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Ulcer Healing Properties of Ethanolic Extract of *P. oleracea* in Streptozotocin-induced Diabetic Rats

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Abstract

Portulaca oleracea (*P. oleracea*) has been reported to have both antidiabetic and antiulcer activities. The present investigation evaluates the gastric ulcer healing properties of ethanolic extract of *P. oleracea* in streptozotocin-induced diabetic rats. Streptozotocin (STZ, 50 mg/kg intraperitoneal) was used to induce diabetes mellitus in rats. Aspirin (200 mg/kg, intraperitoneal) was administered for initial 7 days to induce gastric ulcerations in the diabetic rats. The ethanolic extract of *P. oleracea* was administered in different doses (100, 150 and 250 mg/kg, orally) in STZ-induced diabetic rats. Various biochemical markers of blood and tissue origin were also estimated to compare the ulcer healing potential of this extract. Of all the three doses of *P. oleracea* tested, the dose of 250 mg/kg exhibited dose-dependent significant ulcer healing property along with the better antidiabetic property as compared to other two doses. The 50% ethanolic extract of *P. oleracea* can be beneficial in the treatment of gastric ulcers and diabetes.

Key-Words: Ethanolic extract, Diabetes, Ulcer, *P. oleracea*

Introduction

Diabetes mellitus has been described as a metabolic disorder usually associated with hyperglycemia, which brings about complications, that affects multiple organs particularly if it persists for a long time either in type 1 or type 2 diabetes mellitus (Rodrigues & Motta, 2012). Gastric mucosal damage has also been associated with diabetes (Morsy et al., 2010). Diabetic patients are prone to acute gastric injury and impaired ulcer healing which could aggravate to acute stress-induced gastric lesion (Konturek et al., 2010; Harsch et al., 2003). It is reported that severe gastric inflammation or ulcer can affect gastric motility in diabetic patients (Boehme et al., 2007). Healing impairment of chronic ulcers in diabetes mellitus has been attributed to release of proinflammatory cytokines such as tumor necrosis factor-α and the diminished activity of the mucosal antioxidative system (Brzozowska et al., 2004). Helicobacter pylorus, an organism implicated in ulcer has been noted to have a high prevalence amongst diabetics (Tseng, 2012).

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Many indigenous Indian tropical medicines have been found useful in successfully managing the diabetes and ulcer. Recently Sharma et al., 2010 studied the antihyperglycemic and antioxidant potential of polysaccharide fraction from *P. oleracea* seeds against streptozotocin-induced diabetes in rats. Previously, authors have reported significant antidiabetic potential of *P. oleracea* seeds in streptozotocin (STZ)- induced hyperglycemia and oxidative stress (Sharma et al., 2009). Arun et al., 2010 reported the antiulcerogenic effect of ethanolic extract of *P. oleracea* experimental study. In the continuation of the study, there is no report of antiulcer activity of *P. oleracea* against experimental gastric ulcers with coexistence of diabetes in rats. Using aspirin for the induction of gastric ulcer in streptozotocin (STZ)-induced type II diabetes, this study thus initiated to investigate the healing property of gastric ulcer of different dose of *P. oleracea* extracts (POEt) in diabetes.

Material and Methods

Test Animals

Albino mice (20-25g) were obtained from the animal colony of National Laboratory Animal Centre, Lucknow, India. They were maintained in standard environmental conditions and had free access to feed

and tap water ad libitum during quarantine period. The animals were kept fasting overnight but allowed free access to the water. All studies were performed in accordance with the guidance for care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, India (Reg. No. IAEC/NBRI/PH/6-6).

Plant Materials and Preparation of Extract

The seeds of *P. oleracea* were procured from Lucknow (U.P.) India. The seeds were identified taxonomically and authenticated by taxonomist in National Botanical Research Institute, Lucknow, India. The shade dried seeds were crushed, powdered and exhaustively extracted with 10 volumes of 50% ethanol. The extract was filtered, pooled and concentrated on rotavapour (Buchi, USA) and dried in lyophilizer (Laboconco, USA) under reduced pressure to obtain residue (POEt).

