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Original article

Gastroprotective evaluation of *Medicago sativa* L. (Fabaceae) on diabetic rats

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ABSTRACT

Traditional uses for the plant *Medicago sativa* (*M. sativa*) (Alfalfa) (Family: Fabaceae) include liver protection, antioxidant activity, and the treatment of bleeding and digestive issues. This study aims to assess the effect of ethanol extract of *M. sativa* (EEMS) on experimental-induced ulcers in diabetic rats. By pylorus ligation and ethanol administration, gastric ulcers were induced in diabetic rats. Five groups each consisting of six rats in each model were used. All other groups except Group I were made diabetic by giving rats alloxan (140 mg/kg i.p.). Vehicles were given to Group I (normal control) and Group II (diabetes control) rats. Group III (positive control) received ranitidine 50 mg/kg, and Group IV and V received EEMS at doses of 100 and 400 mg/kg, respectively. In the pylorus ligation and ethanol-induced stomach ulcer model of rats, the findings demonstrated that EEMS (100 mg/kg) showed a decreased ulcer index of 2.01 ± 0.41 and was found statistically significant against the diabetes control group ($p < 0.001$) as well as, an ulcer index of 0.68 ± 0.22 by EEMS (400 mg/kg) with a significant reduction in the ulcer index ($p < 0.001$). EEMS (100 and 400 mg/kg) reduce free acidity by 13.16 ± 0.65 mEq/L and 9.83 ± 0.30 mEq/L, respectively. EEMS also showed a protective impact on the liver and kidneys of diabetic rats. Antihyperglycemic action was also discovered in diabetic animals. The findings of the current investigation demonstrated that ethanolic extract of *M. sativa* possesses anti-ulcer activity in diabetic rats. Ethanolic extract of *M. sativa* may be a treatment option for stomach ulcers that also have diabetes.

Abbreviations: ALP, alkaline phosphatase; CNS, Central Nervous System; ATP, Adenosine tri-phosphate; BW, Body weight, dl, Deciliter; g, Gram; $GLUT_2$, Glucose transporter 2; GSH, Glutathione; HDL, High density lipoprotein; i.p., Intraperitoneal; LD_{50}, Lethal dose; LDL, Low density lipoprotein; mEq, Milliequivalent; EEMS, Ethanolic Extract of *Medicago sativa*; N, Normality; NaOH, Sodium hydroxide; DCG, Diabetic Control Group; NCG, Normal Control Group; $NSAID_S$, Non-Steroidal Anti-Inflammatory Drugs; OECD, Organisation for economic cooperation and development; p.o., Orally; PUD, Peptic ulcer disease; ROS, Reactive oxygen species; SGOT, Serum Glutamic Oxaloacetic Transaminase; SGPT, Serum Glutamic Pyruvic Transaminase; TB, Total bilirubin; TC, Total Cholesterol; TG, Triglycerides; TP, Total Protein; VLDL, Very low density lipoprotein; *H. pylori*, *Helicobacter pylori*; MSE, *Medicago sativa extract*.

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1. Introduction

Both diabetes mellitus (DM) and peptic ulcer disease are chronic, debilitating conditions with wide-ranging medical and social effects. Duodenal ulcers and gastric ulcers, which resemble peptic ulcers and are among the most prominent gastrointestinal conditions worldwide, affect 10 % of the world's population (Beiranvand and Bahramikia 2020, Beiranvand et al., 2021, Popa et al., 2021). About 5–10 % of people may get peptic ulcers in their lifetime, and 0.1–0.3 % of people will develop one each year in the general population (Xu et al., 2019). The etiology of this illness is multifaceted and is brought on by a disproportion between the aggressive factors (pepsin, acid, Helicobacter pylori infection, and NSAIDs), the defensive factors (mucus formation, bicarbonate ion, and PGs), and the unique mucosal cell resistance (Karampour et al., 2019; Ahmad et al., 2019, Engevik et al., 2020). Peptic ulcer problems pose a concern to patients with diabetes mellitus (bleeding, perforation) (Tarasconi et al., 2020). Diabetes increases a person's predisposition to many factors that might cause ulcers, including alcohol, stress, ischemia injury, and NSAIDs (Drini 2017). The most noticeable abnormalities that are frequently seen in persons with chronic DM are reduced gastric release (in response to insulin but not to histamine or pentagastrin) and impaired gastric motility. DM has been related to a predisposition to develop a peptic ulcer and a reduction in stomach acid secretion or an increase in acid production (Rafsanjani and Vahedian, 2004, Owu et al., 2012, Chandra et al., 2022).

Multiple organs are impacted by diabetes mellitus, especially if it lasts a long period, whether it is type 1 or type 2 (Rodrigues and Motta, 2012). Long-term diabetics may have autonomic neuropathy and a range of symptoms, including dyspepsia, constipation, stomach discomfort, nausea, diarrhoea, and sluggish gastric emptying (Ko et al., 1999, Bytzer et al., 2002). Diabetes mellitus worsens stomach ulcer healing and increases the mucosa's vulnerability to ulcers (Konturek et al., 2010). However, problems like gastrointestinal bleeding are often linked to peptic ulcers brought on by diabetes mellitus (Pietzsch et al., 2002, Boehme et al., 2007).

According to reports, significant gastric inflammation or ulcers might impair a patient's ability to move their stomach in diabetics (Boehme et al., 2007). Among diabetes patients, Helicobacter pylori, a bacteria linked to ulcers, predominates in the development of ulcers (Quatrini et al., 2001, Tseng 2012). There are now two basic methods for treating peptic ulcers: one involves decreasing stomach acid output, and the other involves enhancing gastric mucosal protection (Chandra et al., 2015). According to many research, *Pterocarpus marsupium, Azadirachta indica,* and *Jasminum sambac* reduced blood glucose levels in type 2 DM mice while concurrently protecting against ulcers (Joshi et al., 2004, Dorababu et al., 2006, Rambabu and Rao, 2014).

Ranitidine an antihistaminic class of drug, blocks the action of histamine on the H_2 receptors found on the surface of the cells that produce gastric acid in the stomach and reduces the amount and concentration of gastric acid in the stomach, which can help to prevent and heal ulcers, as well as relieve symptoms of heartburn and acid reflux (Tripathi 2018, Nautiyal et al., 2023).

Alfalfa (*Medicago sativa*) is a plant of the Fabaceae family that includes therapeutic usage. It has an extensive history of usage as a Homoeopathic and Ayurvedic system of medicine for the treatment of numerous illnesses as well as diseases of the CNS and digestive systems (Bora and Sharma, 2010). In China, North Africa, Russia, and the United States, *Medicago sativa* is used as a food ingredient due to its high concentration of vitamins and bioactive components (Liu et al., 2016). It includes a variety of digestive-aiding enzymes, including amylase, invertase, and pectinase (Elakovich and Hampton 1984). Pharmacological research has shown that *Medicago sativa* extract has anti-diabetic properties (Baxi et al., 2010, Farsani et al., 2016), antioxidant (Gomathi et al., 2014, Sadeghi et al., 2016), anti-inflammatory (Hong et al., 2009), antifungal (Sadowska et al., 2014), cardio-protective (Gomathi et al., 2014), antimicrobial (Chavan et al., 2015, Chegini et al., 2018), anticancer (Gatouillat et al., 2015), hepatoprotective (Liang et al., 2015), anti-coagulant, hypocholestrolemic (Fana et al., 2018), anti-scorbutic (Leavy and Fox, 1935) and in central nervous system (CNS) disorders (Bora and Sharma, 2012). According to phytochemical analysis in literature, *Medicago sativa* contains flavonoids (Larose et al., 2002), alkaloids (Phillips et al., 1992), phytoestrogens (Hong et al., 2011), phytosterols (Abdel-Hafez, 1993), amino acids (Fougere et al., 1991), coumarins (Orr et al., 1993), organic acids (Fougere et al., 1991), phenolic compounds (Newby et al., 1980), proteins (Rechulicz et al., 2014), vitamins (Plaza et al., 2003), digestive enzymes (Becana et al., 1986), triterpenes and saponins (Bialy et al., 1999, Montanari et al., 2016, Gupta and Chaturvedi 2018). Alfalfa tea is used to strengthen the digestive system. Sprouts (of seeds) are used by diabetics (Khare, 2007). According to the literature evaluation, there isn't enough data to make a scientific determination about the effectiveness of *Medicago sativa* in treating experimental stomach ulcers in rats who also have co-occurring diabetes. As a result, the present work's focus is on ethanol extract of *Medicago sativa* on experimental-induced ulcers in diabetic rats.

