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Effect of *Citrus medica* Linn extract on ethylene glycol induced urolithiasis in streptozotocin-induced diabetic rats

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Abstract
The present study evaluated the ameliorative effect of *Citrus medica* Linn. Extract on ethylene glycol-induced urolithiasis in streptozotocin (STZ)-induced diabetic rats. Eighty male Wistar rats were divided into eight groups (I-VIII, N=10). Diabetes was induced with a single intraperitoneal injection of STZ (60 mg/kg) in groups II, IV, VI, and VIII. Urolithiasis was induced in groups III, IV, VII, and VIII using 0.75% ethylene glycol and 1% ammonium chloride. *Citrus medica* Linn extract (200 mg/kg/day) was administered in groups VI, VII, and VIII. STZ-treated rats showed typical diabetic symptoms, significant weight loss, hyperglycaemia, altered haematology, serum biochemistry, and 40-50% mortality. Ethylene glycol exposure led to depression, lethargy, increased urea, creatinine, and renal calcium oxalate crystal deposition. However, it failed to improve hyperglycaemia, weight loss, haematological, or biochemical changes in diabetic rats. Thus, *Citrus medica* Linn. Extract showed potential in mitigating nephropathy caused by urolithiasis but not in reversing STZ-induced diabetic complications.

Keywords: *Citrus medica* Linn, streptozotocin (STZ), urolithiasis, diabetic, rats

Introduction
The World Health Organization (WHO) emphasizes the development of herbal and herbal medications to benefit people worldwide, increase the quality of the results, and reduce drug side effects. According to the organization, approximately 80% of individuals in industrialized countries rely on pharmaceuticals for their health needs (Barrett, 2003) [2].
Citrus medica Linn is a versatile medicinal agent with various therapeutic properties. Leaves have anthelmintic and estrogenic properties, while fruits have antimutagenic, anticancer, antimicrobial, analgesic, insulin secretagogue, antioxidant, and antiulcer properties. Seeds have antidiabetic, hypolipidemic, and estrogenic properties. Ripe fruits are used for various ailments, including urinary problems (Chhikara *et al*., 2018) [4].
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by reduced insulin production, leading to impaired carbohydrate metabolism and hyperglycemia. Types include type 1, type 2, and gestational diabetes. The exact mechanism of type 2 DM is unclear, but it is believed to be due to increased fatty acid concentrations stimulating the serine kinase cascade. DM can cause serious complications like cardiovascular problems and urinary calculi. Factors contributing to DM include improper metabolism, nitrogen waste products, food habits, dehydration, and water usage (Williams *et al*., 2016) [13].
As these challenges continue to affect humans, several animal models are constantly being produced to examine the pathogenic processes and therapeutic approaches. Among all, streptozotocin (STZ) is a drug that has been commonly used to induce diabetes in mouse and rodent experimental models. It was first reported to have a diabetogenic effect in 1963, and since then, numerous studies have been conducted using multiple combinations to induce diabetes. Research suggests that type 1 diabetes may be developed in animals using a single high-dose STZ injection, and few others use multiple doses. Type 2 diabetes can be induced using various standard techniques, such as STZ injection combined with nicotinamide administration.

A high-fat diet combined with streptozotocin induces type II diabetes due to lipotoxicity and insulin resistance in peripheral tissues.

Calcium oxalate (CaOx) urolithiasis induced by ethylene glycol (EG) in rats is frequently used to investigate the cause of kidney crystal deposition (Bano *et al*., 2018) [1]. For the purpose of studying kidney calcium oxalate crystal deposition, this drug has been used alone or in combination with other drugs, such as ammonium chloride. When rats are administered EG, they develop hyperoxaluria, CaOx crystalluria, and occasional deposition of CaOx crystals in the kidneys (Green *et al*., 2005) [5]. Ammonium chloride consumption increases urine acidification and reduces urinary citrate excretion, which may be responsible for increased CaOx crystal formation in the kidneys (Fan *et al*., 1999) [6]. Animal models that develop uroliths naturally and spontaneously are insufficiently utilized in the study of human stone disease. Improved collaboration among urologists, basic scientists, and veterinarians is required to further understand how stones form and to consider potential new preventive and therapeutic treatment options. Diabetes in animal models is not only an important field of research due to the many similarities with humans, but it can also play a key role in veterinary medicine and its progression in animals.

Research is needed to reduce diabetes mellitus suffering and complications while increasing cost-effectiveness. Domestic large animals are often neglected, and allopathic drugs have been used to treat urinary calculi. Medicinal plants are being studied for their antiurolithiatic and antidiabetic properties, but most are not scientifically documented. *Citrus medica* Linn may be effective in treating urolithiasis (Shah *et al*., 2015) [11].

Materials and Methods

The guidelines of Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India, were followed when the experiment was conducted on laboratory animals. The Institutional Animal Ethics Committee (IAEC), College of Veterinary Science and Animal Husbandry, Sararkrushinagar, has given its approval to the current experimental protocol, No VET COLL/IAEC/2022/19/Protocol No 19.

