# Ulcer healing properties of different extracts of *Origanum majorana* in streptozotocin−nicotinamide induced diabetic rats

BP Pimple[1,2*], PV Kadam[3], M J Patil[3]

[1]*P. E. Society's Modern College of Pharmacy, Nigdi, Pune−411044, Maharashtra, India*

[2]*Jawaharlal Nehru Technological University, Hyderabad, Andhra Pradesh, India*

[3]*Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune−411033, Maharashtra, India*

## ARTICLE INFO

## ABSTRACT

**Objective:** The aim of the present investigation was to evaluate the ulcer healing properties of different extracts of *Origannum majorana*, *viz.*, hydrodistilled volatile oil (OMO), methanolic (OMM) and aqueous extract (OMW) in streptozotocin−nicotinamide induced diabetic rats. **Methods:** All the extracts were administered in different doses (100, 200 and 400 mg/kg, *p.o.*) to investigate the ulcer healing potential. Streptozotocin (STZ; 65 mg/kg, *i.p.*) along with nicotinamide (120 mg/kg, i.p.) was used to induce non−insulin dependent diabetes mellitus in rats. Aspirin (200 mg/kg, i.p.) was administered for initial 7 d to induce gastric ulcerations in the diabetic rats. Various biochemical markers of blood and tissue origin were estimated to compare the ulcer healing potential of these extracts. **Results:** The OMO and OMM exhibited dose dependent significant ($P<0.01$) ulcer healing property than the OMW. Additionally, the antidiabetic property of OMO and OMM was better than OMW. **Conclusions:** The OMO and OMM of *Origanum majorana* leaves can prove to be beneficial in the concomitant treatment of gastric ulcers and diabetes.

## 1. Introduction

Diabetes mellitus is one of the most common chronic diseases in almost all countries and continues to amplify in numbers and significance. In 2011, there are 366 million people with diabetes, and this is anticipated to go up to 552 million by 2030. The majority of people with diabetes reside in low− and middle−income countries, and these countries will also notice the maximum rise over the next 19 years[1]. According to another study on adult (aged 20−79 years) diabetic population, the world prevalence of diabetes among adults affected 285 million adults in 2010, and will amplify to 439 million by 2030. There will be an around 70% increase in adult diabetic population in developing countries and a 20% increase in developed countries up to 2030[2]. Despite the availability of effective treatments to prevent or delay major complications, diabetes still places an enormous burden on both patients and the health care system[3].

Experimentally induced type II diabetes using streptozotocin−nicotinamide has been proved to be an excellent model to investigate the efficiency of antidiabetic drugs[4]. 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 streptozotocin− 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[5].

Most of the herbal remedies are safe when used under the guidance of knowledgeable practitioners; the potential for adverse effects or intoxications certainly exists[6]. Numerous plant species have been identified worldwide

*Corresponding author: BP Pimple, P. E. Society's Modern College of Pharmacy, Nigdi, Pune−411044, Maharashtra, India.
   Mobile: +91−997−7830030
   Fax: +91−222−7661314
   E−mail: bhushanppimple@rediffmail.com

for antidiabetic and antiulcer activity. In spite of the presence of antidiabetic medicine in pharmaceutical market, screening of new herbs is still attracting scientists as they include substances that are effective and safe in diabetes[4]. *Origanum majorana* Linn (*O. majorana*) is a bushy shrub belonging to family Lamiaceae. This perennial and herbaceous plant is native to southern Europe and the Mediterranean regions[7]. Conventionally, it is employed for the management of diabetes, asthma, catarrh, insomnia and nervousness[8]. Research has scientifically proven the advantageous effects of the leaves; antioxidant[9], hepatoprotecitve[10], antibacterial[11], antihypertensive[12] and antiplatelet aggregation[13] properties.