2. Materials and methods

2.1. Cultivation and collection of plant material

The plant was grown on rich, friable, loamy soil that was properly drained in the field. The ground was carefully ploughed. Before planting, seeds were given an overnight soak in water. November marked the end of sowing. Thinning is done after the seeds have germination, which takes two to three weeks. The first week of February is the start of flowering. The plant was picked and dried in the shade after blossoming started.

2.2. Identification and authentication of plant material

The aerial parts of the plant were taxonomically recognized and verified at the Department of Botany under Reference No. 2019/SOS/BOT/73 by Dr. Ashok Kumar, Head, Department of Botany, IFTM University, Moradabad.

2.3. Preparation of the extract

The aerial part of *M. sativa* was separated and shade-dried. The plant was then subjected to size reduction. 300 g powder of *M. sativa* was macerated separately with petroleum ether for 24 h at room temperature and the extract was filtered with the help of muslin cloth; marc was then macerated for 24 h at room temperature with chloroform then filtered with the help of muslin cloth. Subsequently, the same procedure was repeated for ethanol. Ethanol extract of *M. sativa* (EEMS) was then subjected to evaporation and subsequent drying (Yield 35.86 % w/w). EEMS was stored in a desiccator for further preliminary phytochemical screening and pharmacological evaluation.

2.4. Preliminary phytochemical screening of the extract

EEMS underwent preliminary phytochemical screening using common chemical procedures to identify phytoconstituents (Trease and Evans, 1987).

2.5. Animals

Wistar albino rats, of 180–200 g, were procured from the IFTM University's Moradabad animal house and housed in a room for the duration of the experiments at a temperature of 25 ± 2 °C, an RH of 45–55 percent, and a cycle of dark and light lasting 12 h each. All the studies were performed per the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Ethics Committee, CPCSEA, India vide resolution No. 2019/837ac/M.Pharm./04. (CPCSEA 2018).

2.6. Acute toxicity study

A single dosage of a chemical or many doses given within 24 h might have adverse effects that are referred to as acute oral toxicity. Changes in the skin, hair, salivation, mucous membrane, piloerection, eyes, lacrimation, sweat, urine incontinence, and feces are among the once-daily cage-side observations (drowsiness, gait, tremors, and convulsion). The two weeks will be used to determine any mortality (OECD 2002).

2.7. Doses selection

For the evaluation of gastroprotective activity, two dose levels (100 mg/kg and 400 mg/kg, p.o.) were chosen, (i.e., one dose being one-fifth of the dose during acute toxicity testing and the other dose being 25 % of the one-fifth dose).

2.8. Pharmacological assessment

Pyloric ligation and an ethanol-induced ulcer model were used in diabetic rats to evaluate the effect of EEMS as a gastroprotective agent. Histopathological analyses of gastric mucosal cells were used to gauge the integrity of the gastric mucosa. Serum biochemical markers were measured to determine the EEMS impact on the liver and kidneys. In diabetic rats, the extract's impact on blood glucose levels was also assessed.

2.9. Induction of diabetes

Alloxan hydrate was injected (140 mg/kg) intravenously to cause diabetes. Animals with blood glucose levels > 250 mg/dl were separated and used for the study (Kumar et al., 2011, Chandra et al., 2022).

2.10. Assessment of blood glucose concentration

Samples of blood were taken from the tail and the glucose concentration in the blood was determined using a glucometer (Accu-Chek, India).

2.11. Pylorus ligation-induced ulcer model

Five groups of six animals each were formed from the species as follows:

Group 1: As a control, normal saline (5 ml/kg) was administered orally.

Group 2: Normal saline (5 ml/kg) p.o. and served as diabetic control.

Group 3: Ranitidine (50 mg/kg) p.o. and served as the reference drug.

Group 4: EEMS (100 mg/kg) served as the test drug.

Group 5: EEMS (400 mg/kg) served as the test drug.

For 7 days, different dosages of the test substance and 50 mg/kg of ranitidine were given orally to each group. Rats were fasted for 18 h while receiving normal saline (5 ml/kg) as the control group (Shay et al., 1945). Rats under anesthesia (Ketamine) were subjected to a pyloric ligation procedure to cause stomach ulcers. One hour before the pyloric ligation, the last drug dose was administered orally, and 4 h later the rats were sacrificed by overdose of anesthetics (Ketamine). The stomach was dissected after being opened along the major curvature and the gastric fluid was poured into a beaker (Umre et al., 2018). Estimates were made for the ulcer index, stomach content's pH, volume, and total and free acidity (Ajaikumar et al., 2005, Jamal et al., 2006, Chandra et al., 2015).

2.11.1. pH of gastric content

The pH of the contents was determined by inserting an electrode from a pH meter into a beaker comprising stomach components.

2.11.2. Volume of gastric content

By carefully emptying stomach contents into the graduated cylinder, the volume of the contents was measured.

2.11.3. Total and free acidity

1 ml of centrifuged and strained gastric output was titrated against 0.1 N Sodium hydroxide using the Toppers reagent and 1 percent phenolphthalein as an indicator to evaluate free acidity and determine combined/total acidity. Total acidity was the combined result of the two titrations (Sachan et al., 2017, Umre et al., 2018).

2.12. Ethanol-induced ulcer model

There were six rats in each of the five groupings of animals.

Group 1: As a control, normal saline (5 ml/kg) was administered orally.

Group 2: As a diabetic control, normal saline (5 ml/kg) was orally administered.

Group 3: As the standard medication, 50 mg/kg of ranitidine was administered orally.

Group 4: EEMS (100 mg/kg) served as the test drug.

Group 5: EEMS (400 mg/kg) served as the test drug.

The rats get all treatments orally. Before the experiments, the animals were denied food for 24 h but were given unrestricted admittance to water. The treatments were given to the rats half an hour before giving them 1 ml of absolute ethanol. The rats were sacrificed an hour after administering ethanol by giving them anesthetics (Ketamine). The stomachs were excised and gently rinsed with saline (0.9 %), inflated with 1 % formalin solution (10 ml), and immersed in the same solution to fix the stomach's outer layer. After 10 min, each stomach is then opened along the greater curvature and examined under a dissecting microscope to assess the formation of ulcers. Ulcer index and percentage of ulcer inhibition in rats were estimated (Choudhary et al., 2013, Safari et al., 2023).

2.13. Effects on the liver

By assessing the aspartate transaminase, albumin, alanine transaminase, total protein, alkaline phosphatase, and bilirubin total, liver function was assessed.