Collection of Test Article and Preparation of *Citrus medica* Linn. (CM)Extract

Citrus medica Linn. (CM) were collected from surrounding region of Palanpur taluka of Banas kantha district Gujarat. The collected plant was identified and authenticated at the Department of Botany, Hemchandracharya North Gujarat University, Patan and Gujarat. *Citrus medica* Linn**.** Fruits were properly cleaned with distilled water, partially dried on paper towels, and then kept in an oven to finish drying. In a mixer grinder, it was reduced to a fine powder. Finally, powdered fruits material sieved through the kitchen strainer and fine powder was collected for extraction. The powder sample was packed in a thimble and kept in soxhlet apparatus. The solvent (methanol) was taken separately for the extraction and the powdered material was siphoned by 3 times. The whole apparatus was kept over a heating mantle and was heated continuously for 8 hours at boiling point of methanol (56 ºC). The extract was concentrated to dryness and the residues were transferred to a sample bottle and were stored for present studies (Bioquest, 2023) [3]. The extract was made at start of the study and properly stored still used. Daily doses of extract were made.

Animals

The 80 Wistar rats were purchased from the Laboratory Animal Facility of Torrent Research Centre, GIDC, Bhat, Ahmedabad, Gujarat, India. Before being enrolled in the experiments, all the rats were subjected to a 15-day acclimatization period. The care and handling of animals was done in accordance with the standards set by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India.

Induction of diabetes in rats

Citrate buffer preparation

In 800 mL of distilled water was mixed with 25.703 g of Sodium Citrate dihydrate in a suitable container and add 2.421 g of citric acid in the solution. Desired pH of 4.5-4.8 was adjusted by using HCL or NaOH and final volume was made 1 Litre by using distilled water (Bioquest, 2023) [3].

Streptozotocin (STZ) preparation

The streptozotocin (STZ) used in the present study was purchased from Sigma Aldrich (India). A single intraperitoneal injection of 60 mg/kg of streptozotocin (STZ) was given to each of total of 40 rats on day to induce diabetes after an overnight fast. The streptozotocin solution was prepared in 1 ml of 50 mM citrate buffer solution (pH 4.5) by dissolving 32.5 mg and then promptly injected into the peritoneum (Furman, 2015) [7] according to the weight of each animal, whereas control animals were injected intraperitoneallyonly with 50 mM citrate buffer solution (pH 4.5). After seven days of STZ injection, the rats were fasted overnight, blood samples were collected for glucose estimation. For the trial rats were considered diabetic whose fasting blood sugar levels exceeded 200 mg/dl.

Induction of urolithiasis in rats

Preparation ethylene glycol and ammonium chloride

The ethylene glycol and ammonium chloride are purchased from Sigma-Aldrich, Inc. Drinking water containing 0.75% (w/v) ethylene glycol was given to rats for 21 days, coupled with 1% (w/v) ammonium chloride during the first five days, in order to induce urolithiasis (Khan *et al*. 2016) [10].

Preparation of doses of *Citrus medica* Linn. (CM)

The dosage formulation was made by mixing distilled water and *Citrus medica* Linn extract to achieve the necessary concentration, and they were then stored at room temperature. The dose was prepared on a daily basis after each formulation was swirled with a magnetic stirrer until a homogenous suspension was achieved. Oral dosage of the formulation was 1ml per 100g of body weight.

The dose of the aqueous extracts of *Citrus medica* Linn used in present study was adopted from the study of El-Alfy *et al*. (2012) [5]. *Citrus medica* Linn was dissolved in distilled water daily and was administered orally with use of oro-gastric cannula to rats at 200 mg/ kg, BW (at 9.00-11.00 a.m. each day) for a 90 days.

Experimental design: (90 Day's)

Group	Induction of urolithiasis	Induction of diabetes	(CM) Dose	No of animals (Rats)
I (Control)	-	-	0	10
II (Streptozotocin Diabetic (STZD) Control)	-	Yes	0	10
III (Ethylene glycol (EG) Control)	EG from 69th day of study onward	-	0	10
IV (Streptozotocin Diabetic (STZD) + Ethylene glycol (EG) Control)	EG from 69th day of study onward	Yes	0	10
V (*Citrus medica* Linn. Extract (CME)	-	-	200	10
VI (STZD + CME)	-	Yes	200	10
VII (EG + CME)	EG from 69th day of study onward	-	200	10
VIII (STZD + EG + CME)	EG from 69th day of study onward	Yes	200	10
Total				80

Clinical observations

Throughout the course of the study, observations for morbidity and mortality were made twice every day. Clinical observation was documented once daily during the acclimatization period. Pre-dose and two hours after-dose clinical assessments were performed at least twice per day of dosing.

Body weights

The body weight of survived rats was recorded at day one of initiation of dosing and thereafter at weekly intervals throughout the study period. Based on the terminal body weight that was recorded prior to necropsy, relative organ weight was determined. All body weights on dosing days were recorded prior to dosing and used for dose volume determinations.

Blood Glucose

The fasting blood glucose rat for Group (I, II, IV, VI and VIII) were recorded on research days 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91. In present study, rats were fasted for 6 hr. prior to measured blood glucose.