One of the recommended mechanisms underlying the complications of diabetes is oxidative stress[14]. A great deal of evidence indicates that exposure to reactive oxygen species leads to lipid peroxidation in cell[9]. At present, there is no reports of antiulcer activity of *O. majorana* 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 work was aimed to investigate the healing property of gastric ulcer of *O. majorana* extracts in diabetes.

## 2. Materials and methods

### 2.1. Plant materials

The fresh leaves of *O. majorana* were obtained from the local markets of Pune, India and were authenticated by Botanical Survey of India, Western Circle, Pune with voucher specimen no. SWK–1.

### 2.2. Preparation of extracts

#### 2.2.1. Methanolic extract and aqueous extracts of O. majorana (OMM and OMW for short)

Fresh leaves were dried in shade and coarsely powdered. The powder was successively extracted in a Soxhlet apparatus with petroleum ether followed by methanol[15]. The OMW was prepared by cold maceration with distilled water for 24 h. The extracts were concentrated in vacuum and were stored at 8–10 °C throughout the study. The yield rates of OMM and OMW were 2.78% and 4.13% (w/w) respectively.

#### 2.2.2. Hydrodistilled volatile oil of O. majorana (OMO)

The fresh *O. majorana* leaves were dried in shade and coarsely powdered. The powder was extracted in Clevenger apparatus. The oil was collected and stored in an air tight container in a refrigerator. The yield rate of oil was 1.32% (v/w) with respect to air dried powder.

### 2.3. Chemicals and reagents

STZ and nicotinamide were obtained from Sigma–Aldrich (St. Louis, MO, USA). Analytical grade methanol (99.9%, v/v) and other chemicals were procured from Merck (Whitehouse Station, NJ, USA).

### 2.4. Animals

Swiss albino mice (20–25 g) and rats [(200±20) g] of either sex were obtained from the Yash farms, Pune, India. The animals were kept in polyethylene cages in the departmental animal house at (26±2) °C and relative humidity 40%–55% with 12 h light and dark cycles. The animals were fed with standard pellet chow diet (Hind liver) and were allowed free access to water. All the experimental protocols for animal care procedures were approved by the Institutional Animal Ethical Committee with a protocol number MCP/IAEC/02/2009.

### 2.5. Acute toxicity studies

Swiss albino mice weighing 20–25 g were used for the study. The animals were equally divided into four groups. Before the study, mice were fasted overnight but were allowed free access to water. First group served as control and received 10 g/L carboxymethyl cellulose (CMC) in distilled water. While the other three groups received 2 g/kg *p.o.* dose of OMO, OMM and OMW extract respectively. The extracts were suspended in 10 g/L CMC solution in distilled water as they were not easily soluble in water.

The animals were observed for 5 min once every 30 min till 2 h and then at 4, 8 and 24 h to detect any change in the autonomic or behavioral response and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and then were further observed daily for 14 d for mortality[16].

### 2.6. Experimental induction of non–insulin–dependent diabetes mellitus (NIDDM) and gastric ulcers in rats

Type II diabetes was induced by injecting a single i.p. dose of nicotinamide (120 mg/kg) followed by i.p administration of STZ (65 mg/kg) in 0.1 mol/L citrate buffer (pH 4.5)[17,18]. The fasting blood glucose level was estimated after 72 h post STZ administration. Rats exhibiting blood glucose concentration more than 145 mg/dL were considered diabetic and were included in the study. All the diabetic rats included in the study were administered with aspirin (200 mg/kg, *p.o.*) for the initial 7 d.

### 2.7. Experimental procedure

The animals were divided into 13 groups each containing six. A total of 76 rats (6 normal and 72 diabetic) were used throughout the study. Group 1 contained normal rats which were neither intoxicated nor treated with drugs but received 10 g/L CMC solution. Except the normal, remaining 12 groups contained diabetic rats, which were later intoxicated with aspirin (200 mg/kg, *p.o.*) for initial 7 d of the 28 d study. Group 2 represented STZ+aspirin control group and rats housed in this group did not received any treatment with drugs. Animals in group 3 were treated with glibenclamide (0.25 mg/kg, *p.o.*) after intoxication with STZ and aspirin. Group 4 rats were treated with ranitidine (2.5 mg/kg, *p.o.*). Group 5, 6 and 7 were treated with an oral dose of 100, 200 and 400 mg/kg, respectively of OMO suspended in CMC. Group 8, 9 and

10 were supplied with an oral dose of 100, 200 and 400 mg/kg, respectively of OMM suspended in CMC. Similarly, group 11, 12 and 13 were treated with an oral dose of 100, 200 and 400 mg/kg, respectively of OMW suspended in CMC.