Preliminary Phytochemical Screening and High Performance Thin Layer Chromatography (HPTLC) Analysis

Preliminary qualitative phytochemical screening of POEt gave a positive result for steroids, carbohydrates, triterpenoids, resins, flavonoids, and tannins. On the basis of preliminary phytochemical test, HPTLC analysis was done to quantify the flavonoids. Quercetin and POEt were spotted using a Camag Linomat IV spotter on the pre-activated silica gel 60/UV254 HPTLC plates (Merck, India) as a stationary phase. Plates were eluted with toluene: ethyl acetate: formic acid (5:15:0.5) as a mobile phase. After development, the plates were dried and densitometrically scanned at wavelength 330 nm (Win Cats software, CAMAG, Switzerland), recorded the peak area and prepared the calibration curve by plotting peak area against concentration of quercetin applied.

Induction of Diabetes

Diabetes was induced in rats by injecting a freshly prepared solution of streptozotocin (STZ, 50 mg/kg body weight, intraperitoneal [i.p]) in 0.1M citrate buffer (pH was 4.5). Fasting blood glucose concentration was measured after 72 hour of STZ injection to confirm the induced diabetes. The rats with blood glucose level above 140 mg/dL were considered to be diabetic and were used in the experiment. The animals were kept fasting over night for dosing as per experimental design. All the diabetic rats included in the study were administered with aspirin (200 mg/kg, orally [p.o.]) for the initial seven days.

Experiment Design

After induction of diabetes, rats were divided into seven groups equally as follows. Except the Group I, the remaining groups contained diabetic rats, which were later intoxicated with aspirin (200 mg/kg, p.o.) for initial 7 days of the 28 days study.

Group I: Normal group	**Rats of this group received only vehicle solution.**
Group II: STZ + Aspirin control	Diabetic rats received only vehicle solution
Group III:	Diabetic rats treated with POEt 100 mg/kg bw /day orally in 2% gum acacia.
Group IV:	Diabetic rats treated with POEt 150 mg/kg bw /day orally in 2% gum acacia.
Group V:	Diabetic rats treated with POEt 250 mg/kg bw /day orally in 2% gum acacia.
Group VI	Diabetic rats treated with Omeprazole in 50 mg/kg (Sigma, USA)
Group VII	Diabetic rats treated with Tolbutamide in 10 mg/kg (Sigma, USA)
bw=body weight, i.p.=intraperitoneal, POEt=ethanolic extract of *P. oleracea*, STZ=Streptozotocin	

Ulcer Index

Ulcer index was 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: 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1 mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1-2 mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; ++++, ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscularis still remaining unaffected and pooled group ulcer score was then recorded (Rao et al., 2000).

Measurement of Fasting Blood Glucose Levels and Serum Insulin Levels

Fasting blood samples were drawn on 1st day after single administration of POEt and after 7 and 14 days by tail vein puncture under mild ether anesthesia in Eppendroff's tubes containing 50 ml of anticoagulant (10% trisodium citrate solution) from the normal and STZ-induced diabetic rats.

All the animals were sacrificed by decapitation after recording the final body weight. Blood was collected and serum was separated by centrifugation at 5000 rpm for 10 minutes for insulin assay by enzyme-linked immunosorbent assay (ELISA) technique (Sharma et al., 2010).

Glucose Tolerance Test

After overnight fasting, on the day the animals were sacrificed, a zero-minute blood sample was taken from tip of tail vein of all the rats. The rats of all groups were given glucose (2 g/kg) thirty minutes after dosing and blood samples were collected at 30 and 90 minutes for the measurement of glucose levels by single touch glucometer after the administration of glucose (O'Brien et al., 1985).

Antioxidant Activities

Lipid Peroxidase

Lipid peroxidase (LPO) product malondialdehyde (MDA) was estimated using 1,1,3,-tetrahydroxypropane as the standard and is expressed as nmols mg-1 of proteins (Ohkawa et al., 1979).

Superoxide Dismutage Activity

Superoxide dismutage (SOD) was estimated by following the procedure of (Kakkar et al., 1984). The inhibition of reduction of nitro blue tetrazolium to blue colored formozan in presence of phenazine meta-sulphate 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 the rates of reaction by 50% in one minute under the defined assay conditions and the results have been expressed as units (U) of SOD activity mg-1 of protein.

