

Combined Effect of *Syzygium cumini* Seed Kernel Extract with Oral Hypoglycemics in Diabetes Induced Increase in Susceptibility to Ulcerogenic Stimuli

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Abstract

Diabetes has been reported to increase propensity to peptic ulceration through its effect both on offensive and defensive mucosal factors. Seeds of *Syzygium cumini* have been reported to have both antidiabetic as well as ulcer protective effects. The present study evaluated the antidiabetic effects of ethanolic extract of dried seed kernel of *Syzygium cumini* (200 mg/kg) and its comparative effect on gastric ulceration with standards Acarbose (5 mg/kg) an α -glucosidase inhibitor and natural standard Quercetin 50 mg/kg and 100 mg/kg. The study also investigated the combined effect of *Syzygium cumini* seed kernel extracts with oral hypoglycemic agents in diabetes induced ulcer. The development of diabetic ulcers was assessed biochemically. Diabetes was induced by high fat diet/low dose streptozotocin (35 mg/kg). The protective effects of *Syzygium cumini* in rats with type 2 diabetes mellitus were investigated by oral glucose tolerance test. Single and repeated dose study was performed in normal and diabetic rats. Diabetic rats were exposed to gastric ulceration by indomethacin and ethanol induced models to study the role of oral hypoglycemics in diabetes induced increase in susceptibility to ulcerogenic stimuli. *Syzygium cumini* and its combination with standard showed significance in reducing the elevations of gastric ulcer index in the induced models and restored the depleted antioxidant defenses (superoxide dismutase, catalase and total proteins activities) caused by indomethacin administration. It was concluded that *Syzygium cumini* along with Acarbose represents a potential therapeutic option to reduce the risk of Diabetes induced gastric ulcerogenic stimuli in type 2 diabetic patients.

Keywords: Diabetes; Gastric ulcer; *Syzygium cumini*; Acarbose; Quercetin

Introduction

Acute gastric inflammation and ulcer disease occur with high prevalence in patients with type 2 diabetes mellitus, and are strongly correlated with the duration of diabetes. Also, peptic ulcers related to diabetes mellitus are more severe with slow healing rate and often associated with complications such as gastrointestinal bleeding [1,2]. However, little attention has been paid to the incidence and healing rate of peptic ulcer in diabetes because peptic ulcers among diabetics are considered infrequent [3]. Previous studies demonstrated that streptozotocin-diabetic animals have increased vulnerability of the gastric mucosa to various ulcerogens such as ischemia/reperfusion, stress, ethanol and non steroidal anti-inflammatory drugs [4-7]. The mechanism underlying the increased susceptibility of gastric mucosa in diabetic animals is multi factorial and includes alteration of gastric motility [8], impairment of duodenal bicarbonate secretion [9], attenuation of angiogenesis and dysfunction of capsaicin sensitive neurons involved in the protection of gastric mucosa [10]. However, increased production of reactive oxygen species and pro inflammatory cytokines seems to play a major role [7,11-13]. Antiulcer evaluation was done by using Indomethacin and ethanol induced ulcers in experimental animals.

Syzygium cumini also called Eugenia jambolana (EJ) has been reported to have hypoglycemic effects both in experimental models and clinical studies. EJ seed apart from hypoglycemic activity has been reported to have anti-inflammatory [14], neuropsychopharmacological, antibacterial [15], anti-oxidant [16] and anti-diarrhoeal effects [17]. EJ seed kernel decreased the oxidative stress in diabetic rats, which in turn may be due to its hypoglycemic property. Reported the ulcer protective effect of ethanolic extract of E. jambolana

seeds (EJE) and the effect seemed to be due to its predominant action on mucosal defensive factor and antioxidant effect [18].

This was encouraging to conduct the present study in order to evaluate the protective effects of the EJE alone and in combination with Acarbose in rats with type 2 diabetes mellitus exposed to models which cause ulcerogenic stimuli. To study the role of oral hypoglycemics in diabetes induced increase in susceptibility to ulcerogenic stimuli and the effect of *Syzygium cumini* seed kernel extract on antioxidant defense status in stomach homogenate with the selected dose of 200 mg/kg/day as per Aditi Chaturvedi [18]. Quercetin used as natural standard for comparing anti ulcer property, as our intension is with the use of only one synthetic drug, decrease the progression of complication Diabetes induced ulcers. So *Syzygium cumini* was compared with quercetin in treating ulcers. Also the possible results underlying these protective effects were investigated.