Clinical pathology

The survived rats were fasted overnight prior to blood collection and necropsy. Blood was collected from all experimental groups on the 91th day of study from the retro-orbital plexus with the help of a heparinised capillary tube in serum vial for clinical chemistry and in K3-EDTA for haematology. Haematological parameters *viz*., Haemoglobin estimation (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (TLC) and Differential Leucocyte Counts (DLC) were analysed. Clinical chemistry parameters like Glucose, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT), Creatinine, Urea Nitrogen (BUN), Cholesterol, LDL (low-density lipoproteins), HDL (High-density lipoproteins), Triglycerides, Total Protein, Albumin, Calcium and Phosphate were analysed from sample collected on 91th day.

Statistical Analysis

The statistical analysis of data generated on various parameters were subjected to statistical analysis using one-way analysis of variance (ANOVA).

Results and Discussion

The result obtained from the present study have been described and discussed broadly under the following headings.

Symptomatology

All the rats were observed for development of clinical and behavioural symptoms throughout the experimental period of 90 days. The control rats of group I (GI) did not show any abnormal clinical signs or behaviours. However, STZ treated rats of Groups II (GII), IV (GIV), VI (GVI) and VIII (GVIII) polyuria, polydipsia and dehydration. Polyuria was appreciated by wetness of bedding material and polydipsia was appreciated by consumption of water from the bottom. Polyuria, polydipsia and dehydration were noted from 3rd day of study and consistently reported throughout the study periods in survived rats in GII, GIV, GVI and GVIII. Similarly, ethylene glycol treated rats (Group III (GIII), and group VII (GVII)) showed depression, lethargy and decreased feed intake towards end of study.

Mortality

All the rats were observed twice daily for mortality during experiment. In the present study, no mortality was noted in Group I (Control), III (EG control), V (CME) and VII (EG + CME). However, STZ treatment induced mortality in GII, GIV, GVI and GVIII. In Group II (STZD control) four rats (24,32,43, and 55 day of study), Group IV (STZD + EG control) five rats (16, 23, 24, 31, and 33 day of study), Group VI (STZD + CME)) five rats (10,16, 16, 24, and 26 day of study) and Group VIII (STZD + EG + CME) five rats (10,15, 20, 20, and 33 day of study) were found dead.

Body weight

The summary of the body weight of eight groups is presented in Table 1. In the present investigation, STZ treatment induced significant reduction in body weight in GII, GIV, GVI, and GVIII, while GIII, GV, and GVII body weights were comparable to control. Statistically significant reduction in body weights of STZ treated groups were noted from 4th weeks onwards and trends was continued until end of the study. In the present investigation loss of body weight in GVI (STZD + CME) or GVIII (STZD + EG + CME) was not ameliorated by CME treatment. For GIII (EG control), GV (CME), and GVII (EG + CME) there were no statistically significant difference in the body weight, when compared with control. In intergroup comparison, GI (control) to GV (CME)), GII (STZD control) to GVI (STZD + CME), GIII (EG control), to GVII (EG + CME)) and GIV (STZD + EG control) to GVIII (STZD + EG + CME)) there were not any significant changes found. Bodyweight data clearly indicated that CME unable to ameliorate loss of body weight caused STZ treatment.

Clinical pathology

Haematology

The mean of the haematology data of eight groups is presented in Table 2. In the present study, RBC, Hb and PCV

values were decreased in STZ treated Group GII (STZD Control), GIV (STZD + EG Control), GVI (STZD + CME), GVIII (STZD + EG + CME), when compared with the control group. The RBC HB and PCV values of GIII (EG control). GIV (STZD + EG control) and GVII (EG + CME) were comparable to control. MCH value was decreased in GIII (EG control), GIV (STZD + EG control), GVI (STZD + CME) and GVII (EG + CME). In the present study, thrombocytopenia was noted in STZ treated GII (STZD Control), GIV (STZD + EG Control), GVI (STZD + CME), GVIII (STZD + EG + CME), when compared with the control group. While GIII (EG control) and GVII (EG + CME) showed thrombocytosis. Leucocytosis with absolute increased in neutrophils counts were noted in all groups (GII, GIII, GIV, GVI, GVII and GVIII) except GV when compared with the control group.

In intergroup comparison, (Group GI (control) to GV (CME), GII (STZD control) to GVI (STZD + CME)), GIII (EG control), to GVII (EG + CME) and GIV (STZD + EG control) to GVIII (STZD + EG + CME)) there were no any significant changes found in haematological parameter. Inter comparison data clearly indicate that CME treatment could not protect or ameliorate streptozotocin and/or ethylene glycol induced abnormalities.

Fasting blood glucose

The summary of the Fasting blood glucose level data of four groups is presented in Table 3. Fasting blood glucose levels were monitored weekly for each of the four experimental GII (STZD Control), GIV (STZD + EG Control), GVI (STZD + CME), GVIII (STZD + EG + CME), with all diabetic rats had been fasted for 6 hours prior to analysis.

