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IN-VITRO AND *IN-VIVO* ANTI-OXIDANT AND ANTI-DIABETIC EVALUATION OF CLADODES CRUDE EXTRACT AND SOLVENT FRACTIONS OF *OPUNTIA ELATIOR* MILL (CACTACEAE)

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Keywords:

Antioxidant, Antidiabetic, α-amylase, α–glucosidase, Streptozotocin, *Opuntia elatior*

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ABSTRACT: Objective: Ariel part of *Opuntia elatior* (Cactaceae) has been used in folklore health systems to treat diabetes in America, Mexico, and India. However, the anti-diabetic potential of cladodes of this medicinal plant is not scientifically validated and authenticated. The present study aimed to evaluate *in-vitro* and *in-vivo* anti-oxidant and anti-diabetic potential of methanol extracts and solvent fractions of *O. elatior* cladodes. **Methods:** The methanol extracts and solvent fractions of *Opuntia elatior* cladodes were evaluated at different concentrations (12.5-400 µg/ml) for anti-oxidant activity by using DPPH method and different doses (200 and 400 mg/kg body weight) for anti-diabetic potentials in staptozotocin induced diabetic albino rats. The extracts were administered for three weeks in different groups. **Results:** The acute toxicity study of *Opuntia elatior* cladodes extract, and fractions did not show mortality in the animals at the limit dose of 2000mg/kg during the observation period. The outcome of the present study indicates that extract and different fraction shows potential anti-oxidant activity. Cladodes also extract significantly decreases elevated level of blood glucose in dose dependant manner and also caused to reverse of the cholesterol, triglyceride, HDL, and LDL values when compared to untreated diabetic rats. **Conclusions:** The result indicates the beneficial effects of *Opuntia elatior* cladodes extract by inhibiting α–amylase, α–glucosidase, scavenging diphenyl-2-picryl-hydrazyl (DPPH) free radicals and improving serum lipid profile levels. The cladodes crude extract of *Opuntia elatior* are effective in lowering blood glucose and improving insulin levels in diabetic rats. The claimed traditional use as anti-diabetic has scientific ground.

INTRODUCTION: One of the foremost causes of mortality and morbidity in humans is none other than diabetes mellitus [1].

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According to an estimation of the International Diabetes Federation, approximately 375 million people are suffering from diabetes, and this may double by 2030, in India 42 million people are suffering from diabetes, which is expected to increase to about 65 million people by the year 2025 [2].

Diabetes mellitus represents a serious, chronic heterogeneous group of a metabolic disorder caused by an absolute or relative lack of insulin

and/or reduced insulin activity or inherited and/or acquired deficiency in production of insulin which ends up in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism [3-4]. Chronic hyperglycemia is associated with dysfunction of the heart, eyes, blood vessels, kidneys, nerves etc and it is characterized by symptoms like polyuria and polydipsia [2, 5].

Type 2 diabetes mellitus is considered one of the most recurrent lifestyle diseases. Type 2 is more prevalent than type 1, with about more than 90% of the total diabetic patients suffering from it. Type 2 diabetes (T2DM) is a disease caused by an imbalance between blood glucose absorption and insulin secretion.

Type 2 DM is a non-communicable disease which is leading causes of mortality worldwide due to associated long term side effects as ketoacidosis, hyperosmolarcoma accompanied with chronic disorders, retinopathy, nephropathy, neuropathy, skin complications, as well as increasing cardiovascular risks. Management of DM with none toxic effects remains a challenge for medical system. This results in an increasing search for improved anti-diabetic drugs [6-7].

Few of plant extract utilized in traditional medicine for diabetes received scientific scrutiny and WHO has recommended that this area warrants attention [6, 8]. Despite considerable progress within the management of Type 2 DM by synthetic drugs, the design for natural anti-diabetic plant products for controlling diabetes is goes on. There are many hypoglycemic plants known through the folklore but their introduction into the modern therapy system awaits the invention of animal test system that closely parallel to the pathological course of diabetes in human beings [9-11].

Some medicinal herbs with proven anti-diabetic and related beneficial effects utilized in treatment of diabetes are *Tinospora cordifolia*, *Gymnema sylvestre*, *Casearia esculenta*, *Syzygium cumini*, *Commiphora wightii*, *Gmelina arborea*, *Asparagus racemosus*, *Boerhavia diffusa*, *Sphaeranthus indicus*, *Pterocarpus marsupium*, *Tribulus terrestris*, *Phyllanthus amarus*, *Swertia chirata*, *Glycyrrhiza glabra*, *Gossypium herbaceum*, *Berberis aristata*, *Piper nigrum* [9, 12, 13].