## 2.8. Scoring of ulcer index

On the 28th day, animals were sacrificed by cervical dislocation and the stomach was isolated and incised along the greater curvature and examined for ulcers. The maximum length of each lesion was determined and the sum of the lengths of all lesions in each stomach was expressed as the ulcer index. All the measurements were made by an observer unaware about the treatment the rats were receiving[19]. The inhibition rate of ulcer was determined and mean ulcer score for each animal was expressed as ulcer index[20].

## 2.9. Determination of percent protection

Protection rate (%) = [(STZ+aspirin control Ulcer index) − (drug treated Ulcer index)]/(STZ+aspirin control ulcer index) ×100%.                                                                 (1)

The number of ulcers per stomach was recorded and the percent of ulcer incidence of each group as compared to the STZ+aspirin control was calculated[20].

## 2.10. Estimation of fasting blood glucose and serum insulin levels

The blood glucose levels were estimated on day 0, 14 and 28 day after induction of NIDDM. Blood was collected from the retro−orbital plexus and glucose was estimated using glucostix (One touch Ultra, Johnson and Johnson). Serum insulin was estimated on the 28th day to investigate the secretogouge effect of *O. majorana* extracts[21].

## 2.11. Estimation of mucosal glycoproteins

Samples of gastric mucosal scraping were homogenized in distilled water and treated with 90% (v/v) ethanol to yield a precipitate. The carbohydrates and proteins were estimated in the precipitate of mucosal scrapings. For estimation of proteins, total hexoses, hexosamine and fucose, about 75% of the precipitate were dissolved in 1 mL of 0.1 mol/L sodium hydroxide; whereas, remaining 25% of precipitate was dissolved in 1 mL of 0.1 mol/L sulfuric acid for the estimation of sialic acid. The results were expressed in $\mu$ g/mL. The ratio of total carbohydrate (sum of total hexoses, hexosamine, fucose and sialic acid) to protein has been taken as the index of mucin activity[17].

## 2.12. Superoxide dismutase (SOD) enzyme assay

Estimation of SOD was performed by the established procedure[22]. The magnitude of prevention of reduction of nitro blue tetrazolium in the presence of phenazine methosulphate and NADH to a blue−colored complex (formozan) was measured at 560 nm using butanol as blank.

The results were expressed as units (U) of SOD activity/g wet tissue.

## 2.13. Catalase (CAT) enzyme assay

Decomposition of hydrogen peroxide in presence of CAT enzyme was followed at 240 nm. Results were expressed as units (U) of CAT activity/g wet tissue[23].

## 2.14. Statistical analysis

The above estimations were analyzed statistically by applying One−way analysis of variance (ANOVA) followed by Dunnet's test for multiple comparisons. The differences were considered significant when $P<0.05$. Values are expressed as mean±SEM.

## 3. Results

### 3.1. Effect of O. majorana extracts on ulcer index and protection rate

The diabetic rats on treatment with *O. majorana* extracts for 21 d exhibited noticeable protection of the gastric mucosa against the acid attack. OMO was found to protect the gastric mucosa significantly ($P<0.01$) at a dose of 200 and 400 mg/kg; whereas, OMM showed similar protection at all the doses. OMW at a dose of 400 mg/kg was found to cure the ulcerations significantly ($P<0.01$) only at 400 mg/kg. Dose level of 100 mg/kg of OMO and OMW were not effective enough to treat the ulcers. Table 1 highlights the curative effect of various *O. majorana* extracts on gastric ulceration. Glibenclamide was less effective ($P<0.05$) in protection of ulcers as compared to the OMO and OMW extracts. Table 1 highlights the ulcer index and protection rate in rats.