During the experimental period, a significant increase in glucose levels of blood taken from the caudal vein of rats was observed in GII (STZD Control), GIV (STZD + EG Control), GVI (STZD + CME), GVIII (STZD + EG + CME), when compared to GI (Control). No treatment-related effects of *Citrus medica* Linn were observed.

Biochemistry

The summary of the clinical chemistry data of eight groups is presented in Table 4. In the present study, there was statistically significant increase in serum ALT, AST, ALP, Glucose, triglyceride, cholesterol and LDL level and significant reduction level of HDL in STZ treated GII (STZD control), GIV (STZD + EG control), GVI (STZD + CME) and GVIII (STZD + EG + CME) when it comparable with GI (control). However, no significant changes were found in GGT, TP, albumin, calcium, phosphorus, and magnesium when comparing with GI (control). In this study, there was a statistically significant increase in urea and creatinine levels in GIII (EG Control) and GVII (EG + CME) compared to GI (control). No significant changes were observed in ALT, AST, ALP, GGT, TP, albumin, triglyceride, cholesterol, glucose, calcium, phosphorus, magnesium, HDL, LDL when compared to GI (control). Additionally, no significant changes were observed in biochemistry parameters when comparing Group V (CME) to GI (control). In intergroup comparison, (Group GI (control) to GV (CME), GII (STZD control) to GVI (STZD + CME)), GIII (EG control), to GVII (EG + CME) and GIV (STZD + EG control) to GVIII (STZD + EG + CME)) there were no any significant changes found in clinical chemistry parameters. Inter comparison data clearly indicate that CME treatment could not protect or ameliorate streptozotocin and/or ethylene glycol induced abnormalities. However, when GIII (EG control) was compared with GVII (EG + CME) severity of azotaemia (urea and creatinine levels) was decreased. These findings indicate that CME treatment partially ameliorate azotaemia caused by EG treatment, however no effect on STZ induced clinical chemistry abnormalities.

Table 1: Weekly body weight (gm) (Mean ± SD) in male rats

Day	Group I (Control)	Group II (STZD Control)	Group III (EG Control)	Group IV (STZD + EG Control)	Group V (CME)	Group VI (STZD + CME)	Group VII (EG + CME)	Group VIII (STZD + EG + CME)
0 Day	560.57±44.856^{a} (N=10)	529.68±41.934^{a} (N=10)	528.66±78.392^{a} (N=10)	549.29±32.684^{a} (N=10)	525.61±77.677^{a} (N=10)	536.14±.32.870^{a} (N=10)	538.40±52.494^{a} (N=10)	528.62±34.581^{a} (N=10)
7 Day	560.53±43.479^{a} (N=10)	513.66±42.658^{a} (N=10)	533.11±78.615^{a} (N=10)	527.86±36.224^{a} (N=10)	529.43±78.052^{a} (N=10)	523.64±33.916^{a} (N=10)	548.44±46.824^{a} (N=10)	516.92±33.472^{a} (N=10)
14 Day	565.10±43.676^{b} (N=10)	449.95±42.830^{a} (N=10)	540.44±79.558^{ab} (N=10)	512.19±38.064^{ab} (N=10)	533.60±78.707^{ab} (N=10)	509.69±37.214^{ab} (N=9)	554.19±48.622^{ab} (N=10)	503.12±33.472^{ab} (N=9)
21 Day	570.58±43.900^{c} (N=10)	484.58±41.881^{ab} (N=10)	542.98±80.172a^{bc} (N=10)	486.65±39.852^{ab} (N=9)	538.98±78.426^{abc} (N=7)	497.97±42.633^{abc} (N=10)	560.48±46.508^{bc} (N=10)	477.06±43.552^{c} (N=6)
28 Day	574.06±43.352^{b} (N=10)	473.89±43.072^{a} (N=9)	547.51±79.454^{ab} (N=10)	476.06±35.510^{b} (N=7)	542.89±79.091^{ab} (N=10)	476.64±48.712^{a} (N=5)	566.78±46.508^{b} (N=10)	463.19±49.366^{a} (N=6)
35 Day	574.72±48.670^{a} (N=10)	466.12±44.58^{ab} (N=8)	553.38±80.071^{bc} (N=10)	470.54±33.679^{ab} (N=6)	548.08±79.645^{bc} (N=10)	467.00±48.997^{ab} (N=5)	571.98±48.171^{a} (N=10)	446.57±46.335^{a} (N=5)
42 Day	580.76±45.910^{b} (N=10)	454.28±43.579^{a} (N=8)	555.69±79.372^{b} (N=10)	549.04±30.955^{a} (N=5)	551.77±77.643^{b} (N=10)	461.64±48.498^{a} (N=5)	573.39±47.210^{b} (N=10)	434.35±43.436^{a} (N=5)
49 Day	583.26±45.448^{b} (N=10)	435.78±33.108^{a} (N=7)	558.35±79.410^{b} (N=10)	449.49±31.528^{a} (N=5)	556.01±78.517^{b} (N=10)	455.74±47.541^{a} (N=5)	579.29±45.714^{b} (N=10)	430.79±42.781^{a} (N=5)
56 Day	584.88±46.530^{b} (N=10)	415.06±10.080^{a} (N=6)	559.31±76.922^{b} (N=10)	442.04±32.300^{a} (N=5)	558.40±78.371^{b} (N=10)	450.63±46.589^{a} (N=5)	586.70±48.829^{b} (N=10)	426.23±41.843^{a} (N=5)
63 Day	590.14±45.712^{b} (N=10)	408.82±9.933^{a} (N=6)	562.42±77.672^{b} (N=10)	432.64±31623^{a} (N=5)	562.32±77.068^{b} (N=10)	446.87±45.894^{a} (N=5)	591.15±48.055^{b} (N=10)	422.79±41.293^{a} (N=5)
70 Day	592.55±46.223^{b} (N=10)	404.73±9.830^{a} (N=6)	565.85±78.368^{b} (N=10)	429.40±31.472^{a} (N=5)	565.78±75.865^{b} (N=10)	443.26±45.372^{a} (N=5)	595.15±47.826^{b} (N=10)	419.45±40.677^{a} (N=5)
77 Day	593.36±48.768^{b} (N=10)	400.01±9.635^{a} (N=6)	568.76±77.169^{b} (N=10)	424.49±31.153^{a} (N=5)	569.10±76.865^{b} (N=10)	440.00±44.537^{a} (N=5)	599.47±50.613^{b} (N=10)	416.15±40.064^{a} (N=5)
84 Day	592.53±47.358^{b} (N=10)	396.05±9.591^{a} (N=6)	571.14±76.997^{b} (N=10)	420.14±30.841^{a} (N=5)	573.51±75.368^{b} (N=10)	436.40±43.869^{a} (N=5)	602.38±50.710^{b} (N=10)	412.88±39.134^{a} (N=5)
91 Day	594.27±45.477^{b} (N=10)	390.72±9.492^{a} (N=6)	572.60±77.837^{b} (N=10)	414.51±29.835^{a} (N=5)	575.17±74.703^{b} (N=10)	432.85±43.214^{a} (N=5)	607.10±51.354^{b} (N=10)	409.68±38.543^{a} (N=5)