A traditional medicinal plant, widely distributed in the Rajasthan, known as Cactus, Prickly pears or Hathlo thore, Botanically identified as *Opuntia elatior* Mill. of family Cactaceae. *Opuntia elatior* is a large, succulent shrub, trunk-forming, segmented cactus that may grow upto 5–7 m with a crown of possibly 10 ft in diameter and a trunk diameter of 1 m [9] Cladodes are green to blue-green, bearing few spines up to 2.5 cm (0.98 in) or maybe spineless [14-17].

O. elatior, whole or their parts like phylloclade, fruit, stem, flowers, leaves, and thorns were reported for their traditional-medicinal uses. *O. elatior* is traditionally used in different disease conditions like burning sensation in the stomach, abscess & wound, diphtheria [18-20], anaemia, hyperglycemia, antihyperlipidemic [21-23] analgesic, anti-inflammatory, anticancer and hyper cholesterolemic, anti-oxidant, antiulcer, antiviral, diuretics [24], asthma, cough, refrigerant, gonorrhea, Ophthalmia [25] Antileukemic [26] immunomodulatory, antiasthmatic, improve platelet function, neuro-protective, wound healing, monoamino-oxidase inhibitor and nutritional important *etc.* by tribal of Rajasthan [27-30]. The phytochemical analysis of the Opuntia cladodes showed the presence of flavonoid, carbohydrate, tannin, protein, and pectin compounds. The cladodes of *Opuntia elatior* (Cactaceae) have been used in the treatment of Diabetes mellitus in American and Indian folk-medicine without any scientific verification for safety and efficacy [31–34]. Thus, the objective of the present study is to evaluate the *in-vitro* and *in-vivo* anti-oxidant and anti-diabetic activity of cladodes crude extract and solvent fractions.

MATERIAL AND METHODS:

Plant Collection, Identification and Authentication: The cladodes of *Opuntia elatior* was collected from Sariska forest Alwar district, Rajasthan, India, in November 2019. The collected plants material were botanically identified and authenticated by Dr. L. K. Sharma, Department of Botany, Raj Rishi Govt. College, Alwar (India). The voucher specimens (002-APC/2019) are deposited in herbarium. The crude drug were cleaned, dried in shade and coarsely powdered. The powdered samples were kept in airtight container for further studies.

Chemicals and Drugs: Streptozotocin, Glibenclemide, DPPH and Methanol were purchased from Sigma-Aldrich Chemicals, St. Louis. Chemical kits for estimation of blood glucose, cholesterol, triglyceride, LDL and HDL were purchased from Erba Diagnostics, Mannheim.

Animals: The male albino rats (Wistar strain weighing 150-180 g) and albino mice (weighing 20-30 g, Age 6-8 weeks) were procured from animal house of the Department of Pharmacology, Alwar Pharmacy Pharmacy, Alwar (Raj) India. They were kept at 27 ± 3 ºC (Relative humidity: 65% ± 10% and light / dark cycle for 12 h. All the animals were fed with rodent pellet diet and water was allowed *ad-libitum* under strict hygienic conditions. Institutional Animal Ethics Committee (IAEC) approved all the protocol of study (Reg. No. 963/PO/Re/S/06/CPCSEA).

Preparation of Plant Crude Extract: Cleaned, dried cladodes were weighed by digital weighing balance and a total of 80 g of coarsely powdered cladodes were used for extraction. An extract is prepared using the hot continous method. 80 g coarse cladodes powdered were defatted with 250 ml petroleum ether (50-60 ºC) using soxhlet apparatus. The dried residue (mark) obtained after defattation was packed in soxhlet apparatus and extracted with 250 ml methanol. Extraction was continued until a drop of outcome from the siphon tube, when taken on TLC plate and sprayed with conc. H_2So_4 does not give a spot. Light greenish brown extract obtained and solvent was evaporated first at water bath than under reduced pressure.

Fractionation of Crude Extract: Fractionation was performed by dissolving crude methanol extract in distilled water and partitioned with chloroform (1.5l × 3 times), ethyl acetate (1.5 l × 3 times) respectively. The fractions were subjected individually to evaporation using rotary evaporator.