**Table 1**

Effect of *O. majorana* extract on ulcers index and protection rate of diabetic rats ($n = 6$).

| Group | Treatment | Ulcer index | Protection rate (%) |
|---|---|---|---|
| 1 | Normal | – | – |
| 2 | Streptozotocin+aspirin control | 62.1±3.3 | – |
| 3 | Glibenclamide | 51.5±2.3[*] | 17.07 |
| 4 | Ranitidine | 15.4±1.2[**] | 75.20 |
| 5 | OMO, 100 mg/kg | 44.8±3.6[*] | 27.86 |
| 6 | OMO, 200 mg/kg | 25.3±2.1[**] | 59.26 |
| 7 | OMO, 400 mg/kg | 21.2±1.8[**] | 65.86 |
| 8 | OMM, 100 mg/kg | 33.4±2.9[**] | 46.22 |
| 9 | OMM, 200 mg/kg | 30.1±3.1[**] | 51.53 |
| 10 | OMM, 400 mg/kg | 24.5±2.2[**] | 60.55 |
| 11 | OMW, 100 mg/kg | 57.3±3.8[ns] | 7.73 |
| 12 | OMW, 200 mg/kg | 50.1±4.1[*] | 19.32 |
| 13 | OMW, 400 mg/kg | 48.2±2.7[**] | 22.38 |

[**]$P<0.01$; [*]$P<0.05$; [ns]not significant. The difference was considered to be significant when $P<0.05$ compared to STZ+aspirin control group. OMO, OMM, and OMW represent hydrodistilled volatile oil, methanolic extract and aqueous extract of *O. majorana*, respectively.

### 3.2. Effect of different O. majorana extracts on fasting blood glucose levels in diabetic rats

The blood glucose was estimated after regular intervals to determine the antidiabetic effect of *O. majorana* extracts. The OMO and OMM were significantly ($P<0.01$) effective in lowering the elevated fasting blood glucose levels; whereas, the aqueous extract (100 and 200 mg/kg) and ranitidine were completely ineffective in lowering the blood glucose level. Table 2 shows the effect of *O. majorana* extracts on fasting blood glucose levels in diabetic rats.

### 3.3. Effect of O. majorana extracts on serum insulin and glycosylated hemoglobin

The groups receiving ranitidine, OMM 100 mg/kg and all doses of OMW did not show any alteration in STZ–induced depletion of serum insulin. On the other hand, each dose of OMO and OMM exhibited elevation of serum insulin which was comparable to glibenclamide ($P<0.01$). Except ranitidine, OMW 100 mg/kg and OMW 200 mg/kg groups, all the treated groups significantly ($P<0.01$) depleted the STZ–induced elevated haemoglobin A1c (HbA1c) level. Table 2 furnishes the details of effect of *O. majorana* on serum insulin and HbA1c.

**Table 2**

Effect of different *O. majorana* extracts on blood indexes and antioxidant enzymes in diabetic rats ($n = 6$).

