International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR |Vol. 14 | Issue 3| Jul - Sep 2026

www.ijamscr.com

DOI : https://doi.org/10.61096/ijamscr.v14.iss3.2026.1496.1507

ISSN: 2347-6567

Phytochemical Evaluation and in Vivo Antidiabetic Activity of Methanolic Extract of *Artemisia Afra*

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Published on: 31.08.2026
Published by: Futuristic Publications

Abstract: The aim of this study was to investigate the antidiabetic activity and mechanism of action of methanolic leaf extract prepared from *Artemisia afra*. Since this claim has not been investigated scientifically, the aim of this study was to evaluate the antidiabetic effect and phytochemical screening of alloxan induced diabetic Mice.

The present study aimed to investigate the *in vivo* anti-diabetic activity of leaves of methanolic extract of *Artemisia afra*. It showed the blood glucose lowering activity of 80% Methanolic crude extract and solvent fraction were studied in animal models: Hypoglycemic mice model, oral glucose loaded mice model, dose-treated Alloxan-induced diabetic mice model. The effect of the crude extract on diabetic was studied and the strongest activity was shown by Crude extract fraction (89.60% inhibition at 1000 µg/mL) compared to the standard acarbose having 97.19% inhibition at 1000 µg/mL. The crude leaf extract of *Artemisia afra* showed significant blood glucose-lowering effect on hypoglycemic mice and oral glucose loaded mice. In Alloxan-induced diabetic mice model, the crude leaf extract fraction significantly decreased the fasting blood glucose level after 14 days of treatment.

The results demonstrated the potential antidiabetic activity of *Artemisia afra* extract through α-amylase inhibition and reduction of blood glucose levels. The crude leaf extract showed significant blood glucose-lowering activity in hypoglycemic and oral glucose-loaded mice. The claimed traditional use as antidiabetic has scientific ground.

Key words: Diabetes mellitus, *Artemisia afra*, Alloxan, Anti diabetic activity.

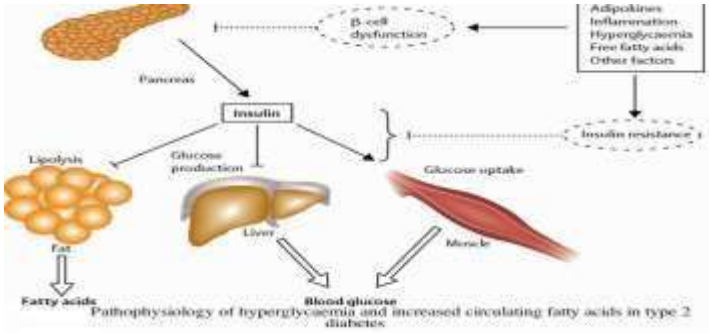
1. INTRODUCTION

1.1 Diabetes Mellitus (DM): Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The etiology of diabetes is a complex interaction of genetic and environmental factors. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders. It is an endocrinological syndrome abnormally having high levels of sugar in the blood. Since the disease

prevails in both genders and in all age groups, the general public has a concern about its control and treatment[1].

1.2-Classification of DM: Diabetes is classified by underlying cause. The most common forms of diabetes are categorized as **Type 1**, or insulin-dependent diabetes mellitus (IDDM) - an autoimmune disease in which the body's own immune system attacks the pancreatic beta cells, rendering it unable to produce insulin and **Type 2**, or non-insulin-dependent diabetes mellitus (NIDDM) - in which there is resistance to the effects of insulin or a defect in insulin secretion.

