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Antidiabetic, antihyperlipidemic effects and quantification of bioactive compounds by HPLC from *Crassula ovata* leaves extracts on streptozotocin induced diabetic Wistar rats

Research Article

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Abstract

Background: *Crassula ovata* have been recommended in ethno medicines for the treatment of diabetes. In this research the scientific claim is studied and validated. Objective: Objective of the current study has been focused to assess the traditional use of *Crassula ovata* to heal diabetes, hyperlipidemia and to check the presence of constituents responsible for these actions. Methods: The approved plant's ethanol, acetone, and chloroform extracts were prepared by maceration. For in vivo activity, the model of streptozotocin induced diabetes mellitus in rats was chosen, wherein antidiabetic parameters such as body weight, urine volume, blood glucose level, and lipid profile parameters were evaluated. Quantification of bioactive compounds was done by a high-performance liquid chromatographic technique using diode-array detection. Results: The ethanolic extract noticeably depleted increased blood glucose levels and has positive effects on altered lipid profile after administering a dose of 200 mg/kg orally compared with oral hypoglycemic drug metformin. HPLC analysis of ethanolic extract identified several secondary metabolites in *Crassula ovata*, including Gallic acid, Rutin, Luteolin, Caffeic acid, Catechin, Kaempferol and Quercetin. These compounds have been reported for their potential antidiabetic effects. The analytical data on the chemical composition supported its traditional use in diabetes management. Conclusion: From the results, it can be concluded that, *Crassula ovata* ethanolic extract can be ideal for the treatment of diabetes and hyperlipidemia. Further studies are required to locate the active pharmaceutical ingredient for the said actions.

Keywords: *Crassula ovata,* Antidiabetic Activity, Streptozotocin, RP-HPLC, Ethanolic extract, Histopathology.

Introduction

Numerous health advocacy organisations, including the World Health Organisation (WHO), recognised that diabetes mellitus is an epidemic that is spreading throughout the world. Within the next 25 years, diabetes is predicted by the WHO to rank among the major causes of death and disability worldwide. The findings are concerning: in 1985, 30 million persons worldwide were diagnosed with diabetes; by 1995, that figure had increased to 135 million; and if current trends continue, the World Health Organisation expects that by 2025, there would be over 300 million diabetics worldwide(1). According to a population survey conducted by the Indian Council of Medical Research, China leads the world in diabetes cases, estimated at 98.4 million, followed by India, with 65.1 million people (2). In order to reduce the burden of diabetes, population-based initiatives that address the modifiable diabetes risk factors particularly obesity and physical inactivity are required. Rising rates of diabetes risk factors will only make the issue worse. Furthermore, problems among individuals with diabetes are common. These complications add to the diabetes's ever-increasing expenses while also having a major impact on the disease's morbidity and death (3). Even though anti-diabetic medications are available on the pharmaceutical market, using medicinal herbs to treat diabetes is frequently effective. Throughout the world, important therapeutic choices for the treatment of this condition include herbal medications and plant components with negligible toxicity and no adverse effects. The majority of studies have shown how beneficial medicinal herbs with hypoglycaemic characteristics are for the treatment of diabetes. The most often used herbal active components in the treatment of diabetes are alkaloids, phenolic acid, flavonoids, and tannins (4). For example Gallic acid, reduces glucose and glycosylated haemoglobin levels while raising insulin levels to have anti-hyperglycaemic actions (5). Tannin enhances insulin secretion and beta-cell activity in the pancreas. Lipid per-oxidation and

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metal ion chelation are inhibited by quercetin, an antioxidant that works through plenty of mechanisms linked to the elimination of oxygen radicals. Indeed, hypoglycaemic plants work by raising insulin secretion, increasing the absorption of glucose by muscle and fat tissues, inhibiting the absorption of glucose from the intestine, and preventing the liver's cells from producing glucose (4). Jade plant, or *Crassula ovata*, is a medicinal herb that has long been used to alleviate diabetes symptoms. The *Crassula ovata* plant is a popular element in Asian cultures, especially in China (about 700 AD). Diabetes symptoms were treated with *Crassula ovata* plant by physician (6). It is known from the traditional usage of several people in North East India (particularly in Manipur) that *Crassula* ovata is primarily used to treat infections and diabetes. They used to consume the plant's leaf juice to treat diabetes (7). The results of the phytochemical screening show that the plant extracts contained protein, carbohydrates, terpenoids, phenolic acid, saponin, phytosterol, and steroid. The significance of these chemicals' anti-diabetic qualities is implied by their existence. *Crassula ovata* have been used in ethno medicines for the treatment of different ailments (8).