| Group | Treatment | Fasting blood glucose level (mg/dL) | | Serum insulin ($\mu$ U/mL) | HbA1c (mg/dL) | SOD (U/g of wet tissue) | CAT (U/g of wet tissue) |
|---|---|---|---|---|---|---|---|
| | | 0th day | 28th day | | | | |
| 1 | Normal | 78±7.6 | 81±6.4$^{**}$ | 15.5±0.62$^{**}$ | 0.51±0.02$^{**}$ | 97.3±4.2$^{**}$ | 27.3±1.1$^{**}$ |
| 2 | Streptozotocin+aspirin control | 254±22.3 | 266±23.1 | 5.1±0.31 | 0.88±0.03 | 48.2±5.1 | 11.3±0.8 |
| 3 | Glibenclamide | 261±18.6 | 85±8.2$^{**}$ | 14.2±0.71$^{**}$ | 0.55±0.02$^{**}$ | 66.4±3.2$^{**}$ | 18.2±0.8$^{**}$ |
| 4 | Ranitidine | 250±20.7 | 228±14.8$^{ns}$ | 5.8±0.45$^{ns}$ | 0.79±0.02$^{ns}$ | 52.6±2.5$^{ns}$ | 24.3±0.9$^{ns}$ |
| 5 | OMO, 100 mg/kg | 261±14.3 | 139±15.7$^{*}$ | 9.2±0.35$^{**}$ | 0.69±0.01$^{**}$ | 58.6±3.6$^{ns}$ | 16.9±1.3$^{*}$ |
| 6 | OMO, 200 mg/kg | 252±24.6 | 100±15.6$^{**}$ | 11.5±0.35$^{**}$ | 0.64±0.02$^{**}$ | 62.3±3.4$^{*}$ | 18.8±1.4$^{**}$ |
| 7 | OMO, 400 mg/kg | 253±24.1 | 95±11.2$^{**}$ | 12.9±0.31$^{**}$ | 0.56±0.03$^{**}$ | 85.3±4.1$^{**}$ | 22.5±2.1$^{**}$ |
| 8 | OMM, 100 mg/kg | 265±12.3 | 135±10.1$^{**}$ | 6.3±0.66$^{ns}$ | 0.72±0.03$^{**}$ | 61.7±3.1$^{ns}$ | 15.1±1.2$^{ns}$ |
| 9 | OMM, 200 mg/kg | 258±15.4 | 120±12.3$^{**}$ | 8.1±0.41$^{**}$ | 0.68±0.04$^{**}$ | 63.3±4.2$^{*}$ | 17.2±0.6$^{**}$ |
| 10 | OMM, 400 mg/kg | 264±20.0 | 98±10.5$^{**}$ | 12.9±0.45$^{**}$ | 0.60±0.04$^{**}$ | 85.4±3.4$^{**}$ | 18.3±0.9$^{**}$ |
| 11 | OMW, 100 mg/kg | 260±20.4 | 210±10.4$^{ns}$ | 5.9±0.35$^{ns}$ | 0.80±0.02$^{ns}$ | 55.3±1.9$^{ns}$ | 12.3±1.1$^{ns}$ |
| 12 | OMW, 200 mg/kg | 256±18.3 | 165±12.4$^{ns}$ | 6.1±0.31$^{ns}$ | 0.78±0.03$^{ns}$ | 58.6±1.8$^{ns}$ | 14.3±1.3$^{ns}$ |
| 13 | OMW, 400 mg/kg | 251±13.2 | 130±11.2$^{**}$ | 6.4±0.18$^{ns}$ | 0.61±0.02$^{**}$ | 76.3±2.9$^{**}$ | 16.4±0.8$^{*}$ |

Values are expressed as mean ± SEM. One–way analysis of variance followed by Dunnett's multiple comparisons test was applied both in normal and non–insulin–dependent diabetes mellitus rat groups. $^{**}P<0.01$; $^{*}P<0.05$; $^{ns}$not significant. The difference was considered to be significant when $P<0.05$ compared to STZ+aspirin control group. OMO, OMM, and OMW represent hydrodistilled volatile oil, methanolic extract and aqueous extract of *O. majorana*, respectively.