2.14. Effects on the kidney

By assessing the levels of uric acid, creatinine, urea, sodium, potassium, chloride, and calcium, the kidney's functionality was assessed.

2.15. Statistical analysis

One-way variance analysis (ANOVA) was utilized to assess the statistical difference between control and experimental data, and Dunnett's test was then performed. The data is offered as mean ± SEM (n = 6). Significant data had a probability level (P-value) lower than 0.05. The statistics were done using Graph-Pad Prism software.

3. Results

3.1. Preliminary phytochemical analysis

The results of the preliminary phytochemical analysis are presented in Table 1.

3.2. Acute toxicity study

According to LD50 investigations, rats were safe up to a dosage of 2,000 mg/kg of body weight. Based on this, further studies were carried out with oral dosage of 100 mg/kg and 400 mg/kg body weight. This finding implies that the extract is both non-toxic and harmless. Normal behavioral patterns were unaltered, and no toxicity or mortality symptoms or indicators were seen (Table 2).

3.3. Pylorus ligation-induced ulcer

The effect of EEMS (Table 3) showed that mean blood glucose concentration in the normal group was 93 ± 4.53 mg/dl on day 1st to 98.5 ± 1.66 mg/dl on day 7th, without much difference throughout the investigation. Blood glucose levels in the diabetic control group (DCG) gradually rose from a mean value of 372.5 ± 1 0.18 on day 1 to 408.66 ± 3.78 on day 7. From the first day of the trial to the conclusion, the blood glucose concentration in the standard and treated groups decreased steadily. which, when compared to diabetic controls, was statistically significant.

3.3.1. Ulcer index and ulcer inhibition (%)

All diabetic control group (DCG) rats who had their pylorus tied developed severe stomach ulcers. Most ulcers were superficial. Very few were piercing. There was bleeding in the stomach (Fig. 1). 3.68 ± 0.52 on the ulcer index. Rats were pre-treated with EEMS extracted from the aerial section of *M. sativa*, and when equated to control rats, the ulcer index was reduced in a dosage-dependent manner. The ulcer index at 100 mg/kg body weight obtained by EEMS was 2.01 ± 0.41. The result was statistically relevant when equated to the diabetes control group ($p < 0.001$). However, an ulcer index of 0.68 ± 0.22 was induced by EEMS at a dosage of 400 mg/kg b.w. The outcome met the criteria for statistical significance at the level of $p < 0.001$. Ranitidine suggestively reduced the risk of developing ulcers (84.70 %), providing an ulcer index of 0.56 ± 0.25. By ligating the Pylorus, 400 mg/kg of EEMS prevented the development of ulcers 81.5 percent of the time (Table 4).

Table 1
Results of preliminary phytochemical analysis.

Category	EEMS
Saponins	+
Phenolic compounds	+
Carbohydrates	+
Tannins	–
Alkaloids	+
Phytosterols	+
Flavonoids	+

(+) Present and (-) Absent.

Table 2
Rat behavior during experiments on acute toxicity.

Parameters	Observations					
	30 min	4 h	24 h	48 h	7 days	14 days
Skin & Fur	NL	NL	NL	NL	NL	NL
Eyes	NL	NL	NL	NL	NL	NL
Salivation	NL	NL	NL	NL	NL	NL
Respiration	NL	NL	NL	NL	NL	NL
Urination(color)	NL	NL	NL	NL	NL	NL
Feces consistency	NL	NL	NL	NL	NL	NL
Somatomotor activity & behavior pattern	NL	NL	NL	NL	NL	NL
Sleep	I	NL	NL	NL	NL	NL
Mucous membrane	NL	NL	NL	NL	NL	NL
Convulsions & tremors	UD	UD	UD	UD	UD	UD
Itching	P	UD	UD	UD	UD	UD
Coma	UD	UD	UD	UD	UD	UD
Mortality	UD	UD	UD	UD	UD	UD

NL = Normal, I = Increased, P = Present, UD = Undiscovered.

Table 3
Effect of EEMS on Blood glucose concentration (mg/dl) during pylorus ligation in diabetic rats.

Groups	Blood glucose concentration (mg/dl)	
	1st Day	7th Day
Normal Control	93.00 ± 4.53	98.50 ± 1.66
Diabetic Control	372.50 ± 10.18[a]	408.66 ± 3.78[a]
Ranitidine	324.33 ± 5.10	276.33 ± 4.12***
EEMS 100 mg/kg	311.33 ± 4.54	291.66 ± 4.24***
EEMS 400 mg/kg	317.5 ± 4.09	263.00 ± 4.90***

Values are represented by the mean ± standard error of the mean (n = 6). Performed an analysis of variance followed by a Dunnett's test on a DCG; *** Significance is indicated by the value $p < 0.001$ and [a]$p < 0.001$ (v/sNCG). EEMS = Ethanolic extract of *M. sativa*.

3.3.2. Gastric juice volume

In the diabetes control group, the production of gastric juice drastically increases (Table 5). Gastric juice volume rose from 2.2 3 ± 0.10 ml in the normal control group to 2.65 ± 0.15 ml. While ranitidine and EEMS 400 mg/kg considerably ($p < 0.001$) decreased the amount of gastric juice (1.26 ± 0.10 ml & 1.33 ± 0.13 ml, respectively).

3.3.3. pH of gastric juice

The DCG saw a significant decrease in the gastric juice pH. Gastric juice pH in the NCG was 2.36 ± 0.10, whereas it decreased to 2.18 ± 0.16 in the diabetic control group (Table 5). While the gastric juice pH rose considerably ($p < 0.001$) with ranitidine and EEMS 400 mg/kg (4.11 ± 0.10 & 3.93 ± 0.19).

3.3.4. Free acidity

Pylorus ligation increases free acidity in the diabetic control group (DCG) to 22.16 ± 1.83 mEq/L from 18.83 ± 1.49 mEq/L in the normal control group (NCG) (Table 5). Pre-treatment of

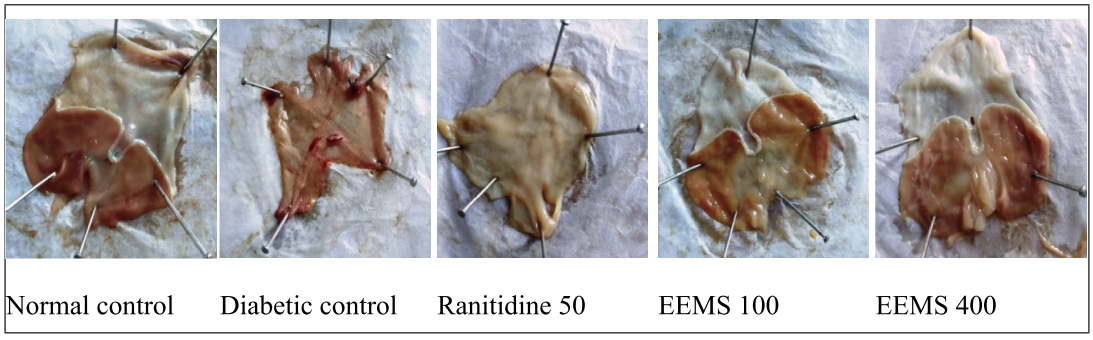

Fig. 1. Macroscopical pictures of rat's stomach using pylorus ligation-induced ulcer.

Table 4
Effect of EEMS on ulcer index during Pylorus ligation-induced gastric ulcer in rats.