Because of this, we planned a study to evaluate *Crassula ovata* anti-diabetic impact on rats that had been exposed to streptozotocin. Thus, the purpose of the current study was to look into the herb *Crassula ovata* antidiabetic properties. Additionally, using HPLC-DAD analysis, the chemical makeup of the *Crassula ovata* was assessed in order to offer a deeper understanding.

Materials and methods

In its meeting on October 16, 2023, the IAEC of Pravara Rural College of Pharmacy, Pravaranagar, accepted the experimental protocol; proposal no.1942/ PO/Re/S/17/CPCSEA/2023/01/28.

Drugs and chemicals

Metformin (Merck, Germany) and streptozotocin (Biogenuix Medsystems Pvt. Ltd., New Delhi). Other chemicals that were purchased from PCL, India included petroleum ether, chloroform, ethanol, methanol, acetone, ethyl acetate, sodium citrate dihydrate, benzene, citric acid, hydrochloric acid, sodium chloride, potassium chloride, and calcium chloride of analytical grades. We bought our Accu Check glucometer from Roche Diabetes Care Inc.

Plant material

Dr. Wabale Anil Sopanrao, Head of Department of Botany, PVP College of Arts, Science and Commerce, Pravaranagar, Maharashtra, India, authenticated the entire *Crassula ovata* plant, which was obtained from the local market in Rahata, District: Ahmednagar, Maharashtra, via letter number PVPC/ Bot/2023-24/202 dated November 1, 2023.

Preparation of Plant Extract

The *Crassula ovata* leaves was dried under a shadow, after drying the 100g coarse powder of plant was extracted by maceration separately to avoid the loss of thermolabile constituents with different solvents like ethanol, acetone and chloroform for 72 hours with frequent agitation and stirring (9). Extraction efficiency was increased by maceration process. After macerating for 72 hours, the extract was filtered. and the rotary evaporator was used to concentrate the extract. Before being used in the experiment, the obtained crude extract was kept refrigerated in an airtight bottle (10).

Acute toxicity study

The OECD 2022 guideline no. 425 was followed in conducting the study. Female Albino mice weighing 24-35gm (11–12 weeks) were used in this investigation. They separated the albino mice into five groups. A 0.5% w/v carboxy methyl cellulose solution was used to induce a vehicle control in the first group of mice (n = 3 groups). Second were subsequently split into twelve subgroups for graded dosages of the medication *Crassula ovata* at 5, 50, 300, and 2000 mg/kg (n=3/ group) for ethanol, acetone, and chloroform extract. Mice were studied for 24 hours after receiving a single oral dose of the medicines, and then once a day for 14 days (13). The mice were weighed and examined for signs of aggression, asthenia, hypoactivity, hyperactivity, piloerection, hyperventilation, yellowing or loss of skin fur and hair, drowsiness, and mortality (14).

Induction of Experimental Diabetes

For the experiment, healthy male Wistar rats weighing between 250 and 300 gm were selected following a week-long period of acclimation. Male rats were used in diabetes research because they are more sensitive to streptozotocin (STZ) than female rats. After STZ treatment, female rats tend to have higher glucose levels, lower insulin levels, and a worse survival rate than male rats. Female rats are also more likely to develop a more severe form of diabetes than males (23). Single intraperitoneal administrations of STZ (55 mg/ kg) combined with 0.05 M citrate buffers (pH 4.5) were given to experimentally develop diabetic mellitus following an overnight fast. To prevent hypoglycaemia mortality, rats were administered oral glucose solution containing 5% w/v (2 ml/kg/BW) 24 hours after diabetes mellitus was induced (15). On the fifth day after the STZ injection, a blood glucose test was used to confirm the development of diabetes. In order to be employed in experiments, animals with fasting blood glucose levels greater than 250 mg/dl were classified as diabetics (16).

Experimental Design

The twelve groups of six experimental rats each were randomly assigned, and the animals received treatment for twenty days.

Group I Normal control (NC) non diabetic rats were kept on a regular diet and with unlimited access to water, Group II Diabetic control (DC) which diabetes was induced by injecting Streptozotocin (STZ) and was an untreated group rats were kept on a regular diet and with unlimited access to water, Group III positive control (PC) diabetic rats given the normal dose of 150

mg/kg b.w. of metformin, Group IV COEE50 diabetic rats treated with *Crassula ovata* ethanolic extract 50mg/kg, Group V COEE100 diabetic rats treated with *Crassula ovata* ethanolic extract 100mg/kg, Group VI COEE200 diabetic rats treated with *Crassula ovata* ethanolic extract 200mg/kg, Group VII COAE50 diabetic rats given a 50 mg/kg dose of *Crassula ovata* acetone extract, Group VIII COAE100 diabetic rats given a 100 mg/kg acetone extract of *Crassula ovata,* Group IX COAE200 *Crassula ovata* acetone extract 200 mg/kg was administered to diabetic rats, Group X COCE50 diabetic rats treated with *Crassula ovata* chloroform extract 50mg/kg, Group XI COCE100 diabetic rats treated with *Crassula ovata* chloroform extract 100mg/kg, Group XII COCE200 diabetic rats treated with *Crassula ovata* chloroform extract 200mg/kg.