Pathophysiology of type 1 diabetes



Pathophysiology of Hyperglycaemia and increased circulating Fatty acids in type-2 diabetes

Diabetes diagnostic criteria			
Condition	2 hour glucose	Fasting glucose	HbA_{1c}
	mmol/l(mg/dl)	mmol/l(mg/dl)	%
Normal	<7.8 (<140)	<6.1 (<110)	<6.0
Impaired fasting glycaemia	<7.8 (<140)	≥ 6.1(≥110) &<7.0(<126)	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥6.5

1.12-Alloxan induced diabetes mellitus[20]

Much of the evidence for the induction of diabetes mellitus comes from the studies of alloxan which produces diabetes in experimental animals. Alloxan appears to selectively destroy the islets of Langerhans by oxidant production. Current evidence suggests that the selective cytotoxicity of alloxan is due to the function of three factors: efficient uptake, oxidant production by redox coupling of the drug with intracellular reductant (ascorbate and thiols) coupled with low levels of glutathione peroxidase in islets. Several studies on enzymes involved in hepatic glucose metabolism in mice with alloxan induced diabetes have shown well defined changes, which consist primarily of a decrease in the activity of glucokinase, hexokinase, and an increase in the activity of gluconeogenic enzymes including aminotransferases (AST and ALT) and glucose-6-phosphatase.

The prediction of biological activity of the chemical compounds present in the extract will also support the *invitro* results after its phytochemical analysis. Herbal drugs were expelled mild side effects reported in literatures. The present study attempts to develop a novel plant-based antidiabetic drug, which will be evaluated by using *Invitro* and *In vivo* methods.

PLANT PROFILE

Artemisia afra

PLANT PROFILE:

Artemisia afra

Kingdom **:** Plantae
Family **:** Asteraceae
Order **:** Asterales
Genus **:** Artemisia
Species **:** A. afra

MATERIALS AND METHODS

Instruments: Following instruments were required for the study:

List of Instruments used for study

Name of the instrument	Source
Centrifuge	Dolphin
Digital weighing balance	Horizon
Glucometer	Horizon
Heating mantle	ASGI®
Refrigerator	Videocon
Soxhlet extractor	ASGI®
Condenser	ASGI®
Burette stand	Dolphin
Round bottom flask	ASGI® , Amar
Mixer	Videocon
Oven	ASGI®
Water bath	ASGI®
Stirrer/glass rod	ASGI®
Watch glass	ASGI®
Whatmann filter paper	Manipore microproducts, Ghaizabad.
Butter paper	ASGI®
Spatula	ASGI®
Rubber pipes	ASGI®

a) Plant collection: The leaves of *Artemisia afra* were collected from Local area.

b) Preparation of Coarse Powder and Extraction Technique: The leaves were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder. This powder was

sequentially extracted according to their increasing polarity with petroleum ether, ethyl acetate, methanol respectively. About 500gm of powdered leaves was uniformly packed into a thimble in a Soxhlet apparatus and extracted with 1000 mL Petroleum ether, Ethyl acetate and Methanol, respectively. Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continued for 1-2 hours for each solvent. The excess solvent was evaporated and the dried extracts were kept in a refrigerator at 4ºC for their future use in phytochemical analysis and pharmacological screenings.

In vitro antidiabetic activity of *Artemisia afra* leaves extracts

Alpha-amylase inhibition assay: The α-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method.50 The crude and solvent fractions of *Artemisia afra* were dissolved in buffer ((Na2HPO4/ NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 50 to 1000 mg/mL. A volume of 200 mL of α-amylase solution (Molychem) (2 units/mL) was mixed with 200 mL of the extract and was incubated for 10 minutes at 30 °C. Thereafter, 200 mL of the starch solution (1% in water w/v) was added to each tube and incubated for 3 minutes. The reaction was terminated by the addition of 200 mL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-DNSA solution) and was boiled for 10 minutes in a water bath at 85°C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Agilent Technologies). The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 mL of the buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (Bayer) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The inhibition of α-amylase was expressed as percentage of inhibition and was calculated by the following equation: Inhibition (%) ¼ [(Ac -Acb) (As Asb) / (Ac -Acb)] × 100, where Ac is the absorbance of control; Acb is the absorbance of control blank; As is the absorbance of sample; and Asb is the absorbance of sample blank. The % α-amylase inhibition was plotted against the extract concentration and the IC_{50} values were obtained from the graph.