Groups	Ulcer index	Ulcer inhibition (%)
Normal Control	2.53 ± 0.52	–
Diabetic Control	3.68 ± 0.20[a]	–
Ranitidine 50 mg/kg	0.56 ± 0.25***	84.70 %
EEMS 100 mg/kg	2.01 ± 0.41**	45.30 %
EEMS400 mg/kg	0.68 ± 0.22***	81.50 %

Values are represented by the mean ± standard error of the mean (n = 6). Performed an analysis of variance followed by a Dunnett's test on a DCG; ** Significance is denoted by the value p < 0.01, *** Significance is denoted by the value p < 0.001 and [a]p < 0.05 (v/s Normal Control). EEMS = Ethanolic extract of *M. sativa.*

animals with EEMS resulted in a dosage-dependent reduction in free acidity when compared to a diabetic control. Pre-treatment of animals with EEMS caused a dosage-dependent reduction in free acidity when compared to a diabetic control. EEMS at dosages of 100 and 400 mg/kg reduce free acidity by 13.16 ± 0.65 mEq/L and 9.83 ± 0.30 mEq/L, respectively. The outcome was statistically noteworthy as compared to the DCG (p < 0.001). The greatest notable decrease in free acidity was found to be caused by ranitidine (7.66 ± 0.49 mEq/L). The result achieved statistical significance when compared to the diabetes control group (p < 0.001).

3.3.5. Total acidity

Pylorus ligation results in a little difference in total acidity between the DCG and the NCG, 32.16 ± 1.88 vs. 30.66 ± 1.89 mEq/L, respectively (Table 5). When compared to diabetic control, pretreatment of animals with EEMS led to a dose-dependent reduction in total acidity. EEMS 100 mg/kg dosage reduces total acidity by 24.66 ± 1.05 mEq/L. The outcome was statistically significant when equated to the DCG (p < 0.001). Ranitidine and EEMS 400 mg/kg were shown to significantly lower total acidity by 14.33 ± 1.30 and 12.83 ± 0.87 mEq/L, respectively. The outcome was statistically significant when compared to the diabetes control group (p < 0.001).

3.3.6. Renal function test

According to the results of the renal function test (Table 6), the concentration of creatinine significantly raised in the DCG (1.08 ± 0.46 mg/dl vs. 1.95 ± 0.23 mg/dl), and it significantly decreased in the groups receiving ranitidine (50 mg/kg) and EEMS (400 mg/kg) treatments when equated to the diabetic control group. Urea levels considerably rose in the diabetes control group (53.73 ± 3.74 mg/dl) equating to the normal control group (36.75 ± 4.16 mg/dl). It was significantly decreased in the ranitidine and EEMS (400 mg/kg) treated groups compared to the diabetes control group. Comparing DCG to EEMS (100 mg/kg) experimental groups, urea changes were not statistically significant. The level of uric acid was substantially higher in the diabetic control group (7.21 ± 0.37 mg/dl) compared to the normal control (4.61 ± 0.40 mg/dl), and it was significantly lower in the high dosage (5.33 ± 0.41 mg/dl) compared to the diabetic control. No discernible changes were seen between the ranitidine and low-dosage treatment groups. Compared to normal control rats, diabetic control rats' serum concentrations of sodium (128.51 ± 0.96 mEq/L), chloride (79.8 ± 4.27 mEq/L), and calcium (6.93 ± 0.49 mg/dl) were all considerably (p < 0.05) lower while potassium (5.78 ± 0.27 mEq/L) was significantly higher (Table 7). When compared to diabetes control, treating with extract and conventional medications considerably (p < 0.05) raised the concentration of these electrolytes (except for K+, which fell dramatically).

3.3.7. Liver function test

Blood levels of SGPT and SGOT in DCG rats were considerably greater (p < 0.05) than those in NCG throughout liver function tests. In diabetic rats given EEMS and ranitidine treatment, the level of SGPT and SGOT was markedly reduced (p < 0.05). Total protein levels in DCG were 5.78 ± 0.22 g%, compared to 6.16 ± 0.20 g% in NCG, with no discernible difference. Compared to the control rats with diabetes, the ranitidine and EEMS-treated group was shown to have considerably higher concentrations of total protein. The albumin level in the DCG was significantly reduced as compared to the NCG (p < 0.05). All of the treated groups saw a signif-

Table 5
Effect of EEMS on pH, total acidity, free acidity, and gastric juice volume during Pylorus ligation-induced gastric ulcer in albino rats.

Groups	Volume (ml)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)
Normal Control	2.23 ± 0.10	2.36 ± 0.10	18.83 ± 1.49	30.66 ± 1.89
Diabetic Control	2.65 ± 0.15a	2.18 ± 0.16	22.16 ± 1.83	32.16 ± 1.88
Ranitidine 50 mg/kg	1.26 ± 0.10***	4.11 ± 0.10***	7.66 ± 0.49***	12.83 ± 0.87***
EEMS 100 mg/kg	2.15 ± 0.06*	2.95 ± 0.20*	13.16 ± 0.65***	24.66 ± 1.05**
EEMS 400 mg/kg	1.33 ± 0.13***	3.93 ± 0.19***	9.83 ± 0.30***	14.33 ± 1.30***

Values are shown using the mean ± standard error of the mean (n = 6). Dunnett's test after the analysis of variance was performed with the DCG; Significance indicated as **p < 0.01, *p < 0.05, ***p < 0.001 and [a]p < 0.05 (v/s NCG). EEMS = Ethanolic extract of *M. sativa.*

Table 6
Effect of EEMS on renal function test (uric acid, creatinine and urea) of rats in pylorus ligation induced ulcer model.

Groups	Uric acid (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Normal Control	4.61 ± 0.40	1.08 ± 0.46	36.75 ± 4.16
Diabetic Control	7.21 ± 0.37[a]	1.95 ± 0.23[a]	53.73 ± 3.74[a]
Ranitidine 50 mg/kg	5.98 ± 0.23***	0.98 ± 0.10***	41.28 ± 0.20
EEMS 100 mg/kg	6.46 ± 0.19**	1.51 ± 0.22*	45.91 ± 2.91**
EEMS 400 mg/kg	5.33 ± 0.41***	0.83 ± 0.21***	40.26 ± 1.31***

Values are represented by the mean ± standard error of the mean (n = 6). Dunnett's test after the analysis of variance was performed with the DCG; Significance indicated as **p < 0.01, *p < 0.05, ***p < 0.001 and [a]p < 0.05 (v/s NCG). EEMS = Ethanolic extract of *M. sativa*.

icant increase in albumin levels (p < 0.05). There were no notable changes in the TB and ALP levels across the groups (Table 8).

3.3.8. Evaluation of effects of EEMS on the histology of stomach mucosa

A histological analysis was done to determine how EEMS affected the stomach mucosa. According to (Fig. 2), the results of HE staining demonstrated that pre-treatment with EEMS (400 mg/kg) reduced the pathological changes brought on by ethanol in the stomach. Edoema, leukocyte infiltration to the sub-mucosal layer, and stomach epithelial degradation were some of these modifications. The DCG, in contrast to the NCG, had significant epithelial cell loss, inflammation, and the development of hemorrhagic patches on the stomach tissue. The stomach mucosa was comparably better protected by ranitidine (50 mg/kg), as seen by a decrease in the ulcer area, a decrease in sub-mucosal edema, and a lack of epithelial cell loss.