Preliminary Phytochemical Screening of Cladodes Crude Extracts: Standard Preliminary phytochemical qualitative analysis of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as Reducing sugars, Alkaloids, Steroidal compounds, Phenolic com- pounds, Cardiac glycosides, Flavonoids, Saponins, Tannins and Anthraquinones using standard procedures [35-36].

Acute Oral Toxicity Study: The acute oral toxicity studies of crude extracts were carried out as per the guidelines of the Organization for Economic Co-operation Development (OECD), draft guidelines 423 adopted on 17th December 2001 [37]. An acute toxicity of MEOE (Methanol Extract of Opuntia elatior) was carried out in 5 female albino mice (25-30 g, 6–8 weeks). The mice were divided in to three groups (n=6). All mice were fasted (food but not water) for 3 h before and 1 h after administration of the extract. A limit dose of 2000 mg/kg was given for the first mice.

On the basis of result, four additional mice were dosed sequentially. The animals were housed separately and observed for the manifestation of gross behavioural and physical toxicities like changes in skin, urination, lacrimation, reduction in feeding activity, excitation, paw licking, increased respiratory rate, decreased motor activity, diarrhea, weight loss and paralysis continuously for the first 30 min and intermittently for 4 h, over a period of 24 h and later followed for 14 days for an interval of 24 hrs for any lethality.

Experimental Group and Dose: In the experiment, a total of 30 rats were divided into 5 groups (n=6) for the oral/i.p. administration of extracts/drugs or vehicle **Table 1**.

TABLE 1: GROUPING AND DOSE OF DRUG FOR ANTI-DIABETIC ACTIVITY

Group	Drug and Doses
Streptozotocin -induced diabetes	
Group I	Normal Control (Group NC, Received 0.5% CMC)
Group II	Diabetic Control Streptozotocin (60mg/kg i.p)
Group III	*O. elatior* 200mg (oral) and inducing material(i.p.)
Group IV	*O. elatior* 400mg (oral) and inducing material(i.p.)
Group V	GLC 5mg/kg (oral) and inducing material(i.p.)

Induction of Diabetes in Rats: Streptozotocin (STZ) dissolved in 0.1 M citrate buffer (pH=4.5) was injected in rats intraperitoneally at dose of 60 mg/ kg body weight for induction of diabetes. After 72 h of STZ injection, the rats were screened for DM. Rats that showed FBG level >200 mg/dl were used for the study.

Biochemical Estimation: Serum samples from all the experimental rats were collected for estimation of biochemical parameters, serum glucose (GOD-POD method), cholesterol (CHOD-PAP method), triglycerides (GPO-Triender method), HDL and LDL [29].

Statistics: All values are expressed as Mean ±SEM. The differences were compared using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. P values < 0.05 were considered as significant.

Results:

Preliminary Phytochemical Screening: Preliminary phytochemical screening was done for the cladodes crude extract of *O. elatior* resulted in the presence of Alkaloids, Saponins, Tannins, Phenols, Flavonoids, and Steroids.

Terpenoids and Glycosides were not present in the phytochemical screening **Table 2**.

TABLE 2: PHYTOCHEMICAL SCREENING OF CLADODES CRUDE EXTRACT

S. no.	Secondary Metabolites	Test Results
1	Alkaloids	+
2	Saponins	+
3	Tannins	+
4	Terpenoids	+
5	Phenols	+
6	Flavonoids	+
7	Glycosides	+
8	Steroids	+
9	Anthraquinones	+

***In-vitro* Antioxidant Potential: DPPH Free Radical Scavenging Activity:** The experiment was performed to assess the anti-oxidant potential of MEOE by scavenging the DPPH free radicals. The better anti-oxidant activity was reflected by, the lower IC_{50} (Concentrations that led to 50% of inhibition) values. The concentration of ascorbic acid, cladodes crude extracts, and solvent fractions varied from 12.5 to 400 µg/mL. MEOE and solvent fractions exhibited significant anti-oxidant activity as the extract contains Vitamin C. Among extracts, the highest inhibitory activities were shown by the crude cladodes methanol extract (MEOE) with an IC_{50} value 5.53 ± 0.54 µg/mL (56.21% inhibition at 400 µg/mL) using Ascorbic Acid as reference standard displaying IC_{50} value 3.56 ± 0.94 µg/mL (94.64% inhibition at 400 µg/mL). There was a dose-dependent increase in the percentage of free radical scavenging potency for all concentrations tested. The IC_{50} values of aqueous fraction, chloroform fraction and ethyl acetate fraction were not significant. The potential of the MEOE was found to be less than that of the reference anti-oxidant value; however, the MEOE has demonstrated significant DPPH radical scavenging potential **Table 3** and **Fig. 1**.

