ANTIULCEROGENIC EFFECT OF ETHANOLIC EXTRACT OF PORTULACA OLERACEA EXPERIMENTAL STUDY

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Summary

Gastroprotective effect of 50% ethanolic extract of *Portulaca oleracea* (POE) Portulacaceae have assessed in different gastric ulcer models in rats. POE (50, 100 and 150 mg/kg body weight) was administered orally, twice daily for 5 days for prevention from ethanol (EtOH) and 10 days for prevention of acetic acid induced ulcers. POE showed dose dependent inhibition of ulcer index in ethanol and acetic-acid induced ulcers. POE prevents the oxidative damage of gastric mucosa by blocking lipid peroxidation and by significant decrease in superoxide dismutase, and increase in catalase activity. The ethanolic POE showed significant gastric ulcer protective effect in doses of 50-150 mg/kg, when given twice daily for 5 days against gastric ulcers induced by ethanol (EtOH), aspirin (ASA), cold restraint stress (CRS) and pyloric ligation (PL). POE showed dose dependent decrease in ulcer index (UI) against ulcers induced by: (i) ethanol (control UI: 24.5±3.0 mm2/rat, POE% decrease 62.4 -86.1%, PB 0.05 to PB 0.001); (ii) aspirin (control UI: 14.2±1.8, POE% decrease 28.9 – 77.5%, PB 0.1 to PB 0.001); (iii) cold restraint stress (control UI: 23.2±3.1, POE% decrease 38.4 – 73.27%, PB 0.2 to PB 0.001); and (iv) pylorus ligation (control UI: 20.1±2.4, POE% decrease 36.8 -82.1%, PB 0.1 to PB 0.001). Our results show that POE possesses significant gastroprotective activity which might be due to gastric defence factors.

Keywords: *Portulaca oleracea*; Anti-ulcer; Antioxidant; Gastroprotective; Lipid peroxidation

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Introduction

Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood ulcers are essentially due to imbalance between offensive and defensive factors[1]. Ulcer therapy is now mainly focused on limiting the deleterious effects of offensive acid secretion, but the search for new safer alternative drugs have rekindled the interest in cytoprotective drugs, which protect the gastric mucosa from damaging agents without influencing acid secretion or neutralising intragastric acidity[2]. Although few drugs like sucralfate and prostaglandin analogs, i.e. misoprostol are recognised as cytoprotective agents[3,4] many natural drugs have been reported to possess this activity[1,4,5].

Portulaca oleracea L. (Portulacaceae) is a grassy plant with small-yellow flowers and height of 10-30 cm. Polysaccharide from leaves of *P. oleracea* L. (POP) has been recently studied for physiological and pharmaceutical activities[6]. *P. oleracea* is listed in the WHO as one of the most used medicinal plants and it has been given the term 'Global Panacea'[7,8]. The aerial parts of the plant are used medicinally for alleviating pain and swelling, and as an antiseptic[9]. A recent report indicated that an extract of P. oleracea accelerates wound healing and treatment of indomethacine and phenylbutazone-induced ulcers[10].

Material and methods

Plant materials and extraction

Plant material of *P. oleracea* was collected from the Lucknow, U.P. India. The plant material were identified taxonomically and authenticated by National Botanical Research Institute, Lucknow. The shade dried seeds *P. oleracea* L. were ground in a high speed disintegrator to obtain a fine powder; then they were extracted in a soxhlet apparatus with 50% ethanol. Briefly, the organic solvent was volatilized and pretreated dry powder was obtained (20.0 g).

Animals

Albino rats (150-175 g) and albino mice (20 - 25 g) were obtained from the animal colony of National Laboratory Animal Centre, Lucknow.

They randomly distributed into various groups and housed in cages (6 per cage) and maintained under standard conditions i.e. 26 ± 2 °C and relative humidity 44 - 56% and 10 h light: 14 h dark cycles each day for one week before and during the experiments. All animals were fed standard rodent pellet diet (Amrut, India) and drinking water *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA).