**Table 3**

Effect of *O. majorana* extracts on gastric mucosal glycoproteins.

| Group | Treatment | Total hexoses ($\mu$ g/mL) | Hexosamine ($\mu$ g/mL) | Fucose ($\mu$ g/ml) | Sialic acid ($\mu$ g/mL) | TC ($\mu$ g/mL) | TP ($\mu$ g/mL) | TC:TP |
|---|---|---|---|---|---|---|---|---|
| 1 | Normal | 2 865±121 | 1 755±33 | 307±14 | 117±9 | 4 788±129 | 6 112±210 | 0.78±0.05 |
| 2 | Streptozotocin+aspirin control | 2 117±117 | 1 480±45 | 202±17 | 54±8 | 3 987±248 | 7 310±220 | 0.55±0.03 |
| 3 | Glibenclamide | 2 675±126$^{*}$ | 1 685±63$^{*}$ | 274±22$^{ns}$ | 92±5$^{ns}$ | 4 681±147 | 6 610±321 | 0.71±0.06$^{ns}$ |
| 4 | Ranitidine | 2 790±154$^{**}$ | 1 780±33$^{**}$ | 327±29$^{**}$ | 113±9$^{**}$ | 4 826±185$^{*}$ | 6 187±146$^{*}$ | 0.78±0.05$^{ns}$ |
| 5 | OMO, 100 mg/kg | 2 788±105$^{**}$ | 1 708±42$^{*}$ | 287±17$^{*}$ | 81±11$^{ns}$ | 4 751±162$^{*}$ | 6 501±325 | 0.73±0.09$^{ns}$ |
| 6 | OMO, 200 mg/kg | 2 794±93$^{**}$ | 1 721±65$^{**}$ | 301±12$^{*}$ | 96±9$^{**}$ | 4 765±134$^{*}$ | 6 523±245$^{*}$ | 0.73±0.07$^{ns}$ |
| 7 | OMO, 400 mg/kg | 2 810±131$^{**}$ | 1 740±39$^{**}$ | 314±19$^{**}$ | 108±7$^{**}$ | 4 781±177$^{*}$ | 6 210±180$^{**}$ | 0.77±0.06$^{ns}$ |
| 8 | OMM, 100 mg/kg | 2 709±98$^{*}$ | 1 677±47$^{ns}$ | 259±22$^{ns}$ | 84±6$^{ns}$ | 4 706±230$^{*}$ | 6 565±310 | 0.72±0.07$^{ns}$ |
| 9 | OMM, 200 mg/kg | 2 765±115$^{**}$ | 1 691±51$^{*}$ | 288±26$^{*}$ | 91±8$^{*}$ | 4 732±202$^{*}$ | 6 285±194$^{*}$ | 0.75±0.03$^{ns}$ |
| 10 | OMM, 400 mg/kg | 2 784±133$^{**}$ | 1 710±44$^{*}$ | 302±27$^{*}$ | 104±7$^{**}$ | 4 760±214$^{*}$ | 6 225±210$^{**}$ | 0.76±0.09$^{ns}$ |
| 11 | OMW, 100 mg/kg | 2 577±162$^{ns}$ | 1 626±67$^{ns}$ | 241±21$^{ns}$ | 72±9$^{ns}$ | 4 640±157$^{ns}$ | 6 741±354$^{ns}$ | 0.69±0.06$^{ns}$ |
| 12 | OMW, 200 mg/kg | 2 632±181$^{ns}$ | 1 640±50$^{ns}$ | 263±19$^{ns}$ | 81±8$^{ns}$ | 4 681±123$^{ns}$ | 6 770±360$^{ns}$ | 0.69±0.05$^{ns}$ |
| 13 | OMW, 400 mg/kg | 2 690±111$^{*}$ | 1 694±47$^{*}$ | 280±20$^{ns}$ | 93±6$^{*}$ | 4 710±209$^{*}$ | 6 814±240$^{ns}$ | 0.69±0.04$^{**}$ |

Values are expressed as mean ± SEM. One–way analysis of variance followed by Dunnett's multiple comparisons test was applied both in normal and non–insulin–dependent diabetes mellitus rat groups. $^{**}P<0.01$; $^{*}P<0.05$; $^{ns}$not significant. The difference was considered to be significant when $P<0.05$ compared to STZ+aspirin control group. OMO, OMM, and OMW represent hydrodistilled volatile oil, methanolic extract and aqueous extract of *O. majorana*, respectively. TC, total carbohydrates; TP, total proteins.