The results of an acute toxicity study were used to determine the dosage of *Crassula ovata*. The previously mentioned groups underwent examination of several biochemical parameters in order to track the antidiabetic activity of the specimens. A small amount of blood was collected from the tail vein to measure the glucose levels with a glucometer (glucometer was used for instant and rapid analysis using a small amount of blood sample). For every experimental animal, body weight changes were recorded on the pre-study day as well as the first, fifth, tenth, fifteenth, and twentieth day. All of the experimental animals' blood samples were taken at the conclusion of the study, and the plasma was separated and used for the biochemical examination.

Biochemical Analysis

A small amount of blood, a few millilitres (ml), was taken from the retro-orbital sinus on day twenty and placed in EDTA-containing sample tubes. The drawn blood was centrifuged for 10 minutes at 4°C at 2000 g, and the separated plasma layer was utilised for additional estimate. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) are among the lipid values (17).

HPLC Analysis

Exactly 200 mg of dried leaves ethanolic extract of plant, *Crassula ovata* was weighed and dissolved in 5 ml of a (2:1:1 v/v) acetonitrile-methanol-water mixture. It was kept overnight for 12 hours at room temperature in air tight HPLC grade container and after which was placed in a 55°C water bath for ten minutes (11). Furthermore the sample was ultra-sonicated for 10 minutes in order to accelerate the solid-liquid extraction and then filtered through 0.45 um nylon filter. Freshly prepared sample of 20 ul was injected into the HPLC-DAD instrument and detection was carried out at 210 nm to investigate medicinal phytoconstituents from *Crassula ovata* ethanolic extract (12).

Histopathological Analysis

Rats in the experimental group were sacrificed at the end of the twentieth day, and the liver and pancreas were separated for histological examination (18). Sections of the specimens were kept in 10% formalin. Using a microtome, tissue from paraffin-embedded tissue blocks has been cut into 5 µm sections. After being deparaffinized, rehydrated, and stained with haematoxylin and eosin (H&E), the section was put on a glass slide (19). The slides were examined under a light microscope and recorded after being mounted with DPX and covered (20).

Statistical analysis

For every group, the mean ± standard deviation (SD) or standard error of the mean (SEM) data was calculated (21). Graph Pad Prism 10.02; the statistical analysis of all the data was conducted using one-way analysis of variance (ANOVA) and the student t-test. Dunnett's comparison test was used to determine the statistical significance between the drug-treated groups and the negative control group ($p < 0.05$ was considered significant) (22).

Results

Percentage yield of different solvent extracts of *Crassula ovata*

Table 1: Percentage yield of different solvent extracts of *Crassula ovata*

Sr.no.	Extract	% Yield
1	COEE	15.146%
2	COAE	12.458%
3	COCE	13.127%

Acute Toxicity study

Crassula ovata didn't show any mortality in an acute toxicity trial up to 2000 mg/kg. Throughout the investigation, *Crassula ovata* showed no harmful symptoms or indicators. There was no discernible difference in the amount of food, water, sleep, weight, or behavioural changes. Every animal examined under the category of gross pathology displayed no alterations. Consequently, for the primary experiment, we chose two dosages of *Crassula ovata* namely 50 mg/kg, 100 mg/kg, and 200 mg/kg.

Body Weight Analysis

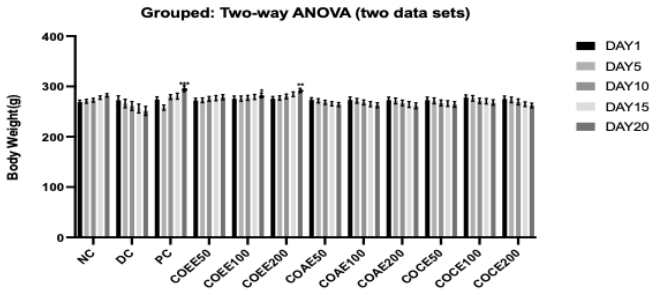
When compared to the control group, the diabetic animals' body weight significantly decreased starting on the fifth day. In contrast, the animals treated with COEE 200mg and Metformin didn't show any decrease in body weight when compared to the control group; instead, the results from COAE, COCE, and COEE 100mg and COEE 50mg were all less significant.(Table 3).(Figure 2).