3.4. Ethanol-induced ulcer method

Without considerable change during the trial, the average blood glucose level in the normal group ranged from 96.83 ± 2.76 mg/dl on day one to 101.33 ± 1.28 mg/dl on day 7 (Table 9). Blood glucose levels in the DCG gradually rose from an average value of 353.66 ± 8.62 on day 1 to 396.83 ± 1.9 on day 7. From the first day of the trial to the conclusion, the blood glucose concentration in the standard and treated groups decreased steadily. which, when compared to diabetic controls, was statistically significant.

3.4.1. Ulcer index and ulcer inhibition (%)

One milliliter of ethanol administered orally to rats resulted in severe stomach erosions with an ulcer score of 2.93 ± 0.31 (Fig. 3), whereas rats given ranitidine and *M. sativa* ethanolic extract displayed a significant decrease in UI, with percentages of inhibition in the groups of animals administered with ranitidine, EEMS 100 mg/kg, and 400 mg/kg, respectively, being 89.4, 46, and 83.6 (Table 10).

3.4.2. Evaluation of the stomach mucosa's response to EEMS using histology

A histological analysis was done to determine how EEMS affected the stomach mucosa. The results of HE staining are shown in (Fig. 4), and they demonstrate that pretreatment with EEMS (400 mg/kg) reduced the pathological changes brought on by ethanol in the stomach. This decreased gastric epithelial degeneration and leukocyte infiltration into the sub-mucosal layer in a dosage-dependent manner. The DCG had significant epithelial cell loss, inflammation, and hemorrhagic patch development on the stomach tissue, in distinction to what was seen in the normal control group. The stomach mucosa was comparably better protected by ranitidine (50 mg/kg), as recognized by a decrease in the ulcer zone, a decrease in sub-mucosal edema, and a lack of epithelial cell loss.

3.4.3. Lipid profile

The blood levels of cholesterol, triglycerides, VLDL, and LDL are affected by the therapy, as shown by the data in Table 11 of the presentation. When compared to NCG, it was shown that this was considerably greater in diabetic control rats (p < 0.05). When compared to values in the DCG, the serum levels of cholesterol, triglycerides, LDL, and VLDL in the two groups that consumed varying doses of the extract were significantly (p < 0.05) lower in an amount of the drug manner, but they were markedly (p < 0.05) higher than those of the NCG. On the other hand, groups treated with extract had substantially greater HDL blood levels (p < 0.05) than did DCG.

Table 7
Effect of EEMS on renal function test (sodium, potassium, chloride and calcium levels) of rats in pylorus ligation-induced ulcer model.

Groups	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Calcium (mg/dl)
Normal Control	138.01 ± 0.21	4.56 ± 0.12	102.93 ± 0.28	11.21 ± 0.3
Diabetic Control	128.51 ± 0.96[a]	5.78 ± 0.27[a]	79.80 ± 4.27[a]	6.93 ± 0.49[a]
Ranitidine 50 mg/kg	142.88 ± 0.17***	5.03 ± 0.10*	93.41 ± 3.41**	9.11 ± 0.18*
EEMS 100 mg/kg	140.88 ± 2.37**	5.11 ± 0.04*	98.36 ± 3.11**	8.96 ± 0.12*
EEMS 400 mg/kg	144.96 ± 1.30***	4.83 ± 0.07*	104.83 ± 0.31**	11.41 ± 0.54**

Values are represented by the mean ± standard error of the mean (n = 6). Dunnett's test after the analysis of variance was performed with the DCG; Significance indicated as **p < 0.01, *p < 0.05, ***p < 0.001 and [a]p < 0.05 (v/s NCG). EEMS = Ethanolic extract of *M. sativa*.

Table 8
Effect of EEMS on LFT of rats in pylorus ligation-induced ulcer model.

Groups	Total protein (g %)	Albumin (g %)	ALP (IU/L)	SGPT (IU/L)	SGOT (IU/L)	TB (mg/dl)
Normal Control	6.16 ± 0.20	4.53 ± 0.11	331.43 ± 2.41	86.58 ± 4.87	80.86 ± 9.73	0.68 ± 0.08
Diabetic Control	5.78 ± 0.22	3.58 ± 0.23[a]	274.76 ± 2.30	94.73 ± 3.26[a]	116.36 ± 3.21[a]	0.74 ± 0.01
Ranitidine 50 mg/kg	6.86 ± 0.38*	4.36 ± 0.13*	269.35 ± 8.94**	79.21 ± 1.92*	83.10 ± 3.36***	0.47 ± 0.01
EEMS 100	6.06 ± 0.32	3.95 ± 0.21*	340.15 ± 3.25	87.41 ± 3.54	105.45 ± 1.32*	0.51 ± 0.01
EEMS 400	7.30 ± 0.18**	4.41 ± 0.25*	238.73 ± 1.45**	63.80 ± 6.60***	69.71 ± 0.42***	0.48 ± 0.09

Values are represented by the mean ± standard error of the mean (n = 6). Dunnett's test after the analysis of variance was performed with the DCG; Significance indicated as **p < 0.01, *p < 0.05, ***p < 0.001 and ap < 0.05 (v/s NCG). EEMS = Ethanolic extract of *M. sativa*.