Experimental procedure

POE, suspended in 1% carboxy methyl cellulose (CMC) in distilled water in doses of 50, 100 and 150 mg/kg and Omeprazole, the reference drug, in the dose of 50 mg/kg were administered orally twice daily at 10:00 and 16:00 h, respectively, for 5 days for ulcer protective studies. Further the effective dose of POE 50 mg/kg, b.d for 5 days was used for secretion and mucosal studies, and up to 10 days for ulcer healing study. Control group of animals received suspension of 1% CMC in distilled water.

Anti-ulcer study

The following experimental models were used.

Aspirin (ASA)-induced ulcers

ASP in dose of 200 mg/kg (20 mg/ml) was administered to the animals on the day of the experiment and ulcers were scored after 4 h[11]. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer after histological confirmation as follows[12]: 0, no ulcer; +,pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1–2mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; ++++, ulcer either more than 2mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscularis still remaining unaffected. The pooled group ulcer score was then calculated according to the method of Sanyal et al. (12).

Ethanol (EtOH)-induced ulcers

The gastric ulcers were induced in rats by administering EtOH 1 ml/200 g, 1 h[13] and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored by a person unaware of the experimental protocol, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm2/rat).

Cold-restraint stress (CRS)-induced ulcers

On day 6 to 18 h fasted rats, cold restraint stress was given by strapping the rats on a wooden plank and keeping them for 2 h at 4- 6 °C. The animals were then sacrificed by cervical dislocation and ulcers were scored on the dissected stomachs[14] as described above.

Acetic acid-induced ulcers

The rats were anaesthetized with pentobarbitone (35 mg/kg, i.p.). The abdomen was opened and the stomach was visualized. A cylindrical glass tube of 6 mm in diameter was tightly placed upon the anterior serosal surface of the glandular portion of stomach 1 cm away from the pyloric end. A total of 50% acetic acid (0.06 ml/ animal) was instilled into the tube and allowed to remain 60 s on the gastric wall. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. POE was given in the dose of 20 mg/kg on day 1, orally, twice daily, 4 h after the application of acetic acid and continued either up to 5 or 10 days after induction of the ulcer. The animals were then sacrificed after 18 h of the last dose of drug either on day 6 or day 11 of experiment to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (mm2/rat) of ulcers[15]. Statistical significance was calculated using unpaired Student's t -test.

Pylorus-ligation (PL)-induced ulcers

Drugs were administered for a period of 5 days as described above. On day 6 after the last dose, the rats were kept for 18 h fasting and care was taken to avoid coprophagy. Animals were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period[16]. After 4 h, stomachs were dissected out and contents were collected into tubes for estimation of biochemical parameters. The ulcers were scored as described under ASA-induced ulcers.

Gastric secretion study

The gastric juice was collected 4 h after PL and centrifuged for 5 min at 2000 rpm. The volume of the supernatant is expressed as ml/100 g body weight while total acid output was determined by titrating with 0.01N NaOH, using phenolphthalein as indicator and is expressed either as µEq/ml for concentration or µEq/4 h for output. Peptic activity was estimated following the method of[17] and expressed either as µmol of tyrosine/ml for concentration or µmol of tyrosine/4 h for output. Dissolved muco-substances were estimated in the 90% alcoholic precipitate precipitate of the gastric juice. Protein[18], total hexoses, hexosamine, sialic acid and fucose, the constituents of the above dissolved mucosubstances, were estimated[19]. The results are expressed in µg/ml. The ratio of total carbohydrates (TC, sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity[19]. DNA content was estimated and expressed as µg/ml gastric juice 100 g weight of rats[20].

Estimation of mucosal glycoproteins

Samples of gastric mucosal scraping were homogenised in distilled water and treated with 90% ethanol and were subjected for the estimation of carbohydrates and proteins using the methods described above for gastric juice contents[21.] Statistical analysis of data was done by using unpaired Student's *t*-test.

Cell proliferation

Estimation of DNA in gastric mucosa

Mucosal scraping was homogenized in 2.5 ml of ice cooled 0.6 N perchloric acid (PCA). DNA[22] and protein[18] were then estimated. The concentration of DNA is expressed as µg DNA/mg protein.