### 3.4. Effect of O. majorana extracts on antioxidant enzymes

The SOD enzyme levels were significantly ($P<0.01$) elevated in animals treated with 400 g/kg dose of OMO, OMM and OMW. The groups receiving 200 mg/kg dose of OMO and OMM were effective ($P<0.05$) in restoring the SOD levels to normal. The OMW extracts (100 and 200 mg/kg) did not restore the depleted SOD levels to normal.

The distinct elevation in the CAT enzymes was observed in the groups treated with 200 and 400 mg/kg dose of OMO and OMM. The groups receiving OMW extracts did not exhibit significant change in CAT enzyme level. Table 2 displays the effect of *O. majorana* extracts on antioxidant enzymes SOD and CAT.

### 3.5. Effect of O. majorana extracts on gastric mucosal glycoproteins

The glibenclamide and OMW treated groups were less effective in restoring the mucosal indicators to their normal values. Dose dependant significant ($P<0.01$) effect of OMO and OMM extracts was comparable to ranitidine, a well known antiulcer drug. Overall, the OMW did not restore the aspirin induced alterations in mucosal glycoproteins (Table 3).

## 4. Discussion

Experimental as well as clinical conditions such as antiplatelet or anticoagulant therapy, diabetes, obesity and glucocorticoid treatment are known to impair the normal wound healing process. Specifically, the impact of diabetes on the healing of dermal wound and ulcers has been extensively studied.

Research has also confirmed that NIDDM rats have an amplified vulnerability of the gastric mucosa against different ulcerogens such as ischemic reperfusion damage, stress and non–steroidal anti–inflammatory drugs (NSAIDs)[24]. Various mechanisms that increase the propensity of gastric mucosa of diabetic animals to damage includes the destruction of the antioxidative system in the gastric mucosa, the inhibition of basic fibroblast growth factor production in the gastric mucosa, impaired duodenal $HCO_3^-$ discharge, reduction of angiogenesis and the dysfunction of capsaicin–sensitive afferent neurons engaged in the protection of gastric mucosa[5].

NSAIDs like aspirin are extensively used; however, the chief limitations of their clinical application are serious adverse effects such as stimulation of acute hemorrhagic erosions, aggravating effect on stress ulceration and interference in healing of pre–existing ulceration[24]. Low dose aspirin is widely used for the prevention of vascular events, ranging from mild dyspepsia (31%) to life threatnenig bleeding from ulcers[25]. NSAIDs like aspirin and indomethacin cause mucosal damage[26] by blocking the prostaglandin synthesis, $H^+$ diffusion, increasing acid secretion[27] and decreasing mucous production[28]. Our

present findings are in accordance with earlier reports that ulcers induced by aspirin were aggravated by the STZ–induced diabetic rats. Our work for the first time has proved the application of *O. majorana* in treatment of ulcers in diabetic rats.

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. It is frequently situated in the stomach, proximal duodenum and rarely in the oesophagus and jejunum[29]. OMO and OMM exhibited significant reduction in the ulcer index of diabetic rats, proving that they are a step ahead over the drugs which have either only antiulcer (ranitidine) or antidiabetic (glibenclamide) effects. Diabetes is associated with decrease in antioxidant status, mucin secretion and mucosal cell shedding, glycoproteins without any effect on cell proliferation[17,30]. 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 ranitidine to be effective in gastric ulceration in diabetic rats. OMO and OMM significantly restored the mucosal glycoprotein in diabetic rats. This effect was almost similar to the reference antiulcer drug raniditine. Hence, the antiulcer effect of *O. majorana* in mild diabetic rats was demonstrated .