% scavenging effect = Control absorbance - sample absorbance × 100

Control Absorbance: Each experiment was carried out in triplicate and results are expressed as means % scavenging effect ± SD.

TABLE 3: ANTI-OXIDANT ACTIVITIES OF THE CRUDE EXTRACT AND SOLVENT FRACTIONS

Concentration	Percentage (%) Inhibition of DPPH				
	Aqueous Extract	Chloroform Fraction	Ethyl Acetate Fraction	Methanol Crude Extract	Ascorbic Acid
12.5 µg/mL	3.14±0.16	4.02±0.34	5.32±0.43	8.12±0.34	17.24±0.35
25 µg/mL	5.14±0.43	8.65±0.76	11.32±0.44	14.32±0.54	32.12±0.54
50 µg/mL	10.43±0.21	11.65±0.94	14.32±0.65	20.12±0.54	50.82±0.12
100 µg/mL	17.34±0.45	21.12±0.14	26.12±0.43	34.72±0.82	62.24±0.76
200 µg/mL	24.23±0.12	32.12±0.43	37.32±0.82	45.14±0.32	73.08±0.92
400 µg/mL	35.52±0.62	46.32±0.76	50.43±0.12	56.21±0.15	94.64±0.72
IC_{50} µg/mL	08.84±0.13	7.36±0.64	6.37±0.54	5.53±0.54	3.56±0.94

Notes: Each value of Percentage inhibition of DPPH free radical is presented as means ± S.E.M., n = 3

***In-vitro* α-amylase Inhibitory Activity of MEOE Cladodes and Solvent Fractions:** α -amylase is the key enzyme associated with the hydrolysis of polysaccharides into the disaccharides and absorbable monomers.

In this study, the potential of MEOE cladodes as anti-hyperglycemic was evaluated along with its fractions to inhibit this enzyme; acarbose was used as a positive control **Table 4** and **Fig. 2**.