Blood Glucose Analysis

Blood glucose levels following oral administration of varying dosages of *Crassula ovata*, as shown in (Table. No. 3). Beginning on day 10, rats in Group VI who were administered 200 mg/kg of COEE had a significant ($p < 0.05$) drop in their blood glucose levels when compared to the diabetic control group. As expected, from day 10 forward, the mean blood glucose

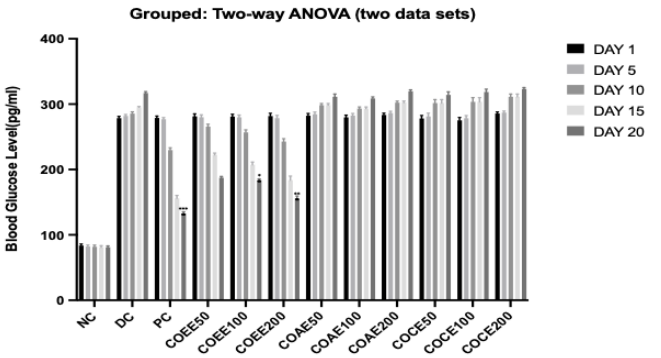
level significantly decreased ($p < 0.05$) in Group IV (COEE50mg) and Group V (COEE100mg) in comparison to the diabetic control rats. Additionally, when compared to diabetic control, all COAE and COCE groups exhibited less significant decreased blood glucose levels ($p < 0.05$) starting on day 10.

Figure 2: Effect of *Crassula ovata* on body weight in rats. The mean ± SEM is used to express all values



A two-way ANOVA test was used to do the group correlation. **, *** denoted a P value less than 0.001 when compared to the typical control group.

Figure 3: Effect of *Crassula ovata* on glucose level

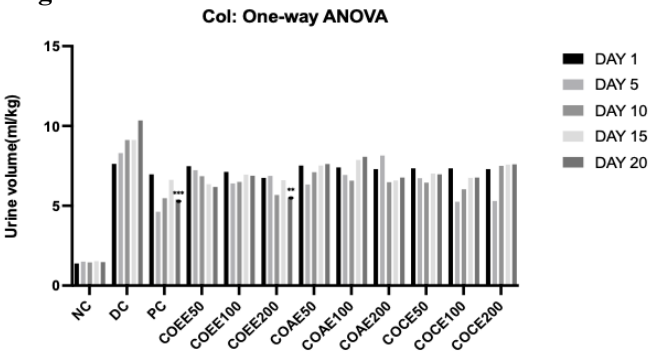


The mean ± SEM is used to express all values. One-way ANOVA was used to measure group correlation. *, **, and ***, respectively, denoted P values less than 0.05 and 0.01 when compared to the normal control group.

Biochemical Analysis

Metformin and higher dose of ethanolic extract have shown a significant reduction in urine volume.