Preliminary phytochemical screening of Methanolic leaves extract of *Artemisia afra*

The Methanolic leaves extract of *Artemisia afra* was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

Molisch's test: Dissolved small quantity of 300mg alcoholic and dried leaves extract powder of *Artemisia afra* separately in 4mL distilled water and filtered. The filtrate was subjected to Molisch's test.

Fehling's test: Dissolved a small portion of extract in water and treat with Fehling's solution.

Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapors.

Test for flavonoids: Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added.

Lead acetate test: To 5mL of extract 1ml of lead acetate solution was added.

Test for tannins: Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.

Test for steroid/terpenoids: Liebermann-Burchardt test: To 1ml of extract, 1mL of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

Test for alkaloids: Dragendorff's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified **Dragendorff's** reagent. **Hager's test:** The extract was

treated with few ml of Hager's reagent. **Wagner's test:** The extract was treated with few ml of Wagner's reagent.

Tests for Glycosides: Legal's test: Dissolved the extract [0.1g] in pyridine [2mL], added sodium nitroprusside solution [2mL] and made alkaline with Sodium hydroxide solution.

Test for Saponins: Foam test: 1ml of extract was diluted with 20mL of distilled water and shaken with a graduated cylinder for 15 minutes.
Test for Anthraquinones: Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1mL of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

Test for Amino acids: Ninhydrin test: Dissolved a small quantity of the extract in few mL of water and added 1mL of ninhydrin reagent.

Test for fixed oils and fats: Press small quantity of the petroleum ether extract between two filter paper.

g) Acute toxicity study: In a research study when a drug is administered to a biological system there will be some interactions may happen. In most cases, these are desired and useful, but many effects are not advantageous. Acute, sub-acute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug. Acute toxicity is involved in estimation of LD_{50} (It is the lethal dose (causing death) to 50% of tested group animals).

LD_{50} (median lethal oral dose): LD_{50} (median lethal oral dose) is a statistically derived oral dose of a substance that can be expected to cause death in percent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of animal (mg/kg).

In this study acute toxicity study was carried out in Mice. The procedure was followed by using OECD 423(Acute toxic class method). The Mice were fasted overnight, prior to dosing. The four dose levels are administered by the help of oral feeding needle over the prior of 24 hours. After the drug has been administered, food may be withheld for a further 3-4 hours in Mice. The purpose of sighting study is to allow selection of the appropriate starting dose for main study.

The test substance is administered to animal in a sequential manner following from the fixed dose levels of 5, 50, 300 and 2000mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, Duration and severity of toxic signs. The animal weight is recorded on weekly once. On the day 14 all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg). Based on the mortality result on 14th day of observation, the doses for *in vivo* study are selected.

In vivo antidiabetic activity of *Artemisia afra* leaves extract in Alloxan induced diabetic Mice.

Prior to the experiment the mice were housed in a clean polypropylene cages (6 Mice/cage) for a period of 7 days under standard temperature (25 - 30^{o}C), relative humidity (45 – 55%), dark / light cycle (12 /12 hrs). The studies were performed with the approval of Organizational Animal Ethics Committee (OAEC) (DAEC/TNA/965/345/16). The animals were fasted overnight and deprived of food for 16 hrs but allowed free access of water.

Chemicals: Alloxan from Loba Chemie. Standard Glibenclamide (Daonil) from Aventis Pharma. methanol (Analytical grade) and 5% Dextrose solution Glucose Estimation Kit from Gluco Dr Super sensor.

Hypoglycemic Test Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g Mice). Group II served as Positive control – Glibenclamide (2mg /kg). Group III served as aqueous Methanolic extract of Artemisia afra– (200mg/kg). Group IV served as Methanolic extract of *Artemisia afra*– (400mg/kg).

Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3mL\100g Mice). Group II served as Positive control – Glibenclamide (2 mg /kg). Group III served as Methanolic extract of *Artemisia afra*– (200mg/kg). Group IV served as Methanolic extract of *Artemisia afra*– (400mg/kg).

All the groups of animals were fasted for 24hr and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been administered to different groups and 30mins later all the groups of Mice were treated with glucose orally at dose 10gm/kg body weight by using oral feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration, blood samples have been collected different time intervals at 30, 60 and 120min.

Induction of diabetes to animals: A single dose (100 mg/kg b.w., i.p.) of Alloxan dissolved in sodium citrate buffer was used for the induction of diabetes in Mice after overnight fasting. After 1 hr of Alloxan administration, the animals were fed ad libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycaemic phase. The animals were stabilized for a week and animals showing blood glucose levels more than 200 mg/dl were selected for the study.

Experimental design: Five groups of six mice in each groups received the following treatment schedule for 14 days.

GROUP I - Normal control (normal saline 10 ml /kg, P.O). GROUP II - Alloxan treated control (100 mg/kg, I.P). GROUP III - Alloxan (100 mg/kg, I.P) + Standard drug Glibenclamide (2 mg/kg, P.O). GROUP IV - Alloxan (100 mg/kg, i.p.) + MEAA.(200 mg/kg, P.O). GROUP V - Alloxan (100 mg/kg, i.p.) + MEAA. (400 mg/kg, P.O)

Plant leaves extract, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II is Alloxan treated control Mice. Group IV and Group V (which previously received Alloxan 100mg/kg) were given fixed doses of Methanolic leaves extract (200 mg/kg, P.O, 400 mg/kg, P.O) of *Artemisia afra* and group III received standard drug Glibenclamide (2 mg/kg,P.O) for 14 consecutive days. (MEAA-Methanolic extract of *Artemisia afra* Leaves).

Collection of blood samples: Fasting blood samples were drawn from retro orbital puncture of Mice at weekly intervals till the end of the study 1, 7, and 14 days.

Estimation of biochemical parameters Serum blood glucose: On 1, 7, and 14 days fasting blood samples were collected and analyzed the blood glucose.

Blood glucose level: The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

Statistical analysis: Statistical analysis was done by using GraphPad Prism 5.0. All the values of Biochemical parameters and body weight were expressed as mean ± standard error mean (SEM). The values were analyzed for statistical significance using one-way analysis of variance (ANOVA), comparison was done by using Dunnett's t-test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.

RESULTS

a) Appearance and percentage yield of MEAA (Methanolic Extract of *Artemisia afra* α-amylase Inhibitory Activities of the Crude Extract and Solvent Fractions.)

Percentage inhibition

Concentration (mg/mL)	Chloroform fraction	Ethyl acetate fraction	Aqueous fraction	Crude extract	Acarbose
50	6.41 + 0.1	15.82 + 0.35	29.16 + 1.11	34.91 + 0.36	57.65 + 0.79
100	11.64 + 0.69	20.04 + 0.11	35.71 + 0.82	41.05 + 1.42	68.10 + 0.46
200	23.14 + 0.45	27.16 + 1.92	42.12 + 0.46	61.19 + 0.98	76.93 + 1.53
400	29.65 + 0.50	46.90 + 0.15	54.81 + 0.53	73.34 + 0.76	88.51 + 0.17
600	38.01 + 0.99	54.14 + 0.64	68.93 + 0.92	81.92 + 0.24	93.06 + 0.26
800	45.15 + 0.81	65.54 + 0.49	75.50 + 0.76	86.41 + 0.19	96.27 + 0.17
1000	53.34 + 0.76	74.77 + 0.12	83.19 + 0.81	89.60 + 0.74	97.19 + 0.92
IC50	31.14 + 0.12	21.80 + 0.71	14.24 + 0.64	7.21 + 0.91	3.34 + 0.14

Abbreviation: IC50, half maximal inhibitory concentration.