Table 2: Hematological parameters (Mean ±SD) in male rats

Parameter	Unit	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VII
RBC	10^6/µL	7.57±0.186^a (N=10)	6.89±0.511^a (N=6)	8.09±0.768^a (N=10)	7.10±0.156^a (N=5)	7.64±0.368^a (N=10)	7.05±0.202^a (N=5)	7.77±0.483^a (N=10)	6.99±0.457^a (N=5)
HGB	gm/dl	16.42±0.365^a (N=10)	14.65±0.911^a (N=6)	16.38±1.530^a (N=10)	14.72±0.472^a (N=5)	16.65±0.616^a (N=10)	14.55±0.245^a (N=5)	16.34±0.998^a (N=10)	14.95±1.133^a (N=5)
HCT	%	39.67±0.986^a (N=10)	36.00±0.678^a (N=6)	40.11±3.521^a (N=10)	36.08±1.299^a (N=5)	39.86±1.366^a (N=10)	36.06±0958^a (N=5)	38.79±2.453^a (N=10)	38.39±0.606^a (N=5)
MCV	fL	52.45±1.052^a (N=10)	52.54±4.759^a (N=6)	49.63±1.575^a (N=10)	50.84±1.140^a (N=5)	52.20±1.052^a (N=10)	51.18±1.462^a (N=5)	50.00±3.339^a (N=10)	55.20±4.594^a (N=5)
MCH	pg	21.71±0.537^a (N=10)	21.29±0.465^a (N=6)	20.26±0.446^a (N=10)	20.75±0.441^a (N=5)	21.80±0.549^a (N=10)	20.65±0.347^a (N=5)	21.03±0.592^a (N=10)	21.40±0.475^a (N=5)
MCHC	g/dL	41.40±0.729^a (N=10)	40.72±2.865^a (N=6)	40.83±0.665^a (N=10)	40.81±0.668^a (N=5)	41.76±0.411^a (N=10)	40.36±0.682^a (N=5)	42.17±2.032^a (N=10)	38.98±3.448^a (N=5)
PLT	10^3/µL	798.80±66.181^a (N=10)	512.56±76.854^a (N=6)	957.10±99.235^b (N=10)	639.04±35.723^a (N=5)	827.80±168.636^a (N=10)	674.19±41.353^a (N=5)	1009.10±94.566^a (N=10)	648.63±56.030^a (N=5)
WBC	10^3/µL	11.47±2.387^a (N=10)	17.13±0.546^{bcd} (N=6)	15.96±5.065^{bc} (N=10)	18.19±0.454^{cd} (N=5)	13.52±2.705^{ab} (N=10)	17.63±0.841^{bcd} (N=5)	20.67±2.770^d (N=10)	27.12±1.258^e (N=5)
Neutrophils	10^3/µL	1.65±0.673^a (N=10)	4.05±0.345^b (N=6)	4.24±2.188^b (N=10)	4.95±0.514^b (N=5)	1.69±0.490^a (N=10)	4.18±0.260^b (N=5)	5.17±0.854^b (N=10)	6.73±0.636^c (N=5)
Lymphocytes	10^3/µL	9.07±1.621^a (N=10)	12.34±0.688^{ab} (N=6)	10.95±4.295^{ab} (N=10)	12.36±0.230^{ab} (N=5)	11.19±2.385^{ab} (N=10)	12.60±0.990^{ab} (N=5)	14.62±1.930^b (N=10)	19.35±1.013^c (N=5)
Monocytes	10^3/µL	0.54±0.247^a (N=10)	0.57±0.084^{ab} (N=6)	0.56±0.176^{bc} (N=10)	0.65±0.101^{abc} (N=5)	0.50±0.134^{ab} (N=10)	0.63±0.102^{abc} (N=5)	0.66±0.214^{cd} (N=10)	0.76±0.256^d (N=5)
Eosinophils	10^3/µL	0.19±0.121^a (N=10)	0.17±0.005^a (N=6)	0.20±0.084^a (N=10)	0.22±0.077^a (N=5)	0.14±0.027^a (N=10)	0.21±0.085^a (N=5)	0.23±0.066^a (N=10)	0.27±0.013^b (N=5)
Basophils	10^3/µL	0.02±0.048^a (N=10)	0.00±0.000^a (N=6)	0.00±0.000^a (N=10)	0.00±0.000^a (N=5)	0.00±0.000^a (N=10)	0.00±0.000^a (N=5)	0.00±0.000^a (N=10)	0.00±0.000^a (N=5)