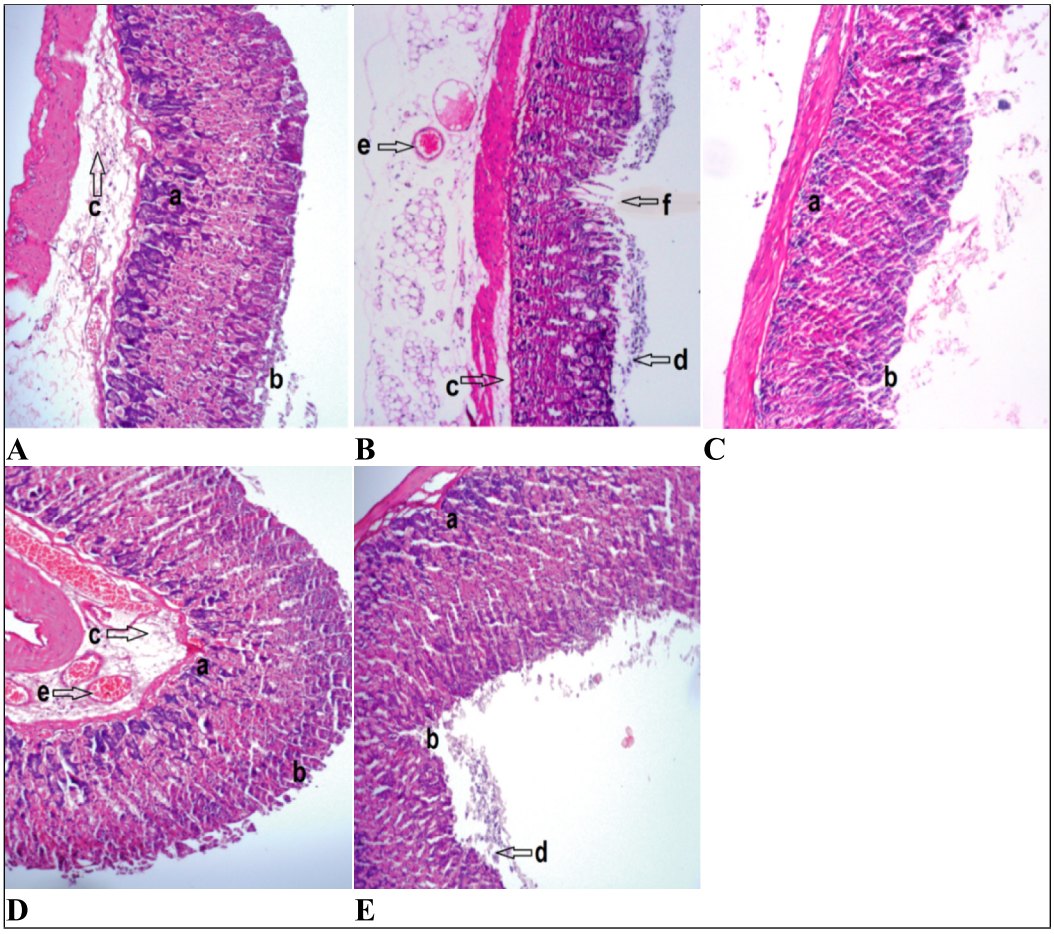

Fig. 2. Wistar Rat stomach tissue from the Pylorus ligation model histopathological images. eosin and hematoxylin dye were applied to histological sections (100X). (A) The normal control groups. (B) Diabetic control Group. (C) Diabetic + Ranitidine 50 mg/kg. (D) Diabetic + EEMS 100 mg/kg. (E) Diabetic + EEMS 400 mg/kg. (a) Gland cell, (b) epithelial cell, (c) significant submucosal edema and leucocyte infiltration, (d) necrosis in confluence in the margin zone, (e) hemorrhage, (f) wide erosion in the mucosa's top half.

Table 9
EEMS's impact on the blood glucose level (mg/dl) in diabetic rats with ulcers brought on by ethanol.

Groups	Blood glucose level (mg/dl)	
	1st Day	7th Day
Normal Control	96.83 ± 2.76	101.33 ± 1.28
Diabetic Control	353.66 ± 8.62[a]	396.83 ± 1.90[a]
Ranitidine	326.83 ± 4.27	314.83 ± 2.38**
EEMS 100 mg/kg	314.83 ± 2.94	287.66 ± 2.40***
EEMS 400 mg/kg	321.66 ± 3.72	264.33 ± 3.11***

Values are represented by the mean ± standard error of the mean (n = 6). Dunnett's test after the analysis of variance was performed with the DCG; Significance indicated as **p < 0.01, *p < 0.05, ***p < 0.001 and [a]p < 0.05 (v/s NCG). EEMS = Ethanolic extract of *M. sativa*.

4. Discussion

A tear in the alimentary canal's mucus layer, which spreads through the mucosa and into the submucosa to develop deeper ulcer disease, causes the gastrointestinal illness known as an ulcer. Acid, pepsin, and the repair of epithelial cells are examples of the aggressive and defensive components that are out of balance and cause peptic ulcers (mucus, formation of PGE. formation of bicarbonate and mucosal blood flow) (Bashir et al., 2014). Over the last several decades, there has been a rise in research aimed at creating safe and efficient anti-ulcer medications, both synthetically and from natural resources. Additionally, herbal medications primarily promote defense mechanisms such as mucin production, cell turnover, bicarbonate secretion, cell mucus, and mucosal blood flow. To

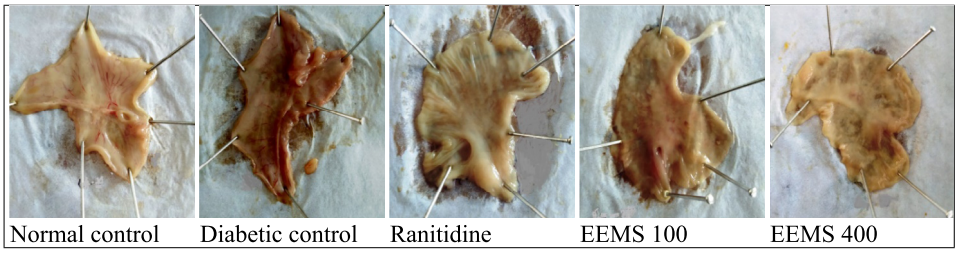

Fig. 3. Representative pictures of stomach rats in ethanol-induced ulcer.

Table 10
EEMS's impact on the stomach ulcer index caused by ethanol in albino rats.

Groups	Ulcer index	Ulcer inhibition (%)
Normal Control	2.06 ± 0.43	–
Diabetic Control	2.93 ± 0.31[a]	–
Standard (Ranitidine)	0.31 ± 0.14[***]	89.40 %
EEMS 100 mg/kg	1.58 ± 0.36[**]	46.00 %
EEMS 400 mg/kg	0.48 ± 0.22[***]	83.60 %

Values are represented by the mean ± standard error of the mean (n = 6). Dunnett's test after the analysis of variance was performed with the DCG; Significance indicated as **p < 0.01, *p < 0.05, ***p < 0.001 and [a]p < 0.05 (v/s NCG). EEMS = Ethanolic extract of *M. sativa*.

find new and unique molecules, the quest for the best anti-ulcer medications has thus expanded to include herbal medicines, which provide superior protection and lower the likelihood of remission (Hussain et al., 2015).

Peptic ulcers are more severe and mostly linked to GIT bleeding issues during the diabetes stage. Peptic ulcer disease (PUD) was shown to be more common in diabetic people than in non-diabetic persons (Garg et al., 2016). The effects of ulcerative medicines or stress on diabetic rats have been found to worsen gastric mucosal sensitivity and prolong the curing of gastric ulcers. Patients with diabetes mellitus have a significant prevalence of