Methods

The experimental protocol used in the present study was approved

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by the institutional Animal Ethical committee. Male Sprague dawley rats weighing between 200-250 g were employed in the present study. They were housed in standard propylene cages (three rats/cage) and maintained under controlled room temperature ($22 \pm 2^\circ\text{C}$) and $55 \pm 5\%$ RH with 12:12 h light and dark cycle. All rats were provided with normal pellet diet (Amrut diet, New Delhi) with water *ad libitum* prior to the dietary manipulation.

Development of high fat diet-fed/low dose streptozotocin treated type 2 diabetic rats

The animals were fed high fat diet (HFD), once a day for 2 weeks followed by IP injection of streptozotocin (35 mg/kg) dissolved in 1 M/L citrate buffer (pH 4.4) after overnight fasting. Blood sample was collected from tail vein and glucose was measured using glucose diagnostic kit (Accucheck, India). The rats with non fasting plasma glucose level of ≥ 300 mg/dl will be considered diabetic [19].

Experimental design

The rats were randomly divided into six equal groups (n=6, each). The first group was the non-diabetic rats, receives saline and vehicle and served as control. All the remaining groups were diabetic rats. Second group was diabetic control. The animals of the third group received oral 200 mg/kg/day of ethanolic extract of *Syzygium cumini* seed kernel (SCSE) [18]. Fourth group treated with Quercetin 50 and 100 mg/kg/day [20]. Fifth group Acarbose 5 mg/kg/day [21] group six SCSE+ Acarbose low dose were given. Drug solution and extract were freshly prepared and administered for a period of 8 weeks.

Oral glucose tolerance test: The animals were fasted for 12 h and then orally administered with 2.0 g/kg glucose. Blood glucose levels were measured at 0, 30, 60 and 120 min after glucose load [19].

Single and Repeated dose study in normal and diabetic rats:

- **Single dose study:** Normal and diabetic rats will be administered with a single dose of *Syzygium cumini* seed kernel extract and the selected oral hypoglycemic agents (OHA). The blood glucose level will be estimated just prior to the administration of *Syzygium cumini* and OHA and at 1, 2 and 4 h after administration. Glucose levels will be estimated using glucose diagnostic kit (Accucheck, India).
- **Repeated dose study:** The same groups (Single dose study) of normal and diabetic animals will be continued with the same dose levels of *Syzygium cumini* and OHA once daily, for 11 days. The glucose levels of all the animals will be measured on 3th, 5th, 7th, 9th and 11th day of the treatment period [22].

Anti ulcer evaluation will be done by following models in experimental animals

Healing of indomethacin induced gastric ulcers, a model to study free radical scavenging activity: The gastric ulcers will be induced by administering indomethacin (5 mg/kg.p.o) for 5 days to the normally fed rats. The animals were then treated with the drug for 5 days after induction of ulcer while the control group will receive only vehicle. The last dose of indomethacin will be considered as 0th day. Rats will be sacrificed on the same day. The stomach will be removed; ulcer score and ulcer index will be determined [23]. The glandular portion of the stomach will be used for the estimation of mucin content [24], total proteins [25] antioxidant factors like superoxide dismutase activity [26], and catalase activity [27].

The number of ulcers is noted and the severity is recorded with the following scores:

0=no ulcer, 1=superficial ulcer, 2=deep ulcers, 3=perforation.

• Evaluation

An ulcer index *UI* is calculated by the formula: $UI = UN + US + UP \times 10^{-1}$

UN=average of number of ulcers per animal, *US*=average of severity score, *UP*=percentage of animals with ulcers [28].

Ethanol induced gastric ulcers: Albino rats will be fasted for 36 h before administration of 90% ethanol (1 ml/200 gm). The drug will be administered 1 h before ethanol administration. One hour after ethanol administration, the animals will be sacrificed, stomach will be isolated and ulcer index will be determined [29].

Estimation of antioxidants in tissue homogenate

Preparation of stomach homogenate: At the end of each experiment, the stomach was homogenized in 20 ml of cold 0.1 N perchloric acid containing 16.8 mg of disodium EDTA and 50 nmol of isopropyl homocholine as an internal standard, using a homogenizer. The homogenate was centrifuged for 20 min with 10,000 rpm at 48°C.