Non-significant (p>0.05)

Table 3: Fasting blood glucose (Mean ±SD) in male rats

Day	Group II (STZD Control)	Group IV (STZD + EG Control)	Group VI (STZD + CME)	Group VIII (STZD + EG + CME)
0 Day	471.5±88.052^a (N=10)	479.80±107.950^a (N=10)	475.20±.99.516^a (N=10)	473.90±88.906^a (N=10)
7 Day	480.60±88.677^a (N=10)	487.60±108.470^a (N=10)	483.50±98.743^a (N=10)	474.90±93.827^a (N=10)
14 Day	480.50±89.470^a (N=10)	492.20±110.967^a (N=10)	473.78±90.828^a (N=9)	461.11±92.931^a (N=9)
21 Day	475.90±87.754^a (N=10)	499.67±115.588^a (N=9)	490.00±97.794^a (N=10)	467.17±86.038a (N=6)
28 Day	473.89±83.156^a (N=9)	480.43±121.264a (N=7)	489.20±92.294^a (N=5)	476.83±74.717^a (N=6)
35 Day	490.13±92.643^a (N=8)	445.33±101.944^a (N=6)	488.00±101.012^a (N=5)	491.60±69.364^a (N=5)
42 Day	486.00±78.316^a (N=8)	416.60±75.761^a (N=5)	4494.80±97.996^a (N=5)	490.40±80.869^a (N=5)
49 Day	484.29±77.519^a (N=7)	428.20±81.060^a (N=5)	492.00±100.027^a (N=5)	497.80±74.429^a (N=5)
56 Day	481.67±60.165^a (N=6)	431.20±81.625^a (N=5)	482.00±104.926^a (N=5)	492.20±64.414^a (N=5)
63 Day	470.00±72.553^a (N=6)	431.20±72.144^a (N=5)	482.62±108.367^a (N=5)	492.20±67.821^a (N=5)
70 Day	481.67±74.471^a (N=6)	433.40±72.848^a (N=5)	491.40±105.070^a (N=5)	502.20±75.284^a (N=5)
77 Day	479.67±67.468^a (N=6)	428.80±85.042^a (N=5)	492.00±101.634^a (N=5)	501.00±76.010^a (N=5)
84 Day	488.17±61.934^a (N=6)	4425.40±81.626^a (N=5)	486.60±103.018^a (N=5)	498.60±68.175^a (N=5)
91 Day	501.83±55.876^a (N=6)	438.60±84.852^a (N=5)	482.40±109.212^a (N=5)	497.40±75.252^a (N=5)