Figure 4: Effect of *Crassula ovata* on urine volume

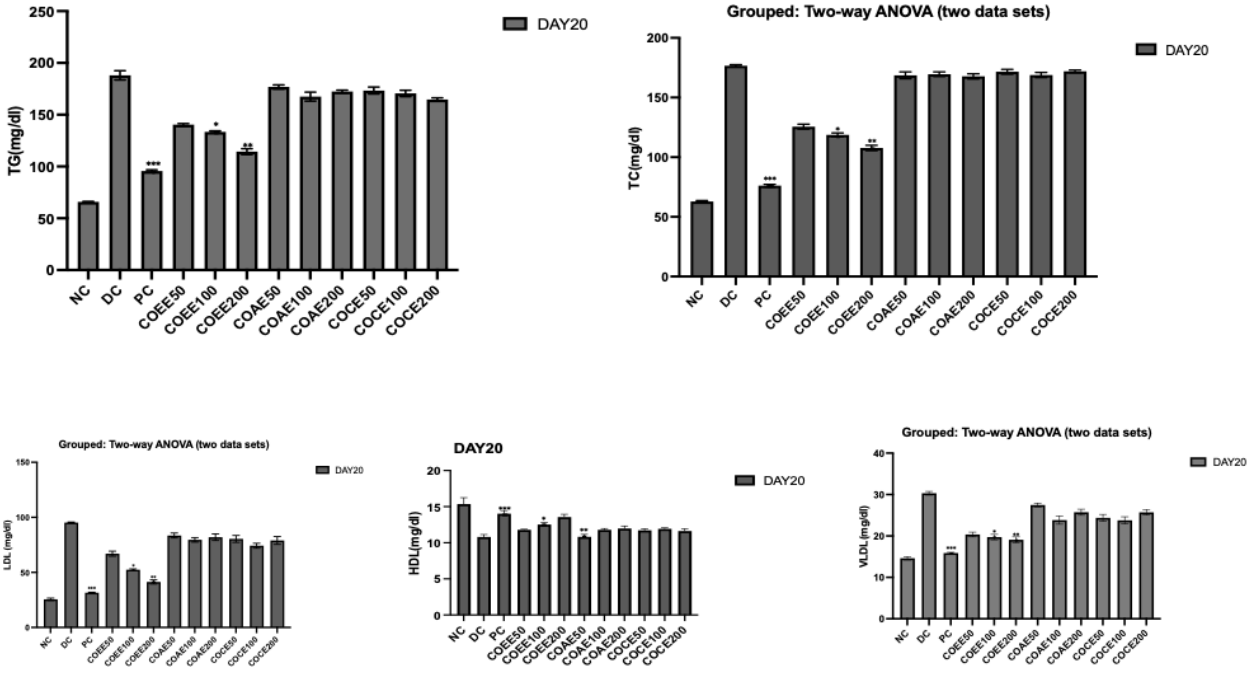


The mean ± SEM is used to express all values. One-way ANOVA was used to measure group correlation. P values less than 0.05 and 0.01 were denoted by ** and ***, respectively, in relation to the normal control group.

Lipid Profile Analysis

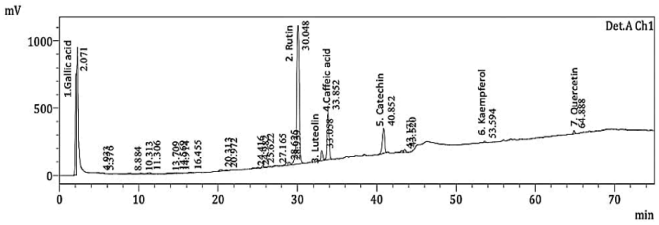
When compared to normal control rats, the diabetes rats had a substantial decrease in HDL and an increase in TC, TG, LDL, and VLDL ($p < 0.001$) (Table no. 4). In contrast to control diabetic rats, COEE100mg and COEE200mg treatment resulted in a significant drop in TC, TG, LDL, and VLDL and an increase in HDL. While treatment with COEE generated significant outcomes, treatment with COAE and COCE did not yield significant outcomes.

Figure 5: *Crassula ovata* impact on the lipid profile LDL, HDL, VLDL, TG, and TC



The mean ± SEM is used to express all values. One-way ANOVA was used to measure group correlation. *, **, and *** denoted a P value less than 0.01, 0.001 in comparison to the usual control group.

Figure 1: HPLC Chromatogram



Chromatogram of *Crassula ovata* ethanolic extract at λ = 210 nm: 1. Gallic acid, 2. Rutin, 3. Lutein; 4. Acidic caffeine 5. Quercetin; 6. Kaempferol; and 7. Catechin.

Table 2: Retention time and peak areas

Peak	Ret. time	Area	Area%	Height	Height %
1. Gallic acid	2.071	5260012	12.441	755268	29.031
2.Rutin	30.048	22106429	52.286	1031566	39.651
3. Luteolin	33.058	1206192	2.853	73352	2.819
4. Caffeic acid	33.852	6118683	14.472	354372	13.621
5. Catechin	40.852	3544809	8.384	184909	7.108
6. Kaempferol	53.594	217944	0.515	12240	0.470
7. Quercetin	64.888	286128	0.677	23739	0.912

Identification of Phytochemical through HPLC UD/ DAD technique

Using an ACE-AR HPLC Column (5ul, 150 x 4.6 mm) for chromatographic separation, detection was carried out at 210 nm in accordance with the compounds' absorption maxima. The injection volume was 20 µL, and the flow rate was 1 mL/min. A constant 25°C column temperature was maintained. Figure 1 shows a chromatogram of an ethanolic extract from *Crassula ovata*. This approach allowed for very good resolution of chemicals of interest in these samples, as the image illustrates.

Discussion

The most serious disease in the world, diabetes mellitus is mostly brought on by a sedentary lifestyle and a diet heavy in calories. Due to improper lipid metabolism, diabetes is also associated with nephropathy and cardiovascular problems. Lowering the high glucose levels with organically generated phytoconstituents has less adverse effects. Natural antioxidant *Crassula ovata* is being researched for a variety of medical purposes. There is no research on the anti-diabetic properties of *Crassula ovata*. Thus, in STZ-induced mice, we evaluated the impact of *Crassula ovata*, a natural phytoconstituent, on lipid levels and antidiabetic properties.

HPLC analysis identified several secondary metabolites in *Crassula Ovata* extract, including Gallic acid, Rutin, Luteolin, Caffeic acid, Catechin, Kaempferol and Quercetin (Fig no.1). These compounds have been reported for their potential antidiabetic effects. The analysis provided data (Table no.2) on the chemical composition of *Crassula ovata* extract, supporting its traditional use in diabetes management.

Table 3: Different solvent extracts on BGL, BW and Vu as an antidiabetic parameters.