Determination of gastric mucin content: Adherent gastric glandular mucous was measured by the method of Ajeigbe et al. [30]. The excised stomach was soaked for 2 h in 0.1% alcian blue dissolved in buffer solution containing 0.1 M sucrose and 0.05 M sodium acetate (pH adjusted to 5.8 with hydrochloric acid). After washing the stomach twice in 0.25 M sucrose (15 and 45 mins), the dye complexed with mucous was eluted by immersion in 10 ml aliquots of 0.5 M MgCl_2 for 2 h. The resulting blue solution was shaken with equal volumes of diethyl ether, and optical density of the aqueous phase was measured at 605 nm using a spectrophotometer. Using a standard curve, the absorbance of each solution was then used to calculate the concentration of the dye and its weight (expressed in mg). The weight of the dye was then expressed over the weight of the stomach.

Estimation of total proteins: Protein concentration was estimated according to the Lowry's method [25], using bovine serum albumin (BSA) as a standard.

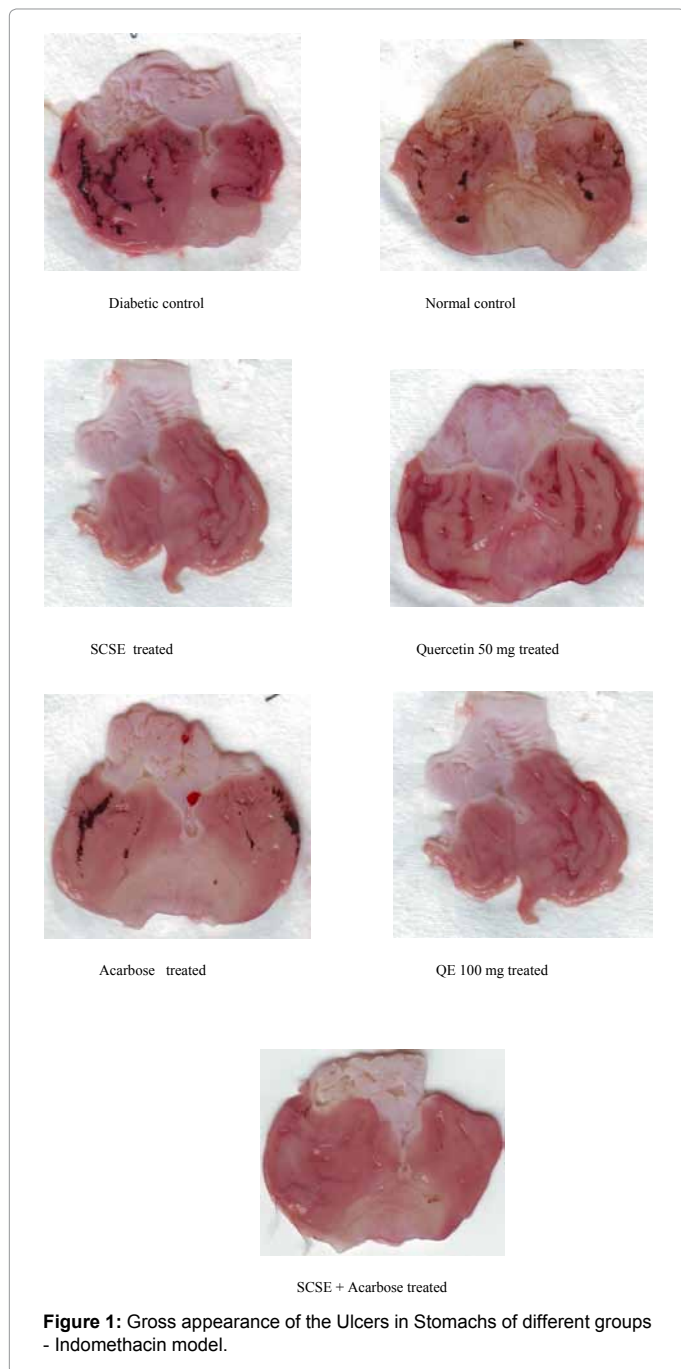
Measurement of superoxide dismutase (SOD) and catalase activity: Stomach homogenate was centrifuged 17,500 g at 4°C, for 10 min. The supernatant was used for the measurement of SOD activity by hematoxylin auto oxidation method [31] and catalase activity by H_2O_2 degradation method [32].