Each value of percentage inhibition of α -amylase is presented as means + standard error of the mean (SEM), n ¼ 3.

α -Amylase inhibitory activity of the crude extract and solvent fractions of *Artemisia afra:* *In Vitro* α -Amylase Inhibition Activity of Crude Extract and Solvent Fractions *In vitro* α -amylase inhibitory study evaluating the percent of α -amylase inhibition as a function of extract concentrations and the IC_{50} values were calculated (Figure). Concentration dependent inhibitions were observed for various concentrations of the tested extracts and the standard. Among the extracts, the crude extract exhibited the lowest IC_{50} of 7.21 + 0.91mg/mL and the IC_{50} values of water fraction, ethyl acetate fraction, and the chloroform fraction were 14.24 + 0.64, 21.80 + 0.71, and 31.14 + 0.12mg/mL, respectively. The standard positive control acarbose showed an IC_{50} of 3.34 + 0.14 mg/mL (Table).

Minimum % Inhibition was found *Artemisia afra leaves* which resemblance to %Inhibition of positive control, So Methanolic extract of *Artemisia afra* contains active constituents of antidiabetic.

Phytochemical studies

Table 7.2: Results of Methanolic extract of *Artemisia afra*

Class of compounds	Tests performed	Results
Carbohydrates	Molisch's test Fehling's test	-
Phenols	Phosphomolybdic acid test	+
Flavonoids	Shinoda test Lead acetate test	+ +
Tannins	Braemer's test	-
Alkaloids	Wagner's Mayer's Dragendroff's test	+ + +
Glycosides	Legal's test Brontranger's test	+ +
Saponins	Foam test	-
Sterols	Salkowski's test	-
Amino acids	Ninhydrin test	-
Terpenoids	Lieberman Burchardt test	+

+Present in moderate amount -Absence

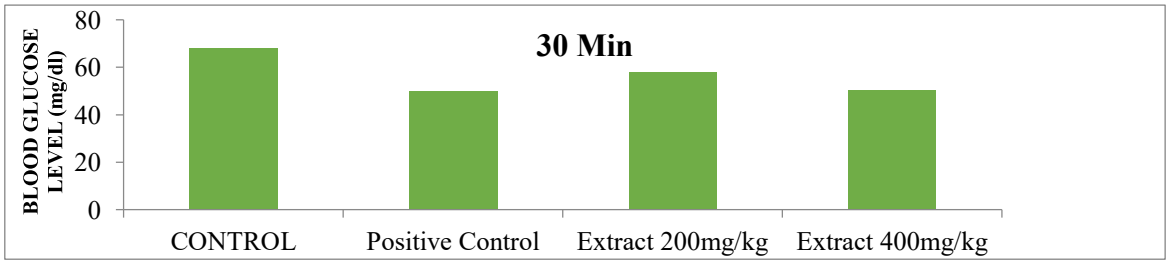
The phytochemical studies results revealed that the Molisch's test no characteristic observation indicated the absence of carbohydrates, by phosphomolybdic acid test blue coloration of the spot indicated the presence of phenols. Shinoda test and Lead acetate test gave pink or red coloration of the solution indicated the presence of flavonoids Flocculent white precipitate also indicated the same. There is no dark

blue or greenish grey coloration of the solution indicated the absence of tannins in the drug. No characteristic observation for steroids and dark pink or red coloration of the solution indicated the presence of Terpenoids. Orange coloration of the spot indicated the presence of alkaloids. Yellow or reddish-brown precipitation indicated the presence of alkaloids. Pink to red Colour solution indicates the presence of glycosides. No layer of foam formation indicates the absence of Saponins. If the response to the test is indicated high it can be note or which indicates that the particular group is present as the major class. If the response is average then note it as indicates the presence in moderate quantity and note it as indicating the presence of only in traces. If no response, the result is considered negative.