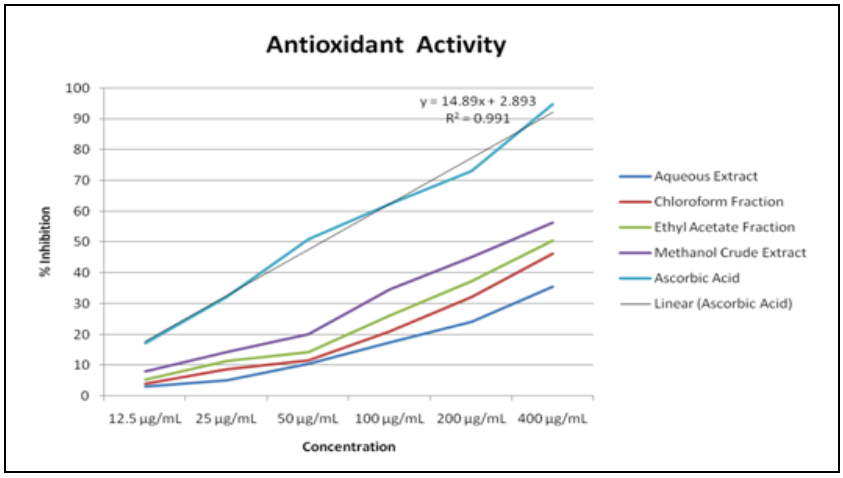

FIG. 1: *IN-VITRO* ANTI-OXIDANT ACTIVITY OF MEOE CRUDE EXTRACT AND FRACTIONS BY DPPH INHIBITION ASSAY

TABLE 4: A-AMYLASE INHIBITORY EFFECT OF THE CRUDE EXTRACT AND FRACTIONS

Concentration	Percentage (%) Inhibition				
	Aqueous fraction	Chloroform Fraction	Ethyl Acetate Fraction	Methanol Crude Extract	Acarbose
25 µg/mL	1.12±0.92	3.21±0.60	15.32±0.75	8.76±0.91	32.76±1.87
50 µg/mL	2.95±0.78	8.54±0.48	33.65±0.12	20.48±0.48	46.92±0.39
100 µg/mL	6.78±0.45	14.65±0.94	48.44±0.67	32.72±0.62	60.58±0.14
200 µg/mL	11.8±0.87	22.87±0.37	62.05±0.82	43.42±0.67	72.71±0.49
400 µg/mL	17.43±0.72	32.54±0.91	68.63±0.28	58.07±0.94	82.62±0.87
IC_{50} µg/mL	13.14±0.63	07.61±0.36	3.32±0.92	4.42±0.86	2.27±0.48

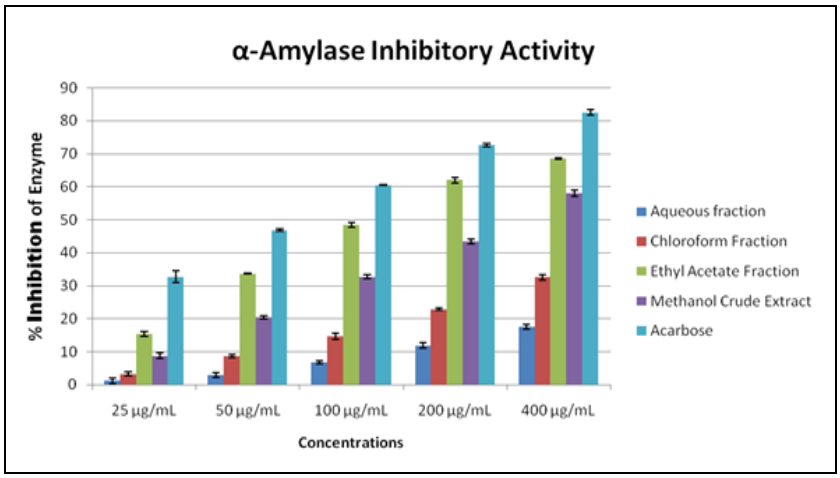

FIG. 2: A-AMYLASE INHIBITORY POTENTIAL OF THE CRUDE EXTRACT AND FRACTIONS

The result showed that aqueous fraction (AF) was inactive while the ethyl-acetate fraction (EAF) was the most potent inhibitor of α- Amylase (IC_{50} = 3.32±0.92) followed by methanol crude extract (IC_{50} = 4.42 ± 0.86) with competing IC_{50} values.

The Chloroform fraction (CF) was found to be very less potent for inhibition of α- amylase. These results suggest that the EAF and MEOE displayed the potential to inhibit this enzyme with greater potency compared to that of standard inhibitor Acarbose (IC_{50} = 2.27±0.48) **Table 4**.

Acute Toxicity Test: The acute oral toxicity study indicated that the crude extract caused no mortality in limit dose of 2000 mg/kg within the first 24 h as well as for the following 14 follow-up days. Physical and behavioral observations of the experimental mice and rats also revealed no visible signs of overt toxicity. This indicates that the extract's median lethal dose (LD50) is greater than 2000 mg/kg.

Oral Sucrose Tolerance in Rats: The methanol extract was evaluated *in-vivo* at three different

concentrations under specified conditions for sucrose tolerance test in rats fed with sucrose. Among these concentrations, the 400 mg/kg displayed potent anti-hyperglycemic activity and significantly prevented a sudden rise in blood glucose levels.

The observations were in line with the results obtained in *in-vitro* enzyme inhibition assays and lead to conclude that the methanol extract inhibited the enzymes responsible for the hydrolysis of sucrose into absorbable sugar in the mice model. The results are shown in **Table 5** and **Fig. 3**.