Statistical analysis

All the data are expressed as mean \pm SEM. Comparisons among the groups were analyzed with one way ANOVA followed by Tukey's multiple comparison test. The *P* value of less than 0.05 was considered to be statistically significant.

Drugs and chemicals

Streptozotocin was obtained from sigma-Aldrich Ltd., St. Louis, USA and quercetin from Himedia laboratories, Bombay, India. Fructose was purchased from El-Nasr Chemical Co., Abou Zaabal, Cairo, Egypt. The extract of *Syzygium cumini* seed kernel was collected from the Green chem. industry, Bangalore. All other chemicals used in the present study were of analytical grade (Figures 1 and 2).



Results

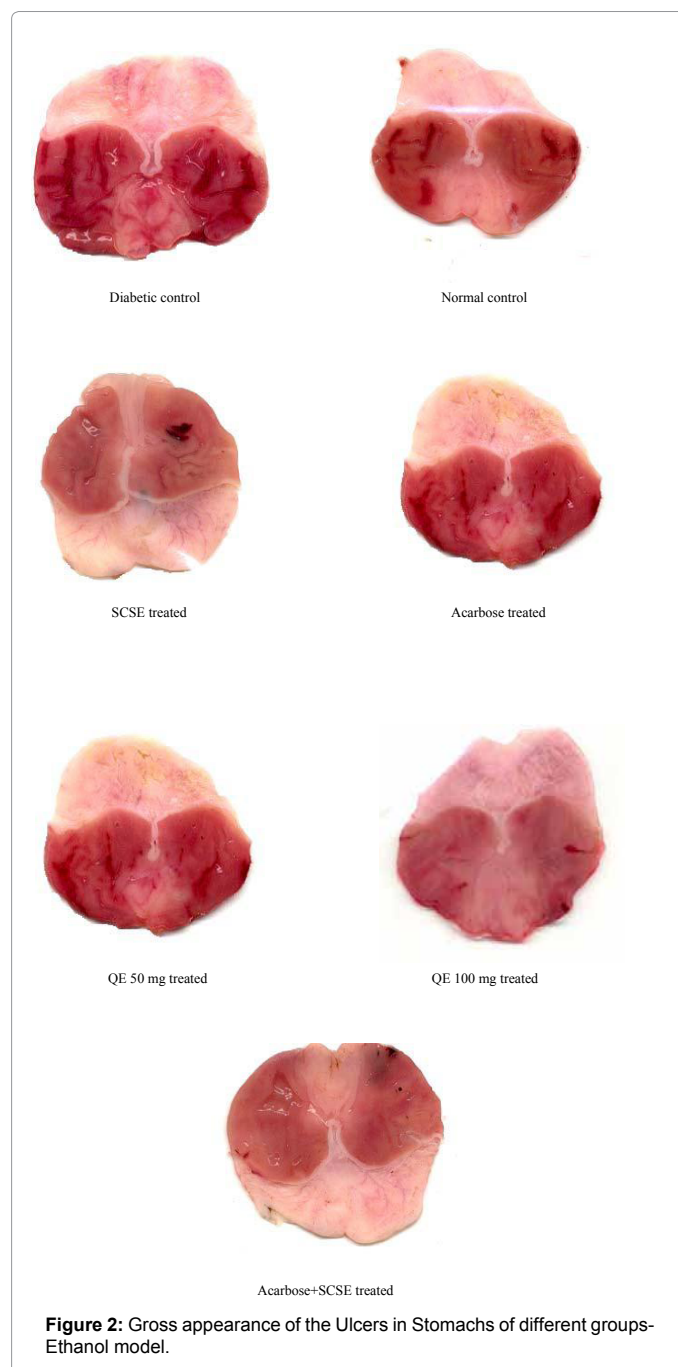
Effect of *S. cumini* seed kernel extract on blood glucose levels in normal and diabetic animals (single and multiple dose study)

After the 2nd week of STZ injection, diabetic rats showed significant increase in blood glucose levels than control rats where as normal rats treated with *S. cumini* seed kernel extract showed decrease in blood glucose level (14.28%) until 2 h in single dose study. Multiple dose study exhibited a significant reduction (28.6-34.2%) in blood glucose level between 9th and 11th day.