Estimation of free radical generation

POE in the dose of 20 mg/kg was given orally, daily for 5 days and on day 6 of experiment, 1 h prior to subjecting the animals to CRS, the animals were then sacrificed and the ulcer index was calculated as described earlier. The fundic part of the stomach was homogenized (5%) in ice cold 0.9% saline with a Potter-Elvehjem glass homogenizer for 30 s. The homogenate was then centrifuged at 800 ×g for 10 min followed by centrifugation of the supernatant at 12,000×g for 15 min and the obtained mitochondrial fraction was used for the following estimations[23,24].

Lipid peroxidase (LPO) activity

LPO product malondialdehyde (MDA) was estimated using 1,1,3,3-tetraethoxypropane as the standard and is expressed as nmol/mg protein[25].

Superoxide dismutase (SOD) activity

SOD was estimated by following the procedure of[26] Kakkar et al., (1984). The inhibition of reduction of nitro blue tetrazolium (NBT) to blue colored formozan in presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using n-butanol as blank. One unit (U) of enzyme activity was defined as the amount of enzyme that inhibits rate of reaction by 50% in 1 min under the defined assay conditions and the results have been expressed as U of SOD activity/mg protein.

Catalase (CAT) activity

Decomposition of H_2O_2 in presence of catalase was followed[28] at 240 nm Beers and Sizer, (1952). One unit of (U) CAT was defined as the amount of enzyme required to decompose 1 mmol of H_2O_2/min, at 25 °C and pH 7.0. Results are expressed as U of CAT activity/mg protein. Statistical analysis was done by Student's t -test.

Statistical analysis

Values were represented as mean±S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newmann Keuls test for the determination of level of significance.

Results

Antiulcer and ulcer healing effects

The ethanolic POE showed significant gastric ulcer protective effect in doses of 50-150 mg/kg, when given twice daily for 5 days against gastric ulcers induced by ethanol (EtOH), aspirin (ASA), cold restraint stress (CRS) and pyloric ligation (PL). POE showed dose dependent decrease in ulcer index (UI) against ulcers induced by: (i) ethanol (control UI: 24.5±3.0 mm2/rat, POE% decrease 62.4 -86.1%, PB 0.05 to PB 0.001); (ii) aspirin (control UI: 14.2±1.8, POE% decrease 28.9 – 77.5%, PB 0.1 to PB 0.001); (iii) cold restraint stress (control UI: 23.2±3.1, POE% decrease 38.4 – 73.27%, PB 0.2 to PB 0.001); and (iv) pylorus ligation (control UI: 20.1±2.4, POE% decrease 36.8 -82.1%, PB 0.1 to PB 0.001) (Table 1). A total of 50% acetic acid when applied to the serosal surface of rat gastric mucosa in the fundal region near to the pyloric end produced chronic

gastric ulcers. POE 100 mg/kg significantly healed ulcers induced by 50% acetic acid after 5 (control UI: 22.28 ± 0.81, healing 36.35%, PB/0.001) and 10 (control UI: 13.88 ± 0.81, healing 60.89%, PB/0.01) days of treatment (Table 2).

Table 1: Effect of ethanolic extract of *Portulaca oleracea* (POE mg/kg, twice daily for 5 days) on ethanol (EtOH, 100%, 1 ml/200 g, p.o., 1 h), aspirin (ASA, 200 mg/kg, p.o., 4 h)-cold restraint stress (CRS) and pylorus ligation (PL) induced gastric ulcers in rats

Treatment (mg /kg)	Ulcer index			
	EtoH	ASA	CRS	PL
Control	24.5±3.0	14.2±1.8	23.2±3.1	20.1±2.4
POE 50mg	9.2±4.6a	10.1±2.1	14.3±3.6	12.7±3.6
POE 100mg	6.4±3.6b	7.3±1.8a	8.2±2.1b	6.9±1.4c
POE 150mg	3.4±1.9c	3.2±1.4c	6.2±1.8c	3.6±1.3c
Omeprazole 50 mg	4.3±2.1c	3.5±1.5c	9.2±2.2b	2.7±1.1c

[a] P<0.05; [b] P<0.01; [c] P<0.001 as compared to their respective control. Data are mean±S.E.M. n=6 in each group

Table 2 - Effect of *Portulaca oleracea* Linn. Rhizomes extract (twice daily for five days) on acetic acid-induced gastric ulcer.