TABLE 5: ORAL SUCROSE TOLERANCE TEST IN RATS

Group	Blood Glucose Level (mg/dl)				
	0 Min	30 Min	60 Min	120 Min	180 Min
DW 10	80.32±2.54	182.45±2.44	162.34±6.72	134.67±3.72	130.65±4.68
DC	80.32±2.54	182.45±2.44	178.12±1.65	173.43±0.44	172.65±1.82
GLC5	81.87±1.98	150.82±3.32	86.12±6.10	76.67±5.12	72.67±3.76
MEOE100	82.12±2.12	176.33±4.65 [b]	145.50±9.62 [a]	116.20±5.92	110.43±5.42 [a]
MEOE200	82.42±2.45	172.67±3.82 [b]	126.33±8.08	98.6±3.65 [a]	92.6±3.92 [a]
MEOE400	80.92±3.45	170.26 ±4.65 [b]	104.60 ±4.42 [a]	84.42±3.65	80.8±2.82

Notes: Values are expressed as mean ± SEM (n=6) a P<0.05 b P<0.01 compared to diabetic control.and analyzed by one-way ANOVA followed by Tukey's post test.; Abbreviations: MEOE-Methanol Extract of Opuntia elatior; DW, distilled water; GLC, glibenclamide.

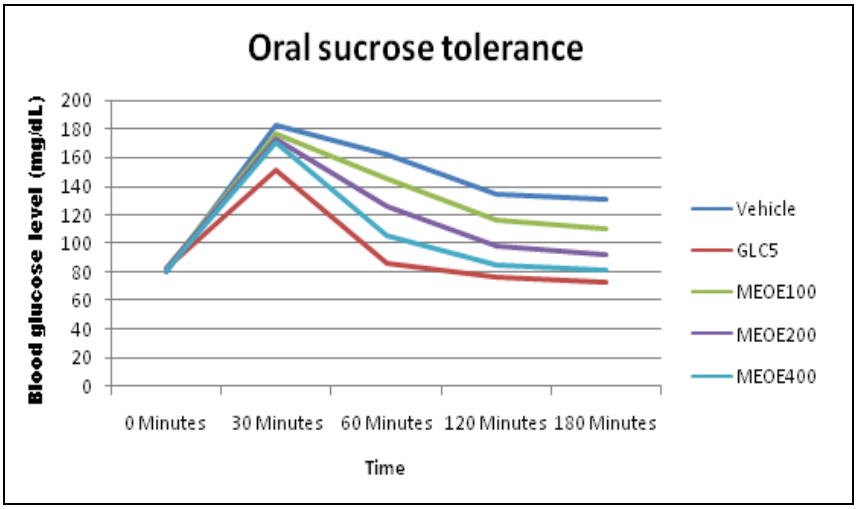

FIG. 3: EFFECT OF *O. ELATIOR* CLADODES CRUDE EXTRACT ON THE BLOOD GLUCOSE LEVEL OF ORAL SUCROSE LOADED MICE

Anti-diabetic activity in Streptozotocin-induced Diabetes Rat model: MEOE on oral administration had significantly lowered the blood glucose level on the administration of MEOE over the experimental period of 21 days.

The experimental animals treated with MEOE with a dose of 200 mg/kg and 400 mg/kg exerted a notable reduction ($P < 0.05$) in blood glucose levels on the 7th, 14th and 21st days for 400 mg/kg dose and 14th and 21st days in case of 200 mg/kg dose respectively in comparison with STZ treated rats. The results were displayed in **Table 6** and **Fig. 4**.

Administration of MEOE 200 and 400 mg/kg for seven-days STC, STG and LDL levels were significantly ($P<0.001$) increased whereas HDL was decreased in diabetic rats compared to normal rats **Table 7**.

TABLE 6: BLOOD GLUCOSE-LOWERING EFFECT OF MEOE

Groups	Blood Glucose Level (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
Normal Control 0.5% CMC (1 ml/kg; p.o)	84.25 ± 2.65	82.18 ± 2.24	82.89 ± 1.54	81.85 ± 2.76
Diabetic Control Streptozotocin (60 mg/kg; b.wt; i.p)	275.78 ±4.20	271.45±3.87	282.19 ± 4.12	310.48 ±2.67
Streptozotocin +MEOE (200 mg/kg, b.wt; p.o)	270.49 ± 3.75	180.25 ± 2.76	138.82 ± 3.22	110.22 ± 2.43
Streptozotocin +MEOE (400 mg/kg, b.wt; p.o)	270.49 ± 3.65	168.25 ± 2.34	127.82 ± 3.12	102.22 ± 1.78
Streptozotocin+Glibinclamide (5 mg/kg, b.wt; p.o)	269.78 ± 4.45	115.85 ± 3.65	100.01 ± 1.87	95.92 ± 1.24