Diabetic rats with single dose of the extract exhibited decrease (14.68%) in blood glucose level until 4 h and then tended to increase and multiple doses showed significant reduction (37.6-40.7%) in blood glucose level between 7th and 11th day.

Effect of SCSE on oral glucose tolerance test in diabetic rats

In Glucose fed rats (2 g/kg), the percentage reduction in blood glucose levels in 2 h was extremely significant with groups treated with SCSE (8.81%) alone and in combination with Acarbose (12.7%) when compared to diabetic control group (Table 1).



Effect of SCSE, QE and Acarbose in stomach homogenate on SOD and catalase activity

Stomach homogenate SOD activity was significantly ($p < 0.05$) low in 10 weeks diabetic rats as compared to age matched non-diabetic rats. Eight weeks treatment with SCSE, QE and Acarbose significantly ($p < 0.05$) restored SOD activity to normal. There was a significant effect of drugs in control rats after 8 weeks of treatment (Table 1).

Stomach homogenate catalase was significantly ($p < 0.05$) high in 10 weeks diabetic rats as compared to age matched non-diabetic rats. Eight weeks treatment with drugs significantly ($p < 0.05$) reverted catalase activity to control values. However when compared to individual drugs, the combination A+SCSE group showed SOD and Catalase values nearest to normal which shows its effectiveness (Table 2).

Measurement of anti ulcer activity in indomethacin and ethanol induced ulcer models

When compared to other groups, SCSE and QE 100 mg are shown to have significant results ($p < 0.05$) in ulcer index and ulcer score when compared to diabetic control. Among which the combination Acarbose+ SCSE is effective than other groups showing significance ($p < 0.001$) for both indomethacin and ethanol ulcer models (Table 3; Chart 1).

Discussion

Metabolic syndrome is characterized by a cluster of pathological changes including obesity, hyper-triglyceridemia, impaired glucose tolerance and insulin resistance. A modified diet (fructose diet) was adopted to induce insulin resistance because the role of fructose in the

development of diabetic complications was well documented [33,34] and injection of a single dose of STZ induced diabetic state is similar to pre-diabetic insulin resistant state in humans [35]. The complication rate and the severity of complications increase as the duration of diabetes increases [36].

Several reports indicated that diabetes mellitus increases the mucosal susceptibility to ulcerogenic stimuli and predisposition to gastric ulceration mainly in NIDDM. The mechanism by which Diabetes induces ulcer is by the loss of pain sensation can result in development of ulcer that heal poorly because of diffuse vascular injury in diabetes and are a major cause of morbidity. Visceral autonomic neuropathy is the most important underlying factor among which is caused due to increase of free radicals [37]. In such conditions of co-occurring diabetes and gastric ulcers, it would be better to manage with drugs that have both anti-diabetic and anti-ulcer activities. This would be cost effective as well as incidences of adverse effects can be minimized.

In our present study taken, *Syzygium cumini* reflects its pharmacological importance in its antioxidant property of Tannins in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing/antioxidant power (FRAP) and thus gastro protective and anti ulcerogenic property [18]. It also shows anti diabetic activity by its effectiveness in inhibiting maltase when compared to the Acarbose control. Therefore the possible mechanism by which this herb acts as an anti-diabetic agent is by inhibiting alpha-glucosidase enzyme [38]. Acarbose, an oral hypoglycemic agent and Quercetin natural drug [39] shows similar alpha-glucosidase enzyme inhibiting action thus selected as standard drugs for the study.

Time	0 min	30 min	60 min	120 min
NC	90 ± 1.3	126 ± 1.2 (↑40.0%)	117 ± 1.4 (↓30.0%)	93 ± 0.9 (↓3.33%)
DC	234 ± 1.9	295 ± 2.26 (↑26.06%)	291 ± 1.5 (↓24.35%)	287.4 ± 2.6 (↑22.64%)
Acarbose	179.2 ± 0.8	208.9 ± 1.6 (↑16.57%) ^a	222 ± 1.02 (↑23.88%) ^a	186.1 ± 0.8 (↓3.85%) ^a
QE 50 mg	166.3 ± 0.9	212.6 ± 1.09 (↑27.84%) ^a	239.9 ± 0.9 (↑44.25%) ^a	196.1 ± 0.78 (↓17.91%) ^a
QE 100 mg	183.3 ± 0.6	212.6 ± 1.05 (↑15.98%) ^a	248.3 ± 0.76 (↑35.46%) ^a	189.9 ± 1.5 (↓3.6%) ^a
<i>S. cumini</i> seed kernel extract	209.1 ± 1.4	241.5 ± 0.6 (↑15.49%) ^a	261.6 ± 0.56 (↑25.10%) ^a	192 ± 0.96 (↓8.18%) ^a
Acarbose+ <i>S. cumini</i> Seed kernel extract	195.5 ± 2.1	227.7 ± 0.76 (↑16.47%) ^a	236 ± 0.11 (↑20.71%) ^a	180.1 ± 0.7 (↓12.77%) ^a