Catalase Activity

Decomposition of H_2O_2 in presence of catalase (CAT) was followed at 240 nm (Aebi, 1952). One unit (U) of CAT was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute, at 25°C and pH 7.0. Results are expressed as units (U) of CAT activity per mg of protein.

Statistical Analysis

All the data were presented as mean ± standard error of mean (SEM) and analyzed by paired t-test using SPSS software package (SPSS, Cary, NC, USA).

Results and Discussion

Effect of *P. oleracea* Extracts on Ulcer Index and Protection Rate

The diabetic rats on treatment with POEt for 21 days exhibited noticeable protection of the gastric mucosa against the acid attack. The POEt was found to protect the gastric mucosa significantly (P<0.01) at a dose of 250 mg/kg and to cure the ulcerations significantly (P<0.01). Table 1 highlights the curative effects (ulcer index and protective rates) of various POEt extracts on gastric ulceration.

Table 1: Effect of POEt on Ulcers Index and Protection Rate of Diabetic Rats

Groups	Treatment	POEt	
		Ulcer Index	Protection Rate (%)
I	Normal	-	-
II	Streptozotocin + aspirin control	28.6±2.9	-
III	POEt, 100 mg/kg	10.3±4.2[a]	25.87
IV	POEt, 150 mg/kg	7.7±3.5[b]	57.43
V	POEt, 250 mg/kg	4.5±1.7[c]	67.61
VI	Omeprazole (50mg.kg)	22.0±2.3	65.01
VII	Tolbutamide (10 mg/kg)	5.5±2.1	25.86

The values represent the means ± SEM for six rats per group.
[a]p<0.05, [b]p<0.01, [c]p<0.001 compared to diabetic control group.

Table 2: Effect of Extract of *POEt* on Blood Glucose Tolerance

Groups	Treatment	Blood glucose (mg/100ml)	
		0 day	28 days
I	Normal	78.99±0.83	79.36±0.9
II	Streptozotocin + aspirin control	79.25±0.91	146.90±1.76
III	POEt, 100 mg/kg	85.15±0.91	97.65±1.14[c]
IV	POEt, 150 mg/kg	83.45±1.09	97.67±1.34[c]
V	POEt, 250 mg/kg	84.03±1.02[b]	96.71±1.15[c]
VI	Omeprazole (10mg/kg)	88.23±0.91[c]	120.81±1.17[c]
VII	Tolbutamide (10mg/kg)	80.48±1.13[c]	82.87±1.66[c]

The values represent the means ± SEM for six rats per group.
[a]p<0.05, [b]p<0.01, [c]p<0.001 compared to diabetic control group. [d]p<0.001 as compared to normal.

Effect of Different Doses of POEt Extracts on Fasting Blood Glucose Levels in Diabetic Rats

The blood glucose was estimated after regular intervals to determine the antidiabetic effect of POEt. The POEt was significantly (P<0.01) effective in lowering the elevated fasting blood glucose levels. Table 2 shows the effect of POEt on fasting blood glucose levels in diabetic rats.

Effect of POEt on Antioxidant Enzymes

The SOD enzyme levels were significantly (P<0.01) elevated in animals treated with 250 mg/kg dose of POEt. The groups receiving 250 mg/kg dose of POEt was effective (P<0.05) in restoring the SOD levels to normal. The distinct elevation in the CAT enzymes was observed in the groups treated with 100, 150 and 250 mg/kg dose of POEt. Table 3 displays the effect of POEt on antioxidant enzymes SOD and CAT.

Table 3: Effect of POEt on Antioxidant Enzymes in Diabetic Rats

Groups	Treatment	Blood glucose (mg/100 mL)		
		LPO	SOD (unit/mg protein)	CAT (unit/mg protein)
I	Normal	6.86 ± 1.26	9.32± 0.75	57.70± 3.71
II	Streptozotocin + aspirin control	15.97 ± 1.56^{de}	4.36± 0.61^{de}	32.13± 2.42^{de}
III	POEt, 100 mg/kg	10.30± 1.48^{b}	6.56± 0.02^{a}	47.82± 3.83^{b}
IV	POEt, 150 mg/kg	8.64± 0.14^{c}	8.36± 0.22^{c}	60.71± 4.18^{c}
V	POEt, 250 mg/kg	8.03± 1.02	9.71± 1.15^{c}	61.91± 4.14^{c}
VI	Omeprazole, 50mg/kg	7.23±.0. 91	5.81± 1.17^{c}	61.91± 4.14^{c}
VII	Tolbutamide, 10 mg/kg	7.25± 0.48^{c}	7.29± 0.04^{b}	59.72± 4.07^{c}

The values represent the means ± SEM for six rats per group.
^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 compared to diabetic control group. ^{d}p<0.01,^{e}p<0.001 as compared to normal.

