (5), mast cell degranulation (6) decrease gastric mucosal blood flow and decrease prostaglandin synthesis (7). The role of acid is questionable but decrease in mucous secretion has been reported during stress induced ulcer (28) EJE afforded ulcer protection in CRS principally by affecting the mucosal defensive factors. Decrease in gastric juice content indicates increase in life span of mucosal cells (28). EJE tended to decrease the gastric juice DNA content indicated an increase in life span of mucosal cells and mucosal resistance. EJE did not affect cell proliferation hence protection afforded by EJE may be because of cell restitution rather than cell proliferation. Pylorus ligation-induced ulcers are thought to be caused due to increased presence of acid and pepsin in the stomach (1). EJE tended to decrease the acid and pepsin secretion in the stomach and increase the TC: P ratio significantly indicating enhanced mucous secretion. Aspirin induced ulcers cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H^+ ions and thus leading to breaking up of mucosal barrier. EJE showed significant protection against aspirin induced ulcer. EJE tended to decrease the acid secretion and increased the mucous secretion which could be capable of preventing back diffusion of H^+ ions. Ethanol induced ulcers are caused by different factors including decreased mucosal blood flow, damage to capillary endothelium and release of arachidonate metabolites specifically LTC_4/D_4, PAF and histamine (4). Increase in mucosal protective factors may be the major factor responsible for the ulcer protection of EJE.

Stress causes both sympathetic and parasympathetic stimulation of stomach leading to local hypoxia (near or actual “ischemia”). The ischemic condition caused an increase in the levels H_2O_2 which in conjugation with O_2 generates OH^- ions which oxidized various cellular constituents such as proteins, membrane lipids and depletes glutathione. Lipid peroxidation causes loss of membrane fluidity and loss of cellular function (29). Treatment with EJE significantly decreased the LPO levels of gastric mucosa against CRS-induced changes in LPO in rats. Reduced glutathione in conjugation with glutathione peroxidase and glutathione S transferase (GST) plays a central role in the defense against free radicals. Thiols such as glutathione are able to bind to reactive free radicals and may influence the physical properties of mucous since its subunits are joined by disulphide bridges. Diethyl malate markedly depletes gastric glutathione and causes severe gastric ulceration indicating the defensive role played by glutathione in ulcer formation (30). Ethanol ingestion has been found to decrease levels of GSH in rats (31). In our present study the decrease in GSH levels by ethanol ingestion was increased by EJE treatment indicating an enhanced anti oxidant status and ulcer protection.

Drugs used in the treatment of acid peptic disorders may be divided into two classes i.e. agents that reduce intragastric acidity e.g. antacids, H_2-receptor antagonists, proton pump inhibitors (PPI) etc and that promote mucosal protection e.g. sucralfate, prostaglandin analogs, colloidal bismuth compounds etc. It is now assumed that drugs ultimately balance the aggressive (acid, pepsin, *H. pylori* and bile salts) and defensive

factors (mucin secretion, cellular mucus, bicarbonate secretion, mucosal blood flow, cell turnover, etc) and lead to successful ulcer therapy (1). Although these drugs have brought about remarkable changes in ulcer therapy, the efficacy of these drugs is still debatable.

Flavanoid diglycosides (32) (Quercetin and Myricetin), hydrolysable tannins (1-0-galloyl castalagin and casuarinin (33) and a triterpene, oleanolic acid (34) were isolated from seeds of *Eugenia jambolana*. The gastric ulcer protective activity of EJE could be because of the presence of flavanoids in the seeds. A further detailed phytochemical study of EJE should be carried out in order to exactly estimate the concentration of various constituents.

Acute toxicity studies of EJE were carried out in live close ranges 100, 200, 500, 1000, 2000 mg/kg. The maximum dose tested being 10 times the dose given for ulcer protective effects and was found to be safe in all the mice without any mortality and behavioural changes. The histological parameters, haematological parameters, liver and kidney function test done in rats indicated EJE to be a safe drug.

The ulcer protective activity of *Eugenia jambolana* may be due to its effects on both offensive and defensive factors. The antioxidant properties of EJE also contribute towards its activity. Further work on other parameters like gastric mucosal expression and release of tumor necrosis factor-α, interleukin-1β, vascular endothelium growth factor, platelet endothelial cell adhesion molecule-1 and the mucosal expression of heat shock protein 70 which are affected in gastric ulcer (35) may throw more light in understanding the mechanism of ulcer protection by EJE.

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