All values are mean ± SEM, n=6, ^aP<0.0001, ^bP<0.001, ^cP<0.05 when compared to Diabetic control (DC) group

Table 1: Effect of *Syzygium cumini* seed kernel extract with Acarbose in OGTT (mg/dl) - Diabetic Rats.

Parameter	Mucin content	Total proteins	SOD	CATALASE
NC	0.89 ± 0.14	22.5 ± 1.7	24.3 ± 1.58	20.3 ± 0.11
D.C	0.09 ± 0.024**	8.7 ± 0.83***	8.08 ± 0.17***	4.56 ± 0.29***
QE 100	0.29 ± 0.14*	15.2 ± 0.80 ^a ***	16.2 ± 1.6 ^a ***	12.6 ± 0.05 ^a ***
QE 50 mg	0.24 ± 0.43*	14.6 ± 0.62 ^a ***	15.75 ± 1.2 ^a ***	10.05 ± 0.09 ^a ***
Acarbose	0.11 ± 0.3**	11.3 ± 1.1***	10.1 ± 0.55***	8.7 ± 0.14 ^a ***
<i>S. cumini</i> seed kernel extract	0.31 ± 0.09*	16.8 ± 1.2***	17.1 ± 0.8**	13.4 ± 0.18 ^a ***
Acarbose+ <i>S. cumini</i> Seed kernel extract	0.54 ± 0.13	17.9 ± 0.6 ^a	19.8 ± 1.1 ^a	15.8 ± 0.07 ^a ***

Results are Mean ± SEM of 6 rats in each group. One-way ANOVA followed by Tuckey's test for multiple comparisons was applied for comparing the Parameters with NC and DC groups. The difference was considered to be significant when *** P<0.0001, ** P<0.001, * P<0.05 when compared to Normal control group and ^aP<0.0001, ^bP<0.001, ^cP<0.05 when compared to Diabetic control group

Table 2: Effect of *S. cumini* and its combination with acarbose on Mucin content (µg/gm), Total Proteins (mg/ml), SOD (U/mg of protein), CAT (U/mg of protein) observed in Indomethacin induced gastric ulcer model.

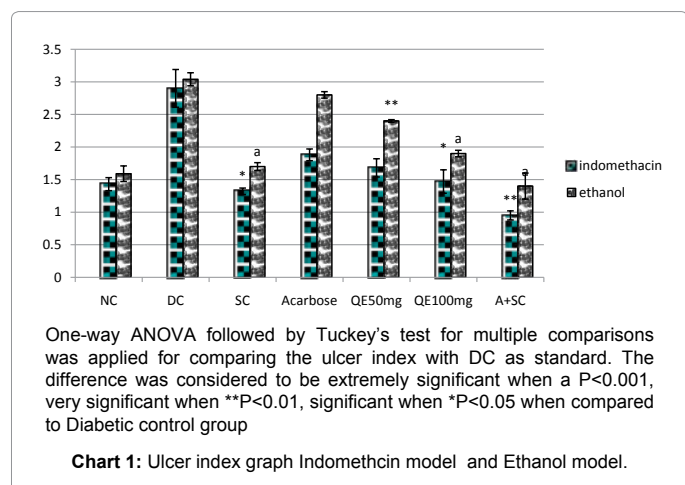
Treatment groups	Ulcer Index	Ulcer Score
NC	1.43 ± 0.10	9.8 ± 0.70
DC	2.9 ± 0.29	19.3 ± 0.76
QE 50 mg	1.68 ± 0.07	14.8 ± 0.55*
QE 100 mg	1.47 ± 0.07*	11.8 ± 0.06***
Acarbose	1.88 ± 0.56	16 ± 1.2
<i>S. cumini</i> seed kernel extract	1.32 ± 0.8*	10.3 ± 0.4***
Acarbose+ <i>S. cumini</i> Seed kernel extract	0.95 ± 0.73**	8.5±1.6***