Parameter	Day	Group 1 Normal Control(Nc)	Group 2 Diabetic Control(Dc) (Stz 55 Mg/Kg)	Group 3 Positive Control (Pc) (Metformin 150 Mg/Kg)	Group 4 Crassula Ovata Ethanolic Extract (Coee50 Mg/Kg)	Group 5 Crassula Ovata Ethanolic Extract (Coee100 Mg/Kg)	Group 6 Crassula Ovata Ethanolic Extract (Coee200 Mg/Kg)	Group 7 Crassula Ovata Acetone Extract (Coae50 Mg/Kg)	Group 8 Crassula Ovata Acetone Extract (Coae100 Mg/Kg)	Group 9 Crassula Ovata Aceton Extract (Coae200 Mg/Kg)	Group 10 Crassula Ovata Chloroform Extract (Coce50 Mg/Kg)	Group 11 Crassula Ovata Chloroform Extract (Coce100 Mg/Kg)	Group 12 Crassula Ovata Chloroform Extract (Coce200 Mg/Kg)
BW(gm)	1	268.95±3.6	272.5±8.85	273.78±5.49	272.09±4.55	275.38±5.5	275.78±4.3	273.21±4.4	273.37±5.61	272.83±5.89	272.88±6.01	277.8±5.36	275.21±5.66
	5	270.41±3.74	266.03±8.45	257.98±5.09***	272.47±4.59	275.78±5.07**	276.95±4.41***	271.54±4.18	271.4±4.87	271.22±5.75	271.21±5.88	276.21±5.63	273.22±5.67
	10	272.62±3.78	261.13±8.79	278.88±4.81***	275.2±4.69*	277.29±4.96**	280.04±4.71***	268.32±4	268.13±4.9	267.13±5.54	267.14±5.85	271.29±5.28	269.22±6.04
	15	277.69±3.43	256.13±8.91	280.58±5.87***	276.79±5.22**	279.03±4.94***	284.39±4.65***	265.6±4.17	264.73±4.96	264.01±5.7	265.83±5.7	270.58±5.39	264.58±4.71
	20	282.22±3.46	251.33±9.1	295.66±6.1***	278.38±5.18**	282.33±4.9***	291.81±4.39***	263.94±4.07	262.55±4.94	261.44±5.82	264.09±5.56	268.08±5.55	262.12±4.82
BGL(gm/dl)	1	83.62 ±2.34	278.66±2.66	278.98 ±2.8***	281.35 ±3.81	280.91 ±3.65	281.87 ±4.21	282.32 ±3.3	279.74±3.36	283.55±2.66	278.12 ±4.6	275.22±4.31	285.74 ±2.27
	5	82.08±2.46	281.94±2.3	276.92±2.56***	279.91±3.74	279.77±3.61	278.62±4.27	284.58±3.39	282.51±3.18	286.37±2.66	281.12±5.05	278.48±4.05	287.09±2.34
	10	81.74±2.67	285.5±2.67	229.65±3.56***	265.65±3.56	256.77±3.94	242.67±4.38*	287.74±3.34	284.28±2.62	290.73±2.67	286.11±4.74	285.41±3.68	293.62±2.69
	15	80.94±2.54	295.16±1.2	155.97±4.37***	222.46±2.73	207.24±4.22*	183.59±6.35***	298.44±2.56	293.12±2.51	302.24±2.48	301.64±5.32	303.47±6.35	311.26±4.18
	20	81.13±1.96	316.98±2.18	132.78±2.45***	187.33±2.05**	183.66±2.05***	155.82±3.61***	311.18±4.25	308.64±2.51	319.71±2.28	314.2±4.61	318.57±4.34	323.24±2.17
Vu(ml/5h)	1	1.38±0.13	7.63±0.22	6.97±.35***	7.48±0.9	7.12±0.4	6.75±0.44	7.52±0.37	7.4±0.35	7.3±0.51	7.35±0.38	7.67±0.56	7.3±0.36
	5	1.5±0.11	8.3±0.44	4.63±0.41***	7.23±0.36	6.4±0.63	6.87±0.33	6.33±0.42	6.93±0.27	8.15±0.71	6.73±0.21	5.25±0.48	5.3±0.53
	10	1.45±0.09	9.12±0.59	5.47±0.63***	6.85±0.32	6.5±0.53	5.68±0.53	7.1±.04	6.57±0.29	6.48±0.59	6.45±0.44	6.03±0.6	7.5±0.24
	15	1.53±0.11	9.63±0.4	6.62±0.16***	7.18±0.37	6.95±.0.28	6.6±0.42*	7.52±0.52	7.87±0.4	6.58±0.56	7.02±0.58	6.75±0.49	7.58±0.45
	20	1.47±0.1	10.35±0.04	5.4±0.16***	6.18±0.08*	6.88±0.29*	5.53±0.1**	7.62±0.73	8.07±0.31	6.77±0.05	6.97±0.47	6.77±0.34	7.6±0.42