Diabetes mellitus is one of the most common chronic diseases in developed countries and continues to amplify in numbers and significance. Experimentally-induced type II diabetes using STZ has been proved to be an excellent model to investigate the efficiency of antidiabetic drugs (Badole SL, Bodhankar, 2010). Recent investigations indicate that peptic ulcers associated to the diabetic condition are more severe and frequently linked with complications like gastrointestinal bleeding. The mechanism behind the enhanced vulnerability of gastric mucosa of STZ-induced diabetic animals to damage is multifactorial; and it includes modification of gastric motility, reduction of angiogenesis and impairment of duodenal bicarbonate secretion and dysfunction of capsaicin sensitive neurons involved in the protection of gastric mucosa (Fouad et al., 2010). Pathologically, an ulcer is a deep necrotic injury disturbing the entire mucosal depth and muscularis mucosae. It is an outcome of a split in the mucosal integrity close to the acid-secreting areas of the gastrointestinal tract. The POEt exhibited a significant reduction in the ulcer index of diabetic rats, proving that they are a step ahead over the drugs which have either only antiulcer (omeprazole) or antidiabetic (tolbutamide) effects. Diabetes is associated with decrease in antioxidant status, mucin secretion and mucosal cell shedding, glycoproteins without any effect on cell proliferation Hence, in diabetes, mucosal defensive factors play a vital role in increasing propensity to gastric ulceration and this may be one of the reasons for omiprazole to be effective in gastric ulceration in diabetic rats. The POEt significantly restored the mucosal glycoprotein in diabetic rats. This effect was almost similar to the reference antiulcer drug omiprazole. Hence, the antiulcer effect of *P. oleracea* in diabetic rats was demonstrated.

Streptozotocin-induced experimental diabetes is a valuable model for induction of type 2 diabetes (Junod A et. al., 1969). STZ diabetic animals may exhibit most of the diabetic complications mediated through oxidative stress (Ozturia et al., 1996). The antidiabetic action of POEt could be either due to inhibition of alpha-glucosidase enzyme (Milo et al., 2006) or stimulation of insulin secretion from the normal pancreatic beta-cells.

The SOD and CAT are two major scavenging enzymes that remove the toxic free radical in vivo. SOD scavenges the superoxide ions produced as cellular by-products and is a major defense for aerobic cells in combating the toxic effects of superoxide radicals (McCord et al., 1971). The CAT reduces H_2O_2 produced by disputation reaction and preventing generation of hydroxyl radicals, thereby, protecting the cellular constituents from oxidative damage in peroxisomes. Reduced activities of SOD and CAT in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and H_2O_2 (Santhakumari et al., 2003). POEt and

tolbutamide-treated rats showed decreased lipid peroxidation that is associated with increased activity of SOD and CAT. POEt has been demonstrated to exhibit antioxidant properties that have been implicated in restoring the integrity of gastric mucosa (Bharti et al., 2010). The POEt effectively elevated the concentration of tissue SOD and CAT enzymes, thereby supporting the process of ulcer healing in diabetic rats.

Aspirin has proven to be a successful drug for the prevention of thrombosis and atherosclerosis. Antiplatelet aggregators like aspirin and clopidogrel are commonly prescribed for prevention of cardiac complications associated with diabetes (Ng et al., 2008).

Conclusion

On the basis of above results, it could be concluded that POEt has a potent antiulcer effect in diabetic rats. It may be stated that this composite extract contains the active antiulcer and hyperglycemic agent(sThese findings could lead identification of novel molecule from *P. oleracea*, which serves as a good adjuvant in the present armamentarium of ulcer and diabetic complications.

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