Ethanol induced ulcer model

Treatment groups	Ulcer Index	Ulcer Score
NC	1.59 ± 0.12	7.5 ± 0.6
DC	3.04 ± 0.10	17.1 ± 0.55
QE 50 mg	2.4 ± 0.02**	13.6 ± 0.73**
QE 100 mg	1.9 ± 0.05***	10 ± 0.2***
Acarbose	2.8 ± 0.05	15.6 ± 1.1
<i>S. Cumini</i> Seed kernel Extract	1.7 ± 0.06***	8.8 ± 0.02***
Acarbose+S. <i>Cumini</i> Seed kernel extract	1.4 ± 0.2***	6.9 ± 0.3***

Results are Mean ± SEM of 6 rats in each group. One-way ANOVA followed by Tuckey's test for multiple comparisons was applied for comparing the Parameters with DC group. The difference was considered to be significant when ***P<0.0001, **P<0.001, *P<0.05 when compared to DC

Table 3: Effect of *S. Cumini* and its combination with Acarbose on Ulcer index and Ulcer score on Diabetic rats. Indomethacin model.



Experimental diabetes had shown to increase the propensity to gastric ulceration with an increase in offensive factors namely acid pepsin secretion and lipid peroxidation and decrease in antioxidant status, mucin secretion and mucosal cell shedding, without any effect on cell proliferation [13,40-42].

Diabetic rats exhibited a significant reduction in antioxidant enzyme activity and increased ulcer index values. These parameters regained to normal values when treated with *S. cumini* and Quercetin. Quercetin has anti oxidant-scavenging activity [43] which delays lipid peroxidation of cell membranes [44] and reduces cu⁺ induced LDL oxidation [45]. *S. cumini* significantly scavenges free radicals [46] and also has potent antioxidant activity *in vitro* [16]. *S. cumini* [18] and Quercetin [47] has proven to protect against the development of diabetic ulcer by restoration of antioxidant enzymes in diabetic rats.

The antiulcer effect of EJE in diabetic rats could be due to decrease in acid-pepsin secretion. There was decrease in propensity to ulceration

only in diabetic rats by correcting blood sugar level and reversing the enhanced acid secretion near to normal level. EJE thus, showed significant antiulcer activity in diabetic rats. Hence, *Eugenia jambolana* could be more effective and economical in diabetes with co-occurring gastric ulcers due to its direct actions both on gastric mucosal offensive and defensive factors or on enhanced acid-pepsin secretion induced by diabetes [18] compared to Acarbose which is decreasing, correcting diabetic parameters only and thus, showing indirect effect on deranged offensive and/or defensive mucosal factors in diabetes.

EJE had significant anti diabetic and anti-ulcer activity in mild diabetes with co-occurring gastric ulcers in rats. However, our results proved that the combined treatment is much effective in treating diabetes induced ulcers than *S. cumini* alone preventing the progression of diabetic ulcers may be due to their anti-oxidant properties on diabetic stomach. Taken together, the overall observed beneficial effect of low dose combination of Acarbose and *S. cumini* in preventing the development of diabetic ulcers may be attributed to their direct ulcer protective action and oxidative stress. The ulcer protective effects of Quercetin have been well reported in basic and clinical studies [48]. Therefore quercetin has been employed as a standard drug in the present study. The ulcer protective effect of low dose combination of Acarbose and *S. cumini* observed in the present study was slightly superior to the effect produced by quercetin in ameliorating diabetes induced ulcerogenic stimuli.

On the basis of the above discussion, it may be concluded that the concurrent administration of *S. cumini* and Acarbose at low doses may have prevented the development of diabetes induced ulcerogenic stimuli by decreasing the gastric oxidative stress, and providing the direct gastro protective action. In addition, their low dose combination strategy may provide synergistic ulcer protective effect against diabetic ulcerogenic stimuli. Therefore, long-term clinical studies demonstrating the rationale of low dose combination of Acarbose and *S. cumini* in curing diabetic ulcerogenic stimuli. Our present study proved effectiveness in the combined effect of *Syzygium cumini* seed kernel extract with oral hypoglycemics in diabetes induced increase in susceptibility to ulcerogenic stimuli.

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