Table 4: Effect of Different solvent extracts on different parameters TG and TC, LDL, VLDL and HDL

Parameter	Day	Group 1 Normal Control(Nc)	Group 2 Diabetic Control(Dc) (Stz 55 Mg/ Kg)	Group 3 Positive Control (Pc) (Metformin 150 Mg/ Kg)	Group 4 Crassula Ovata Ethanolic Extract (Coee50 Mg/Kg)	Group 5 Crassula Ovata Ethanolic Extract (Coee100 Mg/Kg)	Group 6 Crassula Ovata Ethanolic Extract (Coee200 Mg/Kg)	Group 7 Crassula Ovata Acetone Extract (Coae50 Mg/Kg)	Group 8 Crassula Ovata Acetone Extract (Coae100 Mg/Kg)	Group 9 Crassula Ovata Aceton Extract (Coae200 Mg/Kg)	Group 10 Crassula Ovata Chloroform Extract (Coce50 Mg/ Kg)	Group 11 Crassula Ovata Chloroform Extract (Coce100 Mg/Kg)	Group 12 Crassula Ovata Chloroform Extract (Coce200 Mg/Kg)
TC(mg /dl)	20	62.23±0.76	176.64±0.87	76.18±1.07***	125.62 ±2.05*	118.76 ±1.56**	107.73± 2.25***	168.56 ±2.83	169.56 ±1.93	167.76 ±2.15	171.58±2.05	168.88±2.16	172.01±1.05
TG(mg /dl)	20	65.85±0.43	188.04±4.48	95.67±1.17***	140.36 ±1.09*	133.39. 103*	114.34± 2.85***	176.3 ±2.03	167.52 ±4.3	172.39 ±1.33	173.36±3.29	170.57±3.06	164.71±1.6
HDL(mg/dl)	20	14.93±1.11	10.08±0.34	14.35±0.66***	12.51± 0.36**	12.15± 0.4**	13.86±0 .44***	11.01 ±0.43	12.21 ±0.33	12.45 ±0.27*	12.37±0.34	12.63±0.31	12.22±0.48
LDL(mg/dl)	20	25.54±1.07	95.23±049	31.54±0.46***	66.98± 2.29*	52.38± 0.82**	41.31±1 .88***	83.48 ±2.25	79.57 ±2.02	81.95 ±3	80.41±3.15	74.2±2.21*	78.95±356
VLDL(mg/dl)	20	14.61±0.42	30.31±0.44	15.88±0.16***	20.35± 0.57**	19.72± 0.67***	19.08±0 .7***	27.83 ±0.45	23.83 ±1*	25.73 ±0.73	24.36±0.85	23.77±0.86*	25.7±0.69

The animals in the groups that received the appropriate samples underwent 20 days of study, with assessments made on days 1, 5, 10, 15, and 20. The antidiabetic activity of changes in BGL, BW, Vu, TC, TG, HDL, LDL, and VLDL was examined. The parameters exhibited a significant ($p < 0.001$) change in the DC animals during the research period. In comparison to the metformin-treated groups, the BGL, Vu, and BW of the animals showed a substantial decrease ($p < 0.001$) in the COEE200, COEE100, and COEE50 treated groups. According to our research, HDL levels were down in the DC groups while those of TC, TG, LDL, and VLDL increased (Table no. 4). While treatment with COEE produced significant outcomes, treatment with COAE and COCE failed to achieve significant outcomes.

Figure 6: Histopathological Studies of Pancreas Tissue using hematoxylin and observed under a light microscope (with 40x magnification)