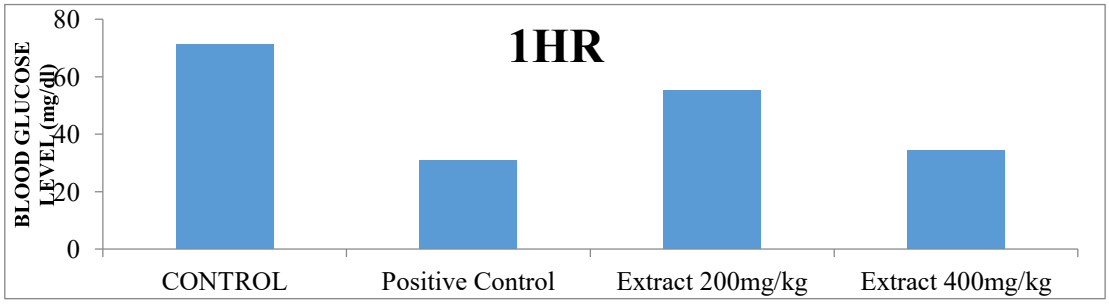
Hypoglycemic Test:

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL (mg/dl)		
		0 min	30min	1hr
CONTROL Carboxymethyl Cellulose (CMC)	0.5%	69.15±2.451	68.14±4.320	71.19±2.129
Positive Control Glibenclamide	2	67.24±3.209	50.15±1.492**	30.96±3.298***
Methanolic Extract of *Artemisia afra*	200	66.87±1.251	57.91±3.482*	55.14±2.101*
Methanolic Extract of *Artemisia afra*	400	66.18±3.420	50.19±3.281**	34.2±1.921***

The glucose levels were analyzed by using glucometer and each value is the mean ± standard error (n= each group consist of 6 animals)($p<0.05$)*, ($p<0.001$)**& ($p<0.0001$)*** as compared to control & positive control group evaluated by one way, ANOVA followed by Dunnet 't' test.



Blood glucose level 30min



Blood glucose level 1hr

The hypoglycemic test results have shown Table No: which indicated Methanolic extract of *Artemisia afra treated* animals 200 & 400, significantly decreased in blood glucose level when compared to control and positive control.

g) *In-vivo* antidiabetic study

Results of the effects of Methanolic extract on blood glucose levels TREATMENT	BLOOD GLUCOSE LEVEL (mg/dl)		
	0 min	30 min	1 hr

Normal control 10 ml/kg P. O	77.29±3.104	73.1±3.219	72.2± 3.917
Negative control	261.1±2.91	267.2±4.1	271.3±2.1
Positive control (Glibenclamide 2mg/kg) P. O	251.18±3.156	136.98±2.4***	113±1.1***
MEAA 200 mg/kg P.O	256±2.1	245.1±2.154**	241.2±1.209**
MEAA 400 mg/kg P. O	260±1.10	170.2±1.72***	158.1±2.9***

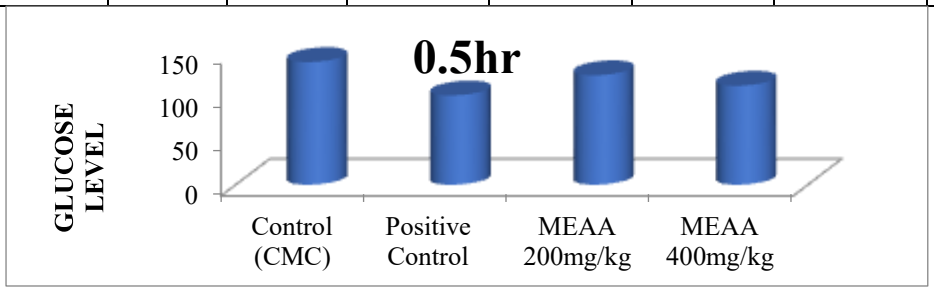
(The values were expressed as mean ± S.E.M. (n=6 animals in each group).