STZ at a dose of 65 mg/kg specifically destroys the beta–cells of islets of Langerhans. However, when administered along with nicotinamide, it causes partial destruction of pancreatic beta–cells. Numerous normal beta–cells are present even after administration of a low dose of STZ and nicotinamide[31]. The antidiabetic action of OMM and OMO extracts could be either due to inhibition of alpha–glucosidase enzyme[32] or stimulation of insulin secretion from the normal pancreatic beta–cells. This is apparent from the fact that these extracts significantly elevated the serum insulin concentration as compared to the OMW. The aqueous extract was however less effective in elevation of serum insulin level. The maintenance of blood glucose level at a nearly normal level could thus possibly protect the NIDDM patient from peptic ulceration[17].

The delay in ulcer healing in diabetic rats was also associated with a significant reduction in the gastric blood flow, which plays an important role in the healing process by supplying oxygen and nutrients and by removing toxic substances from the ulcer area. Earlier reports also reveal that STZ significantly decreases the gastric blood flow[33–39]. Insulin significantly increases gastric mucosal blood flow in diabetic rats. Insulin is essential for cellular proliferation in devitalized tissue. The primary action of insulin in an infected or injured wound resembles that of an enzyme that cleanses wound of exudates and necrotic tissue. Later, insulin serves as a hormone by stimulating tissue adjacent to the layer of necrotic tissue which is greatly injured and incapable to regenerate[40]. Insulin increases metabolism which in turn results in mitosis and cellular proliferation of adjacent tissues. Serum insulin levels in groups treated with OMO and OMM were significantly elevated and were comparable to glibenclamide, suggesting the secretogouge

role of *O. majorana*.

Prolonged elevated blood level of glucose leads to glycosylation of haemoglobin (Hb) which is commonly seen in chronic diabetes[41]. In our present work, when administered for prolonged period (21 d), OMO and OMM were effective in reducing glycosylated Hb level in diabetic rats, indicating its effectiveness in correcting the deleterious effect of diabetes.

SOD and CAT play a vital role in detoxification of superoxide anion and hydrogen peroxide respectively, thereby protecting cell against oxygen free radicals– induced damage[42]. The reactive superoxide radicals are first converted to hydrogen peroxide by SOD. Further, these hydrogen peroxide radicals are scavenged by CAT to prevent the lipid peroxidation resulting from the generation of hydroxyl radicals[43]. Hence, decrease in CAT levels may lead to increase in accumulation of these reactive oxygen species, increasing lipid peroxidation and thus tissue damage. Both OMO and OMM have been demonstrated to exhibit antioxidant properties that have been implicated in restoring the integrity of gastric mucosa[44]. The OMO and OMM extracts effectively elevated the concentration of tissue SOD and CAT enzymes, thereby supporting the process of ulcer healing in diabetic rats.

Throughout the study, the OMO and OMM extracts superseded the OMW in all aspects, which could be attributed to the presence of triterpenoids and phenolic or polyphenolic groups such as tannins or flavonoids. OMO and OMM have been previously reported to contain antioxidants like phenolic terpenoids (thymol and carvacrol), flavonoids (diosmetin, luteolin, and apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin, and thymonin) and triterpenoids (ursolic acid and oleanolic acid)[10,45].

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[25]. Moreover, *O. majorana* was reported to have significant inhibitory effect on blood platelet adhesion, aggregation and secretion[13]. Thus, *O. majorana* can not only serve as an antidiabetic and antiulcer agent but also potentiate the action of aspirin in prevention of cardiac complications.

Further research on derangement in the condition of mucosal defensive factors like cell shedding and tissue damage due to enhanced lipid peroxidation in diabetes may throw more light on the exact mechanism of action of *O. majorana*. Thus, from the study, it can be concluded that the observed beneficial effects of *O. majorana* in diabetic rats with co–existing gastric ulcer might be due to its both antidiabetic (reversing the toxic consequence of diabetes on gastric mucosa) and direct promoting effect on the gastric mucosal defense. To our knowlege, this work for the first time demonstrated that the *O. majorana* extracts stimulated the healing of gastric ulcer in diabetes.

## Acknowledgments

## Conflict of interest statement

We declare that we have no conflict of interest.

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