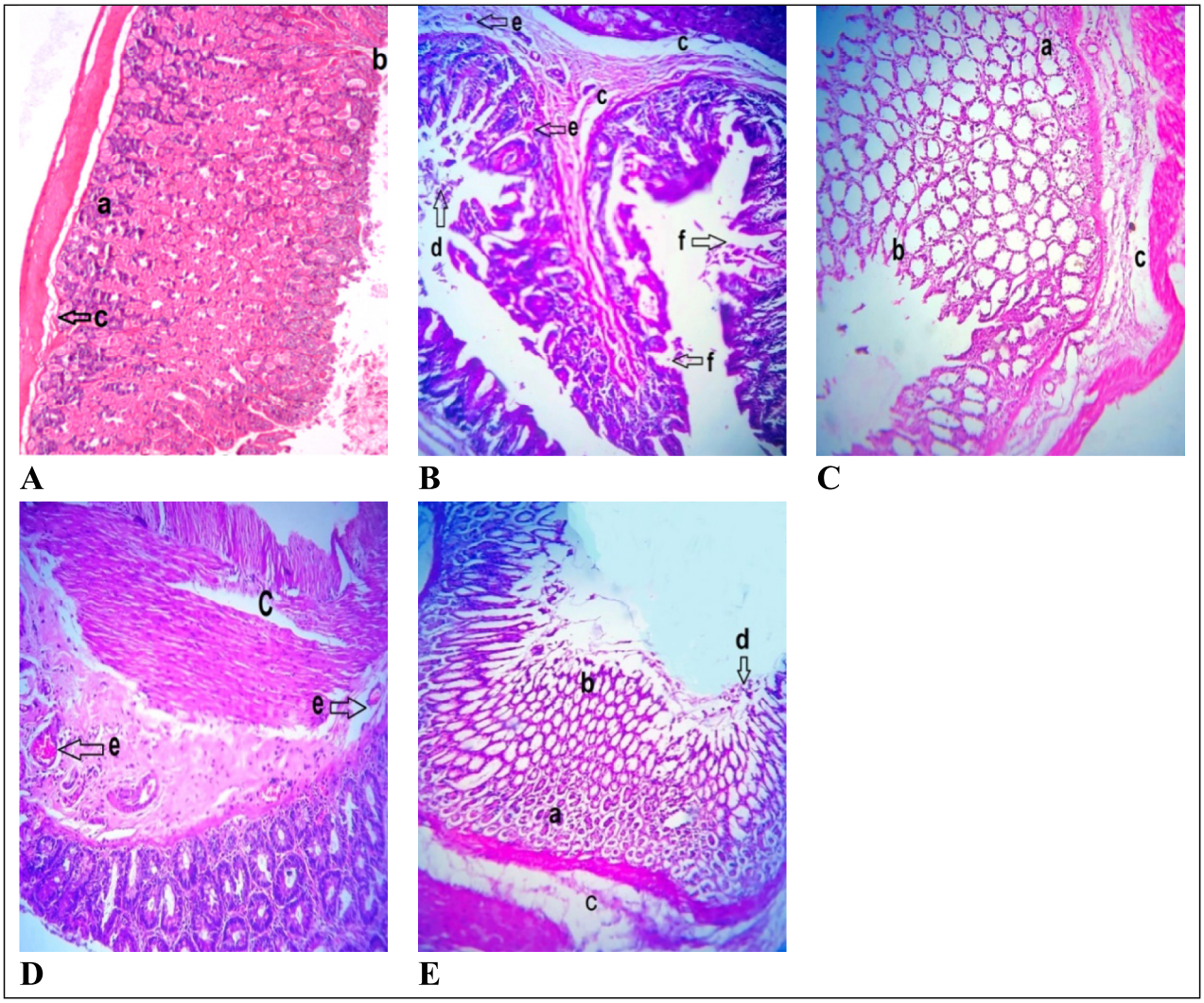

Fig. 4. Histopathological Pictures of stomach tissue of Wistar Rats of Ethanol model. Histological sections were stained with eosin and hematoxylin (100X). (A) The normal control groups. (B) Diabetic control Group. (C) Diabetic + Ranitidine 50 mg/kg. (D) Diabetic + EEMS 100 mg/kg. (E) Diabetic + EEMS 400 mg/kg. (a) Gland cell, (b) epithelial cell, (c) significant submucosal edema and leucocyte infiltration, (d) zone of confluent necrosis on the periphery, (e) hemorrhage, (f) erosion in the upper half of mucosa.

Table 11
Effect of EEMS on lipid profile of rats in ethanol-induced ulcer model.

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal Control	69.94 ± 2.15	54.48 ± 1.82	61.57 ± 3.09	32.5 ± 1.35	27.86 ± 1.14
Diabetic Control	177.45 ± 2.19[a]	183.37 ± 3.83[a]	42.01 ± 1.57[a]	78.08 ± 3.10[a]	64.62 ± 1.39[a]
Ranitidine 50 mg/kg	135.43 ± 2.95[***]	124.7 ± 2.03[***]	83.04 ± 2.01[***]	49.53 ± 2.02[***]	32.08 ± 1.22[***]
EEMS 100 mg/kg	145.66 ± 2.07[**]	151.22 ± 2.49[*]	55.92 ± 2.29[*]	62.97 ± 2.21[**]	38.83 ± 2.16[**]
EEMS 400 mg/kg	124.7 ± 2.54[***]	111.29 ± 4.05[***]	75.34 ± 1.99[***]	43.08 ± 1.03[***]	28.86 ± 1.13[***]

Values are represented by the mean ± standard error of the mean (n = 6). Dunnett's test after the analysis of variance was performed with the DCG; Significance indicated as **p < 0.01, *p < 0.05, ***p < 0.001 and [a]p < 0.05 (v/s NCG). EEMS = Ethanolic extract of *M. sativa*.

ulcer disease and acute stomach inflammation. The stomach mucosa of diabetic rats is more vulnerable to the ulcer-causing effects of medications such as non-steroidal anti-inflammatory medicines (Vimala and Shoba 2014). Autonomic neuropathy, which may cause a range of gastrointestinal signs such as constipation, vomiting, diarrhoea dyspepsia, abdominal pain, and delayed stomach emptying, can occur in people with long-term diabetes mellitus (Harsch et al., 2003). Diabetes mellitus worsens the curing of gastric ulcers and increases the vulnerability of the stomach mucosa to ulcerogenic agents (Konturek et al., 2010). Stomach blood flow, which is essential for healing by delivering nutrients and oxygen to the ulcer area and eliminating toxins, has also been linked to a considerable delay in the recovery of gastric ulcers in diabetic rats (Pimple et al., 2012).

A hydrophilic β-cytotoxin called alloxan damages the insulin-secreting cells in an animal species, leading to chemical diabetes (Djurasevic et al., 2019). Due to its molecular resemblance to glucose, It penetrates cells via GLUT2 transporters, where it inhibits glucokinase and generates ROS (Lenzen 2008). The redox cycle of alloxan GSH-mediated decrease, which decreases dialuric acid and then oxidizes it back to alloxan, produces the reactive oxygen species. The hydroxyl radical, hydrogen peroxide and superoxide anion radical are also involved in the creation of the alloxan radical during this process, as well as the one-electron reduction of alloxan or the one-electron oxidation of dialuric acid. The necrotic death of pancreatic cells is caused by hydroxyl radicals, and alloxan induces diabetes and low blood insulin levels in animal models (Lenzen 2008).

In the current investigation, an effort has been made to look into the potential for *M. sativa* extracts to treat wounds and ulcers in diabetic rats that have been given alloxan injections. MSE has a well-established hypoglycaemic impact that has been studied in the past (Amraie et al., 2015, Farsani et al., 2016). In addition, a dose-dependent anti-hyperglycemic effect was shown in diabetic rats in our current MSE findings. Plasma glucose levels are reduced as a consequence of *M. sativa*'s stimulation of insulin production and enhancement of insulin activity. Prior research found that the presence of *M. sativa* extraction increased insulin secretion by up to three times (Gray et al., 2000).

The results of this investigation demonstrate that *M. sativa*'s ethanolic extract has a gastroprotective effect since it significantly inhibits the growth of ulcers brought on by physical (Pylorus-ligation) and chemical agents (Ethanol). Due to pyloric blockage and subsequent mucosal digesting, increased acid-pepsin buildup results in pylorus-ligation-induced stomach ulcers. According to the pylorus ligature hypersecretion hypothesis, vagus-vagal reflux is brought on by the activation of pressure receptors present in the antral mucosa, which in turn leads to a rise in stomach acid production (Bafna and Balaraman, 2004). The primary reason for gastric ulcers with pyloric ligation is thought to be stress-related increases in stomach HCl output and acid stasis. Because it exposes the body to the building acid of the stomach's exposed lumen, the quantity of secretion is also regarded to have a key role in ulcer development (Hussain et al., 2015). In this procedure, diabetic mice pretreated with EEMS at different dosages had their parameters such as ulcer index, pH, free acidity, total acidity, volume, renal function test, and liver function test assessed. When compared to the DCG, EEMS dramatically reduces the ulcer index, the amount of gastric juice, and the overall acidity, and significantly raises the pH level. EEMS demonstrated a substantial decrease in the ulcer index in diabetic rats, demonstrating their superiority to the drug's (ranitidine) only anti-ulcer impact. Ranitidine may help treat gastric ulceration in diabetic rats because of the crucial role that pepsin and offensive acid production play in the increased tendency to develop gastric ulcers. In diabetic rats treated with alloxan monohydrate, EEMS dramatically reduced total acidity. This impact was virtually identical to that of the ranitidine reference anti-ulcer medication. Thus, this supports *M. sativa*'s anti-ulcer activity in diabetic rats.