Table 4: Biochemical parameters (Mean ± SD) in male rats

Parameter	Unit	Group I (Control)	Group II (STZD Control)	Group III (EG Control)	Group IV (STZD + EG Control)	Group V (CME)	Group VI (STZD + CME)	Group VII (EG + CME)	Group VIII (STZD + EG + CME)
Alanine Aminotransferase (ALT)	U/L	91.97±11.182^a (N=10)	304.11±28.255^c (N=6)	99.72±28.714^a (N=10)	312.15±40.297^c (N=5)	93.40±5.725^a (N=10)	296.76±67.736^c (N=5)	86.74±17.969^a (N=10)	238.57±8.931^b (N=5)
Aspartate Aminotransferase (AST)	U/L	187.04±28.825^a (N=10)	381.53±39.497^b (N=6)	177.16±35.304^a (N=10)	377.16±35.304^b (N=5)	188.61±55.612^a (N=10)	367.50±19.000^b (N=5)	180.42±12.168^c (N=10)	401.92±58.084^b (N=5)
Alkaline Phosphatase (ALP)	U/L	208.71±53.774^a (N=10)	544.04±28.239^b (N=6)	182.14±39.728^a (N=10)	534.30±32.967^b (N=5)	212.32±51.069^a (N=10)	528.28±124.555^b (N=5)	196.46±46.474^a (N=10)	872.83±41.042^c (N=5)
Gamma Glutamyl Transferase (GGT)	g/dL	0.50±0.527^a (N=10)	0.33±0.516^a (N=6)	0.50±0.527^a (N=10)	0.60±0.548^a (N=5)	0.60±0.516^a (N=10)	0.60±0.548^a (N=5)	0.60±0.516^a (N=10)	0.60±0.548^a (N=5)
Total Protein	g/dL	7.02±0.230^a	7.49±0.521^a	7.30±0.663^a	7.17±0.464^a	7.22±0.239^a	7.29±0.608^a	7.11±0.233^a	7.37±.230^a

		(N=10)	(N=6)	(N=10)	(N=5)	(N=10)	(N=5)	(N=10)	(N=5)
Albumin	mg/dl	3.63±0.200^{a} (N=10)	3.43±0.190^{a} (N=6)	3.68±0.187^{a} (N=10)	3.52±0.375^{a} (N=5)	3.73±0.116^{a} (N=10)	3.51±0.108^{a} (N=5)	3.53±0.241^{a} (N=10)	3.50±0.258^{a} (N=5)
Urea	mg/dl	14.30±2.720^{a} (N=10)	22.52±8.479^{b} (N=6)	52.91±9.073^{c} (N=10)	22.46±2.445^{b} (N=5)	14.24±8.618^{a} (N=10)	21.91±5.946^{ab} (N=5)	39.20±8.696^{c} (N=10)	20.20±4.657^{ab} (N=5)
Creatinine	mg/dl	0.82±0.207^{a} (N=10)	1.12±0.522^{ab} (N=6)	1.44±0.512^{c} (N=10)	1.33±0.132^{b} (N=5)	0.69±0.049^{a} (N=10)	1.00±0.300^{b} (N=5)	0.98±0.220^{a} (N=10)	1.00±0.274^{b} (N=5)
Triglyceride	mg/dl	81.11±14.025^{a} (N=10)	430.03±31.329^{b} (N=6)	80.94±14.097^{a} (N=10)	431.30±32.210^{b} (N=5)	86.85±18.024^{a} (N=10)	418.38±25.820^{b} (N=5)	82.62±20.594^{a} (N=10)	425.54±30.328^{b} (N=5)
Cholesterol	mg/dl	86.37±16.840^{a} (N=10)	153.59±11.190^{b} (N=6)	88.37±13.404^{a} (N=10)	151.46±10.190^{b} (N=5)	83.33±9.977^{a} (N=10)	148.32±18.212^{b} (N=5)	88.97±11.905^{a} (N=10)	147.00±12.916^{b} (N=5)
Glucose		91.73±16.168^{a} (N=10)	556.80±48.967^{c} (N=6)	107.81±18.305^{a} (N=10)	512.77±41.835^{bc} (N=5)	85.80±12.777^{a} (N=10)	509.37±22.727^{b} (N=5)	87.45±21.042^{a} (N=10)	499.38±31.064^{b} (N=5)
Calcium	mg/dl	10.86±0.587^{a} (N=10)	10.81±0.522^{a} (N=6)	10.41±0.549^{a} (N=10)	10.62±0.338^{a} (N=5)	10.65±0.428^{a} (N=10)	10.66±0.406^{a} (N=5)	11.17±0.869^{a} (N=10)	10.58±0.396^{a} (N=5)
Phosphorus	mg/dl	12.44±0.963^{a} (N=10)	12.85±0.986^{a} (N=6)	11.02 ±1.632^{a} (N=10)	12.71±0.792^{a} (N=5)	12.30±1.707^{a} (N=10)	12.14±0.997^{a} (N=5)	11.97 ±1.044^{a} (N=10)	12.52±0.775^{a} (N=5)
Magnesium		0.74±0.546^{a} (N=10)	4.62±0.563^{a} (N=6)	4.73±1.396^{a} (N=10)	4.61±0.536^{a} (N=5)	4.53±0.523^{a} (N=10)	4.45±0.134^{a} (N=5)	4.34±0.535^{a} (N=10)	4.23±0.281^{a} (N=5)
HDL	U/L	215.86±8.517^{c} (N=10)	189.60±9.799^{ab} (N=6)	216.83±16.655^{c} (N=10)	181.04±12.577^{a} (N=5)	217.91±13.800^{c} (N=10)	187.78±8.654^{ab} (N=5)	209.85±22.433^{bc} (N=10)	184.34±10.186^{a} (N=5)
LDL		27.09±2.445^{a} (N=10)	56.17±4.092^{c} (N=6)	27.41±3.707^{a} (N=10)	53.18±3.180^{c} (N=5)	26.95±3.483^{a} (N=10)	52.12±6.575^{c} (N=5)	28.17±4.898^{a} (N=10)	49.00±6.703^{b} (N=5)