Treatment and Dose (mg/kg)	5 days treated Ulcer index	% incident of perforation	10 days treated Ulcer index	% inciden perforatio
Control	22.28 ± 0.81	--	18.18 ± 0.81	--
POE 50mg	20.35 ± 0.62	8.66%	11.88 ± 0.85	14.41%
POE 100mg	14.18 ± 0.70[b]	19.30%	7.11 ± 0.87[b]	35.81%
POE 150mg	12.13 ±0.94[a]	45.56%	3.93 ± 0.55[a]	71.68%
Omeprazole 50 mg	11.68 ± 0.59[a]	47.58%	2.18 ± 0.25[a]	84.29%

Values are mean ± SEM for 6 rats. [a]P< 0.001 compared to respective control group. [b]P < 0.01 compared to respective control group.

Effect on acid-pepsin secretion

The effect of POE on various parameters of offensive factors was studied the volume, acid and pepsin secretion in the gastric juice of 4 h PL rats. The mean ± S.E.M. values of control group were: volume 1.89±0.31ml/100 g body weight; acid concentration 129.2±5.8 µEq/ml and output 237.3±23.5 µEq/4h; pepsin concentration 453.4±49.2µmol of tyrosine/ml and output 825.6±90.1µmol of tyrosine/4 h respectively. POE 100 mg/kg decreased the volume (decrease 13.75%, PB 0.05), acid concentration (decrease 1324%, PB 0.01), output (decrease 21.87%, PB 0.01), pepsin concentration (6.57%, PB 0.4), output (4.88%, PB 0.001) while Omeprazole caused inhibition of the above parameters to 6.88%, 19.12%, 23.93%, 37.0% and 40.0% (PB 0.001) respectively (Table.3).

Table 3 Effect of *Portulaca oleracea* (POE) (mg/kg, po, bd for 5 days) on gastric juice volume, acid, pepsin and DNA (mean ± S.E.M., *n* = 8) in 4 h PL rats

Treatment	Volume (ml/100 g)	Acid		Pepsin		DNA (µg/ml)
		Conc. (µEq/ml)	Output (µEq/4 h)	Conc. (µmol/ml)	Output (µmol/4 h)	
Control	1.89±0.31	129.2±5.8	237.3±23.5	453.4±49.2	825.6±90.1	238.2±12.5
POE 150mg	1.83±0.25	112.1±4.6	185.4±28.1	423.6±32.4	785.3±23.8	113.3±14.2b
Omeprazole 50mg	1.76±0.24	104.5±8.5	180.5±16.8	285.3±25.4a	495.3±45.8a	170.3±11.9a

[a]P <0.05 as compered to their respective control. [b]P<0.001 as compered to respective control

Effect on cell shedding and proliferation

Increase or decrease in life span of mucosal cells can be expressed as the amount of DNA present in the gastric juice after test drug treatment the mean± S.E.M. value of control DNA content of gastric juice was: 238.2±12.5µg/ml per 100 g body weight and was decreased significantly both by POE and Omeprazole pretreatment indicating enhancement of life span of mucosal cells (POE 52.43% inhibition, PB 0.05; Omeprazole 28.5% inhibition, PB 0.05) (Table.3).

For cell proliferation study, the effect of POE 100 mg/kg was seen both on the weight of the glandular portion of rat stomach and µg DNA/mg protein which are indicative of any cell proliferation. The control values (mean±S.E.M.) for glandular weight (mg/100 g body weight), mucosal protein and DNA (µg/100 mg wet tissue) and µg DNA/mg protein were: 445±45, 5468±470, 610±38, and 105±9, respectively. POE showed little or no change in all the above parameters (glandular weight 435±39 mg/100 g body weight, 2.2% increase; protein 5892±439µg/100 mg wet tissue, 7.7% decrease; DNA 569±48 mg/100 mg wet tissue, 7.2% increase and mg DNA/mg protein 102±9, 2.8% increase).

Table 4 Effect of *Portulaca oleracea* (POE) on gastric juice mucoprotein and mucosal glycoprotein in 4h PL rats (data are mean±SEM, n=6).
[a]P <0.05 as compared to respective control. [b]P <0.01 as compared to respective control. [c]P <0.001 as compared to respective control.