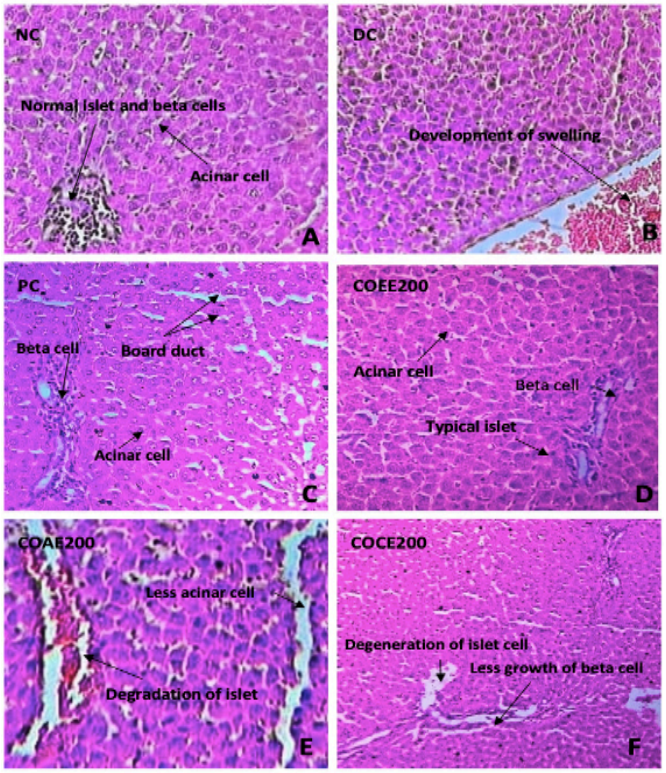
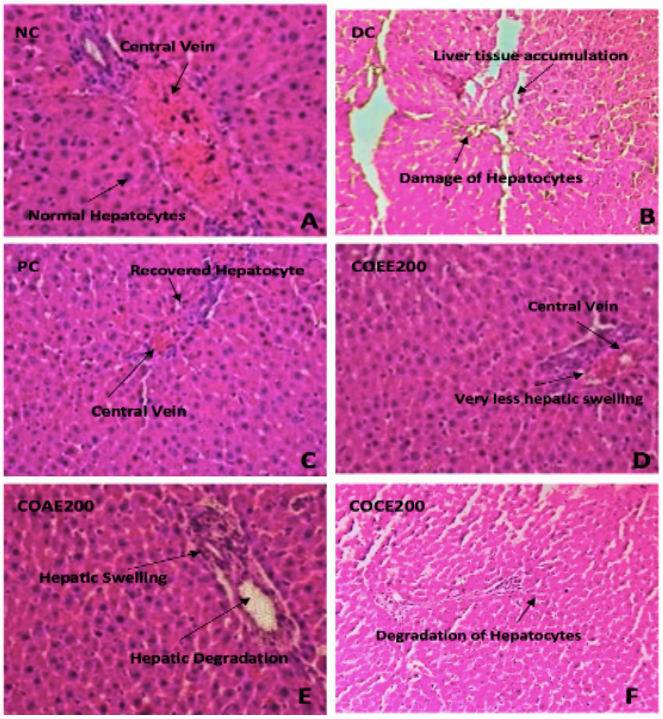


Figure 7: Histopathological Studies of Liver Tissue using hematoxylin and observed under a light microscope (with 40x magnification)



The pancreatic and liver tissue samples' histological observations are displayed in (Figure 6) and (Figure 7). The control group's pancreatic tissue (Figure 6A) displayed spherical Langerhans islets surrounded by lobules. Diabetes (Figure 6B) showed cellular damage to the pancreatic islets, with the islet cells shrinking and degenerating and their vacuoles deforming. PC (Figure 6C) displayed the common aspect development of the Langerhans islets. Acinar damage characterised by cytoplasmic vacuolization and cell withering was found in most acinar cavities. Broad duct intra- and interlobular channels were seen. Group COCE200mg (Figure 6F) showed cellular damage to the pancreatic islets, displaying shrinkage and degeneration of islet cells, while COAE200mg (Figure 6E) showed the degraded, shrunken islets of pancreatic cells. The pancreatic tissues of COEE200mg (Figure 6D) displayed an almost normal islets of Langerhans

structure. Less stringent modifications were made to the acinar cells. The distinction between the endocrine and exocrine parts became becoming more apparent.

According to the liver tissue samples (Figure 7), the NC (Figure 7A) group demonstrated that the liver's normal microscopic examination is made up of normal histological characteristics. The hepatic cells in the diabetic control group (Figure 7B) showed swelling, sporadic necrosis and lipid accumulation known as liver tissue accumulation. Hepatic cells in the metformin-treated group (Figure 7C) showed the recovery of hepatocytes. When compared to the healthy group, the COAE200mg (Figure 7E) tissues of rats showed cellular abnormalities with areas of necrosis, vascular blockage, and cellular degeneration known as hepatic degeration. COCE200 mg (Figure 7F) Hepatocytes showed signs of moderate inflammation, but there was no sign of hepatic digression. The liver showed normal structure of hepatocyte cells in COEE200mg (Figure 7D) with hepatic lobule recovery was noted.

Conclusion

Significant antidiabetic effect was demonstrated by *Crassula ovata* in rats with diabetes mellitus produced by streptozotocin. The recovery of blood glucose levels to normal levels indicates that the larger dose of *Crassula ovata* ethanolic extract has antidiabetic potential equivalent to metformin. For the purpose of identifying pharmacologically active secondary chemicals in *Crassula ovata* leaves, the RP-HPLC method with diode array detection was developed. The ethanolic extract of *Crassula ovata* has the potential to be a useful drug for diabetes. However, more research is needed to identify other plant molecules that have demonstrated activity either singly or in combination, as well as to look into the potential therapeutic applications of these compounds.

A Statement of Competing Interests

The authors declare that none of the work reported in this study could have been influenced by any known competing financial interests or personal relationships.

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