* Significant (p<0.05)

Summary and Conclusion

In the present study, STZ induced diabetic rats of Groups II, IV, VI and VIII showed polyuria, polydipsia and dehydration. Ethylene glycol treated rats Group III, and group VII showed depression, lethargy and decreased feed intake towards end of study. Streptozotocin, at the dose rate of 60 mg/kg body weight, induced marked hyperglycemia in Groups II (STZD control), IV (STZD + EG control), VI (STZD + CME), and VIII (STZD + EG + CME) rats and 40 to 50% mortality was observed in Groups II, IV, VI and VIII diabetic rats. In the present investigation, STZ induced significant reduction in body weight in Groups II (STZD control), IV (STZD + EG control), VI (STZD + CME), and VIII (STZD + EG + CME) rats were comparable to control. Group III (EG control), V (CME), and VII (EG + CME) rats showed no statistically significant difference in the body weight, when compared with control. Haematology parameters of Groups II (STZD control), IV (STZD + EG control), VI (STZD + CME), and VIII (STZD + EG + CME) showed anemia, neutrophiliac leukocytosis and thrombocytopenia. Serum biochemical parameters were statistically significant increased for serum ALT, AST, ALP, glucose, triglyceride, cholesterol and LDL cholesterol level, while significantly decreased HDL cholesterol level.

From the present study, following conclusions are drawn when *Citrus medica* Linn extract given in ethylene glycol induced urolithiasis in streptozotocin-induced diabetic rats

- Streptozotocin, at the dose rate of 60 mg/kg body weight, induced marked hyperglycemia in Groups II (STZD control), IV (STZD + EG control), VI (STZD + CME), and VIII (STZD + EG + CME) rats and 40 to 50% mortality.
- *Citrus medica* Linn extract treatment (@ 200 mg/kg, BW) did not ameliorate abnormalities of STZ induced weight loss, haematological parameters, clinical chemistry parameters and histopathological alterations in groups VI (STZD + CME), and VIII (STZD + EG+ CME).

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Reference

1. Bano H, Jahan N, Makbul SAA, Kumar BN, Husain S, Sayed A. Effect of *Piper cubeba* L. fruit on ethylene glycol and ammonium chloride induced urolithiasis in male Sprague Dawley rats. Integr Med Res. 2018;7(4):358-65. DOI: 10.1016/j.imr.2018.06.005
2. Barrett B. Medicinal properties of *Echinacea*: A critical review. Phytomedicine. 2003;10(1):66-86. DOI: 10.1078/094471103321648692
3. Bioquest. Quest Calculate™ citrate buffer (pH 3.0 to 6.2) preparation and recipe. AAT Bioquest; 2023. Available from: https://www.aatbio.com/resources/buffer-preparations-and-recipes/citrate-buffer-ph-3-to-6-2
4. Chhikara Bawari S, Sah AN, Gupta P, Zengin G, Tewari D. Himalayan *Citrus jambhiri* juice reduced renal crystallization in nephrolithiasis by possible inhibition of glycolate oxidase and matrix metalloproteinases. J Ethnopharmacol. 2023;306(4):116157. DOI: 10.1016/j.jep.2023.116157
5. El-Alfy TS, Hetta MH, Yassin NZ, Rahman RFA, Kadry EM. Estrogenic activity of *Citrus medica* L. leaves growing in Egypt. J Appl Pharm Sci. 2012;2(8):180-185. DOI: 10.7324/JAPS.2012.2831
6. Fan J, Glass MA, Chandhoke PS. Impact of ammonium chloride administration on a rat ethylene glycol urolithiasis model. Scanning Microsc. 1999;13(2-3):299-306. DOI: 10.1016/S0022-5347(05)68922-7
7. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2015;70(1):5-47. DOI: 10.1002/0471141755.ph0547s70
8. Green ML, Hatch M, Freel RW. Ethylene glycol induces hyperoxaluria without metabolic acidosis in rats. Am J Physiol Renal Physiol. 2005;289(3):F536-543. DOI: 10.1152/ajprenal.00025.2005
9. World Health Organization (WHO). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: Report of a WHO/IDF consultation. Geneva: WHO; 2006. ISBN: 9789241594936.
10. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, Tiselius HG. Kidney stones. Nat Rev Dis Primers. 2016;2(1):1-23. DOI: 10.1038/nrdp.2016.8
11. Shah AP, Patel S, Patel K, Gandhi T. Effect of *Citrus medica* Linn in urolithiasis induced by ethylene glycol

model. Iran J Pharmacol Ther. 2015;13(1):35-40. Available from: http://ijpt.iums.ac.ir/article-1-266-en.html
12. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. Int J Med Sci. 2014;11(11):1185-200. DOI: 10.7150/ijms.10001
13. Williams RS, Kozan P, Samocha-Bonet D. The role of dietary acid load and mild metabolic acidosis in insulin resistance in humans. Biochimie. 2016;124:171-177. DOI: 10.1016/j.biochi.2015.09.012

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