Oral treatment	Protein (P)	Total hexoses (A)	Hexose-amine (B)	Fucose (C)	Sialic acid (D)	TC (A+B+C+D)
Mucoprotein (µg/ml)						
Control	451.4±35.6	305.2±21.6	158.3±13.4	55.8±2.9	35.4±2.6	548.3±34.5
POE 150mg	370.1±23.5	398.4±18.9a	170.1±8.3	64.2±3.9	40.1±1.9a	650.2±18.9a
Omeprazole	322.6±21.6a	435.1±32.5b	173.2±13.8	70.1±2.4	48.6±4.2b	743.6±5.8a
Glycoprotein (µg/100mg wt tissue)						
Control	5051±258	2028±215	1658±189	184±9	90±6	4031±270
POE 150mg	4256±14	3150±224a	2043±191a	183±8	110±8a	5563±305b
Omeprazole 50mg	4122±309	2916±265b	2613±340	192±18	127±11c	5981±498

Table 5. Effect of *Portulaca oleracea* (POE) on cell proliferation and weight of glandular portion of stomach in pylorus-ligated rats (data are mean ±SEM, n=6 in each group).

Treatment (mg/kg o.d. x 5 days)	Weight of glandular portion of stomach (mg/100g BW)	Cell proliferation		
		Protein	DNA (µg/100mg wet tissue)	µg DNA /mg protein
Control	445±45	5468±470	610±38	105±9
POE 150mg	435±39	5892±439	569±48	102±9
Omeprazole 50mg	431±38	5842±435	548±45	101±9

Effect on mucin secretion and mucosal glycoproteins

Both POE 150 mg/kg and Omeprazole 50 mg/kg either tended to increase or increased the concentration of individual carbohydrates and total carbohydrates (TC) in the alcoholic precipitate of gastric juice with significant decrease in protein (P) content leading to significant increase in TC:P ratio, a marker of mucin secretion (Table 4). Both POE and Omeprazole showed again similar effect on mucosal glycoproteins content of the mucosa as observed by an increase in TC:P ratio.

Antioxidant effect

Stress significantly caused ulceration (control un-stressed UI 0.0±0.0, stress UI 24.6±4.2, PB 0.001) with concomitant increase in LPO (control 137±12, stress 268±27 nmol MDA/mg protein, PB 0.001) and SOD (control 96.5±10.1, stress 245.7±7.2 U/mg protein, PB 0.001) and decrease in CAT (control 68.7±4.3, stress 29.4±2.5 U/mg protein, PB 0.001). When the animals were pretreated with POE there was significant reversal in the ulcer index, LPO, SOD and CAT levels near to the normal values when compared to the stress group (UI 6.5±2.7, PB 0.001; LPO 78±1.3, SOD 120.8±19.5, CAT 61.8±6.9 (Fig. 1).