The electrolyte concentration and the levels of creatinine, uric acid, and urea in the blood of treated and untreated diabetic animals were used to evaluate the impact of EEMS therapy on the renal function test. Comparing diabetic control rats to normal control rats, there was a noteworthy rise in serum, creatinine, urea, and uric acid in the former group. When compared to DCG, these serum values in the treated groups were significantly reduced. The interaction of alloxan with renal tissue to decrease glomerular functioning may be responsible for the considerable rise in blood concentrations of creatinine, urea, and uric acid in diabetic patients. Treatments using the extract and the control medication, however, resulted in significant decreases. These reductions imply that oral treatment of the crude extract may prevent or significantly reverse renal function impairment brought on by a diabetogenic drug. Creatinine, uric acid, and urea are the proper prognostic markers of renal disease and kidney failure for any harmful chemicals (Usoh and Akpan, 2016). In those with severe diabetes mellitus, excessive lipolysis causes ketosis, followed by acidosis and higher concentrations of urea and creatinine. The kidney ensures that bodily fluids have the right chemical composition by acidifying the urine and getting rid of metabolic waste such as urea, uric acid, and creatinine. When someone has renal illness, their blood levels of these metabolites rise (Sharma et al., 2014). In diabetic control rats, blood levels of sodium, chloride, and calcium were considerably decreased whereas potassium levels dramatically increased. Comparatively to diabetic control groups, treatment with extracts and conventional medications considerably boosted the amounts of various electrolytes (excluding K+, which fell dramatically). The distribution of potassium across the cell membrane, which is largely an intracellular ion, is essential for preserving cardiac and neuromuscular excitability. The capacity of membranes is altered by variations in potassium concentrations that modify the ratio of intracellular to extracellular K^+. Typically, hypokalemia enhances the membrane potential's ability, resulting in hyperexcitability (Usoh and Akpan, 2016). Ranitidine and low dosage extract treated rats had a substantial rise in potassium concentrations (hyperkalemia), which was reversed in high-dose extract treatment, demonstrating a remarkable complimentary activity. Regarding blood calcium, earlier research revealed that diabetes was associated with lower bone mineral content and higher urine calcium excretion (hypocalcaemia) (Fogh-Andersen et al., 1982). The significant decrease in Cl+ (hypochloremia) and Na+ (hyponatremia) levels in DCG rats contrasted to NCG may be due to osmotic diuresis, which causes the succeeding loss of water, and electrolytes brought on by glycosuria (Adrogue et al., 1986). Ketoacidosis is a common condition that causes ketonuria. Alkaline metals like K^+ and Na^+ in serum are decreased as a result of the kidney's effort to buffer the urine.

Serum marker enzyme levels that are higher indicate cellular leakage and a decline in the structural integrity of the liver cell surface (Yakubu et al., 2017). Numerous researchers have noted increased liver enzyme levels in diabetic animals. Because of the increased synthesis of amino acids in hyperglycemic circumstances, higher transaminase levels in the absence of insulin lead to ketogenesis and gluconeogenesis (Baldi and Goyal 2011, Sarfraz et al., 2017). An increase in protein catabolism as a result of insulin deficiency from free radical generation brought on by alloxan induction may also contribute to a decrease in total protein and albumin in alloxan-induced rats. Microproteinurea and

albuminuria are important clinical indicators of diabetic nephropathy (Ajiboye et al., 2014). The *M. sativa* extract has the capacity to reverse alloxan-induced damage in rats' liver and kidneys and to maintain their normal functional condition.

The most popular method for testing novel anti-ulcer medications in animals is ethanol-induced stomach ulcer (Arab et al., 2015). The administration of ethanol lowers the release of nitric oxide, gastric mucus, and bicarbonate while also causing stomach necrotic injury and following inflammatory cell infiltration. Additionally, ethanol decreases stomach blood flow, causes oxidative stress, and increases malondialdehyde formation while lowering glutathione synthesis (El-Maraghy et al., 2015). Absolute alcohol may seriously harm the stomach mucosa and promote neutrophil infiltration, which might be the origin of the inflammatory mediators. Consequently, reducing neutrophil infiltration during inflammation enhanced stomach ulcer healing (Al-Radahe et al., 2013, Al Batran et al., 2013). It has been shown that *M. sativa* extract possesses anti-inflammatory properties (Karimi et al., 2013). It is thought that this plant's anti-inflammatory properties may be responsible for its gastroprotective effects. Antioxidant-producing substances and substances that stimulate the redox system are essential (Klein-Júnior et al., 2012). Consequently, the potent antioxidant action of the plant, which has been well shown in other research, may potentially be connected to the extract's potent ulcer healing action in the ethanol-induced model (Karimi et al., 2013, Gomathi et al., 2014). Various studies have shown that modifications in stomach motility may make a contribution to prevention and grow the gastric lesions. By reducing the amount of gastric irritants on the rugae crest and increasing the mucosal area to which necrotizing chemicals are exposed, relaxation of the circular muscles would protect the stomach mucosa from damage (Al-Radahe et al., 2013, Al Batran et al., 2013). The findings of the current investigation demonstrated that the EEMS might prevent ethanol-induced stomach lesions in diabetic rats.

The second goal was to examine the antihyperlipidemic effects of an ethanolic extract of *M. sativa* in rats that had been ulcerated and made diabetic by alloxan. On the 17th day of therapy, it was found that the diabetic control group had higher LDL, TG, VLDL, and TC and lower HDL than the normal control group. Diabetes causes hyperlipidemia by underutilizing glucose, which is brought on by an excessive mobilization of adipose tissue fat (Akpan et al., 2012). In the diabetic condition, hormone-sensitive lipase is better able to convert stored triacylglycerol into fatty acids, releasing a greater quantity into circulation. This makes it easier for the liver to convert additional fatty acids into cholesterol and phospholipids. Along with excess Triglycerides generated in the liver, cholesterol, and phospholipids may be released in the form of lipoproteins into the blood (Rajaei et al., 2015). Additionally, the inactive lipoprotein lipase breaks down triglycerides, which is a symptom of diabetes. Animals have shown hypertriglyceridemia and a decrease in HDL levels (Pushparaj et al., 2007). Diabetes patients have hypercholesterolemia because insulin blocks HMG-CoA reductase, a crucial enzyme that controls the rate at which cholesterol-rich LDL particles are metabolized (Murali et al., 2002). In the current research, diabetic control rats had elevated plasma levels of CH, TG, VLDL, and LDL, but HDL levels were decreased. Plasma concentration of CH, TG, VLDL, and LDL decreased considerably ($p < 0.01$) after treatment with the plant extract, whereas plasma levels of HDL increased. The plant was discovered to have hypocholesterolemic and antihyperlipidemic action in various earlier research. By increasing the mRNA level of the HMG-CoA reductase and 7-hydroxylase (CYP7Al) gene and decreasing the expression of very-low-density-lipoprotein-recep tor, alfalfa saponin extract (ASE) has been shown to lower cholesterol in laying hens (VLDLR) (Liang et al., 2015, Fana et al., 2018).

5. Conclusion

This research demonstrated for the first time how quickly stomach ulcers in diabetic rats healed when *M. sativa* extract was used. Our findings indicate that the ethanol extract of *M. sativa* has antidiabetic and anti-ulcer activities that may successfully reduce the occurrence and consequences of stomach ulcers in diabetic rats. To elucidate the precise mechanism behind *M. sativa*'s anti-ulcer action in diabetic rats, different pathways are additionally required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Contribution

All the authors have an equal contribution. All the listed authors have read and approved the submitted manuscript.

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