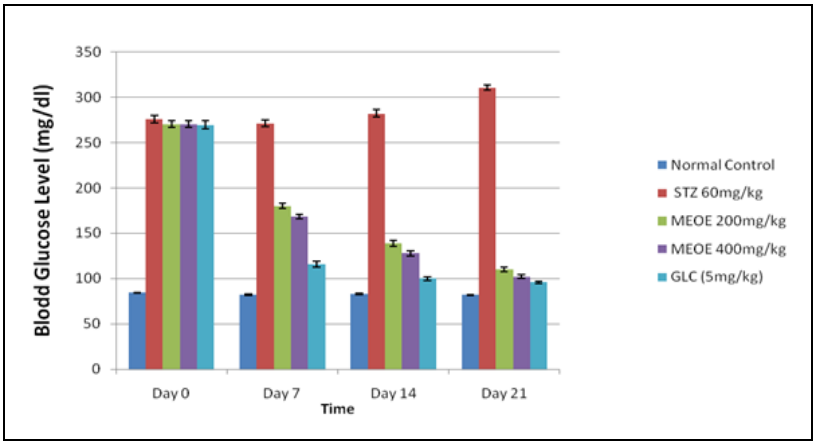

FIG. 4: EFFECT OF MEOE ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

TABLE 7: BIOCHEMICAL PARAMETERS OF NORMAL AND EXPERIMENTAL ANIMALS ON 7TH DAY POST TREATMENT

Groups	Treatment	Serum Lipid Parameters ((mg/dl))			
		Total Cholesterol	Triglycerides	HDL	LDL
I	Normal control	66.34±2.65	84.82±2.72	42.60± 3.72	46.53±4.36
II	Diabetic Control	128.28±4.01	157.24±3.12	23.14 ±5.42	128.23±5.12
III	Diabetic + MEOE 200 mg/kg	96.76±3.32 *	115.60±2.86***	30.14±4.82**	112.34±3.52***
IV	Diabetic + MEOE 400 mg/kg	90.12±1.45 **	98.58±3.18***	34.30±3.72***	82.24±2.65**
V	Diabetic + Glibenclamide 5 mg/kg	78.56±2.32***	92.42±3.34**	38.62±3.34*	64.62±4.37**

Values are expressed as Mean ±SEM; (n = 5), * P < 0.05, ** p < 0.01, *** P<0.001 compared with untreated diabetic rats

Assessment of Serum Insulin Level on MEOE Treatment: This experimental investigation displays the anti-diabetic potential of MEOE where streptozotocin-induced diabetic rats significantly ($p<0.05$) reduced the insulin level, and MEOE could bring the reduced insulin level to near normal limit ($p<0.001$)

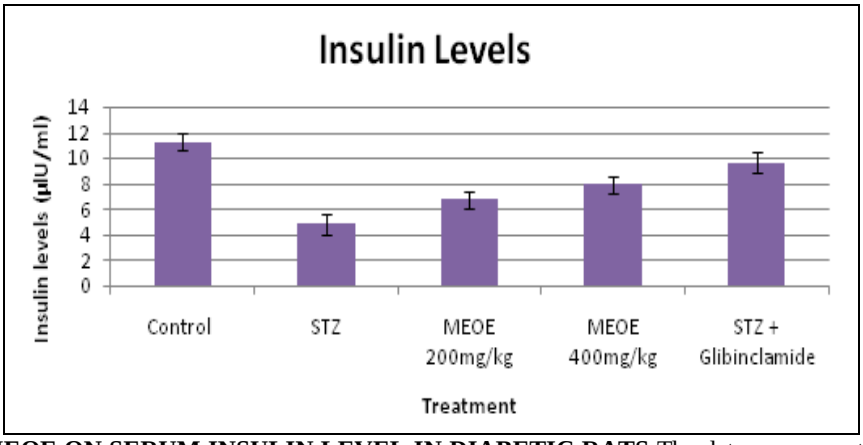

FIG. 5: EFFECT OF MEOE ON SERUM INSULIN LEVEL IN DIABETIC RATS The data are presented as mean ± S.E.M. n = 5 and analyzed using One-way ANOVA followed by post hoc Tukey's test. * P < 0.05 was considered significant as compared to the control group.