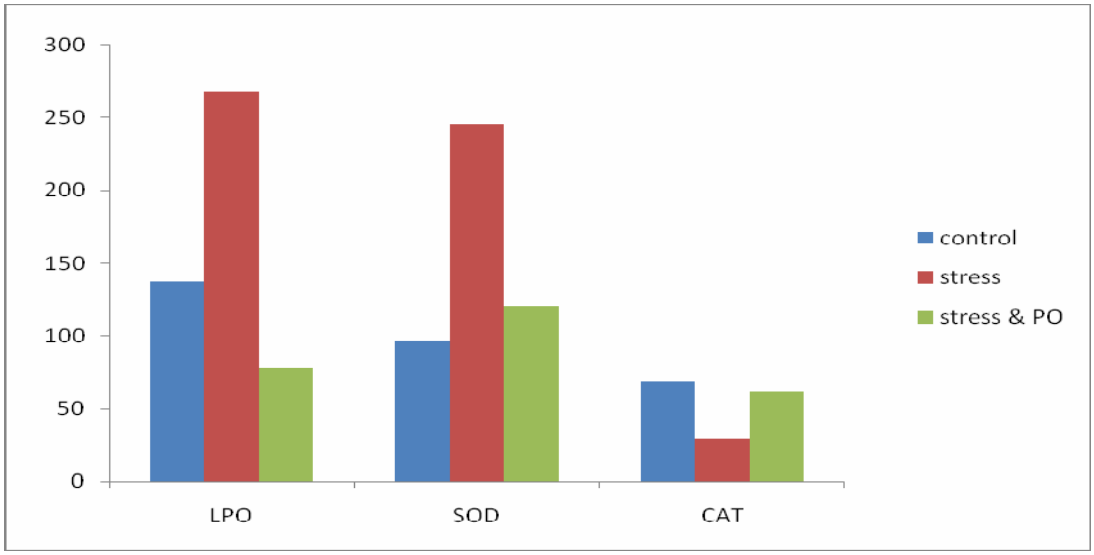

Fig. 1. Effect of stress (2 h) and stress +POE (150 mg/kg, orally, twice daily for 5 days) on LPO, SOD and CAT levels in rat gastric mucosal homogenates. Values are expressed as mean % control, n=6. Mean control ±S.E.M. values are: LPO 78±1.3 nmol MDA/mg protein; SOD 120.8±19.5 U/mg protein; CAT 61.8±6.9 U/mg protein.

Discussion

POE showed significant ulcer protective and healing effects as observed from significant decrease in acute ulcers induced by ethanol, aspirin, cold restraint stress and pyloric ligation and healing of chronic ulcers induced by acetic acid. Gastric ulcer is often a chronic disease and it may persist for 10–20 years characterized by repeated episodes of healing and re-exacerbations. The incidence of ethanol-induced ulcers is predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products[28] and reactive oxygen species[29] resulting in the damage of rat gastric mucosa[30]. Ethanol-induced depletion of gastric wall mucus has been prevented by POE. Ethanol is metabolised in the body and releases superoxide anion and hydro-peroxy free radicals. Ulcers caused by ethanol are due to superficial damage to mucosal cells[31] and damage by NSAIDs are due to decrease in PG synthesis and increase in acid secretion[32].

Pylorus ligation-induced ulcers are due to auto-digestion of the gastric mucosa and break down of the gastric mucosal barrier[33]. Its reported adaptogenic[34] and antimicrobial[35] effects may as well account for part of its anti-ulcerogenic activity. POE significantly decreased the acid and pepsin secretion. Mucus serves as first line of defense against ulcerogens. Mucus is secreted by the mucus neck cells and covers the gastric mucosa thereby preventing physical damage and back diffusion of hydrogen ions[36]. Hence increase in synthesis of mucus may be one of the important contributing factors for ulcer protective role of POE.

The decrease in DNA content of gastric mucosa indicates decreased cell shedding and increased life span of cells[20]. Increase in glycoprotein content of gastric mucosa is evidenced from increase in TC: P ratio of the mucosal cells, which is taken as marker for cellular mucus[21]. This increase was due to increase in muco-polysaccharides, the major constituent of mucus and also which are responsible for viscous nature and gel-forming properties of the mucus.

Ulcers due to stress are both due to physiological and psychological factors[37] and those by pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa[32]. Chronic ulcers by acetic acid are due to increase in volume of acid output leading to subsequent pyloric obstruction and mucosal necrosis[38]. Stress plays an important role in etiopathology of gastro-duodenal ulceration. Increase in gastric motility, vagal over activity[39] mast cell degranulation[40] decreased gastric mucosal blood flow[41] and decreased prostaglandin synthesis[42] are involved in genesis of stress induced ulcers. Acetic acid-induced ulcer better resembles clinical ulcers in location, chronicity and severity and servers as the most reliable model to study healing process[38]. POE significantly healed the penetrating ulcers induced by acetic acid after 5 and 10 days treatment.

The role of free radicals in gastric ulcerations is well documented[43]. POE significantly reduced lipid peroxidation in rat gastric mucosa. SOD scavenges the super oxide radical O_2^-, one of the reactive oxygen species (ROS) responsible for lipid peroxidation[44]. This reaction leads to increase in generation of peroxyl radical $H_2O_2^-$, which is also capable of producing more oxidative damage[45]. CAT and other peroxidases further reduce $H_2O_2^-$. Hence, the anti-oxidant activity in gastric mucosal homogenates observed from decrease in LPO may be due to increase in SOD and CAT levels. During stress LPO and SOD were significantly increased and CAT level was significantly decreased. The increase in SOD was due to increased ROS generation during mucosal damage.

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