TABLE 8: EFFECT OF MEOE ON SERUM INSULIN LEVEL IN DIABETIC RATS

Groups	Insulin(µlU/ml)
Control 0.5% ,CMC (1 ml/kg; p.o)	12.24 ± 0.65
Streptozotocin, (60 mg/kg; b.wt; i.p)	4.82 ± 0.82
Streptozotocin + MEOE, (200 mg/kg, b.wt; p.o)	6.72± 0.66*
Streptozotocin + MEOE (400 mg/kg, b.wt; p.o)	7.94± 0.66*
Streptozotocin + Glibinclamide (5 mg/kg, b.wt; p.o)	9.65 ± 0.76

DISCUSSION: In the present study methanol crude extract and fractions of *Opuntia elatior* cladodes were evaluated for their *in-vitro* as well as *in-vivo* anti-oxidant and anti-diabetic activities *via* DPPH inhibition assay, α-Amylase Inhibitory potential, glucose tolerance test as well as improvement in blood glucose and insulin profile in STZ induced wistar albino rats as compared to

the standard glibenclamide, a hypoglycaemic drug in order to validate their effect. Diabetes being a metabolic disorder, bears a lot of complications if it is not properly managed. Unavailability of suitable anti-diabetic agents and side effects associated with synthetic drug regimes prompt us to search out newer anti-diabetic agents from natural resources. This study sketches out the anti-diabetic potential of medicinal plants from Sariska forest of the Alwar district of India. Preliminary phytochemical analysis showed the presence of secondary metabolites as saponins, phenolic acid, flavonoids, phytosterols, and alkaloids in cladodes of this plant. The anti-diabetic activity in plants may be due to the presence of phenolic and flavonoid compounds. In this study, the crude extract and ethyl acetate fraction of selected plants were found more active than other fractions, indicating that compounds having anti-diabetic activity are polar in nature which established the significant correlations of polyphenolic and flavonoids to lower down the diabetic effect.

Free radical scavenging effect is very important to prevent the deleterious role of radicals in various disorders, including diabetes. DPPH free radical scavenging is an accepted mechanism by which anti-oxidants act to inhibit lipid peroxidation. The DPPH radical scavenging activity of crude extract and EA fraction increased with an increase in concentration **Fig. 1**. In DPPH assay, the extract and EA fraction showed a notable radical scavenging activity in a dose-dependent manner and was significantly different ($p < 0.05$). α-amylase inhibitory activity of the crude extract and solvent fractions of *O. elatior* was in a dose-dependent manner. Most potent inhibition was shown by the ethyl acetate solvent fraction while the aqueous fraction showed the least effect. OSTT is a measure of the body's ability to utilize sugars. At 30 min of sucrose administration, the peak of blood glucose level became higher and then consequently lowered. The postprandial glucose-lowering ability of the extract may be accredited to the embarrassment of glucose absorption, stimulation of peripheral glucose utilization, reduction in glycogenolysis, and gluconeogenesis. This finding gives confident proof that the claimed medicinal plant has anti-diabetic activity. An acute oral toxicity study revealed the non-toxic nature of the methanol extract of cladodes.

STZ causes alkylation of pancreatic deoxy-ribonucleic acid by entering to the β-cell via glucose transporter 2 and induces activation of poly (ADP-ribosylation) that causes depletion of cellular nicotinamide adenine dinucleotide (NAD+) and adenosine triphosphate. As a result, the generation of free radicals causes pancreatic β-cells necrosis [38]. In this study, induction of type 2 diabetes showed significantly increased blood glucose level and decreased insulin level compared to control rats which confirm the induction of diabetes, and it may be due to partial necrosis of pancreatic βcell by STZ. Oral administration of MEOE (200 and 400 mg/kg dose) and glibenclamide to the diabetic rats showed a significant reduction of blood glucose and increase in insulin level than diabetic control rats. Hence, MEOE mediated the above effect possibly due to its preventive effect on STZ-mediated β-cell damage in diabetic rats and thereby increases insulin release.

CONCLUSION: The present study indicated that the cladodes of the plant O. elatior possessed the highest phenolic and flavonoid content and produces strong anti-diabetic and anti-oxidant activities, which were comparable to the commercial anti-dibetic drug Glibenclemide and anti-oxidant ascorbic acid. The results were also verified by inhibition of intestinal α-amylase and free radical scavenging activity by the extracts, contributing to the anti-hyperglycemic and anti-oxidant activity. This seems that the *Opuntia elatior* extract can be used as a natural anti-diabetic and anti-oxidant agent.

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