The experimental results have indicated in the Table the negative control group glucose levels were significantly increased when compared to each other groups. All the groups of animals were affected in diabetes, which indicated blood glucose levels were slight changes in the blood glucose level for normal control group at 7th and 14th days. On day 7th glucose levels were significantly decreased Glibenclamide 2mg/kg treated group when compared with control group at 7th and 14th days. The Methanolic leaves extract of *Artemisia afra* treated groups 200 & 400 mg/kg decreased in a dose-dependent manner when compared with control group but positive control has more anti diabetic activity at 7th day.

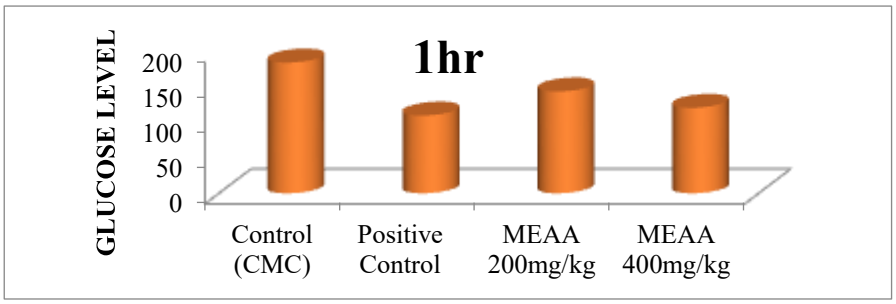
The aqueous Methanolic leaves extract of *Artemisia afra at* the dose level 400mg/kg have equipotent activity when compared with positive control at 7th day. The Methanolic leaves extract of *Artemisia afra 200* and 400 mg/kg have been expressed dose dependent anti diabetic action when compared to control and positive control. On day 14th, Methanolic leaves extract of *Artemisia afra treated* animals 200 & 400 mg/kg significantly decreased and maintained the blood glucose level when compared to control and positive control.

Oral Glucose Tolerance Test:

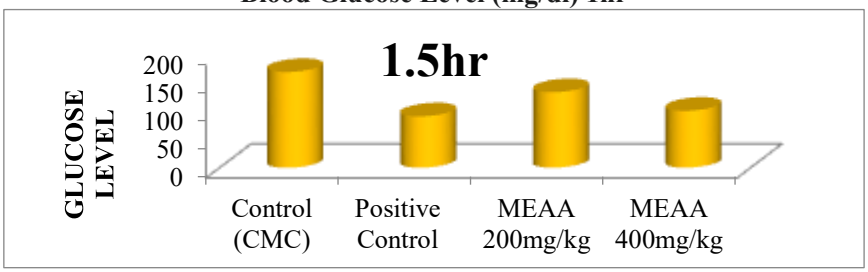
Treatment	DOSE mg/kg	Blood Glucose Level (mg/dl)						
		0 min	0.5hr	1hr	1.5hr	2hr	2.5hr	3hr
Control (CMC)	0.5%	65.01± 2. 164	140.1± 1.352	185.1±2. 151	170.1±12 .41	154.2±4. 121	151.0±1.1 94	130.1±10 .81
Positive Control Glibenclamide de	2	69.10± 0.18	102.0± 2.181**	110.1±3.2 4***	91.21±3. 287***	81.20±1. 921**	75.01±1.2 59***	71.51±2. 910***
MEAA	200	68.14± 5.101	125.1± 2.014	144.1±2.1 15*	134.1±0. 181*	125.1±0. 126*	111.14±0. 26* *	105.0±3. 214**
MEAA	400	68.00± 1.159	113.1±0 .181* *	121±4.14 2**	101.1±4. 296***a	91.30±1. 365*** a	84.21±2.0 6* **a	80.21±31 6***a



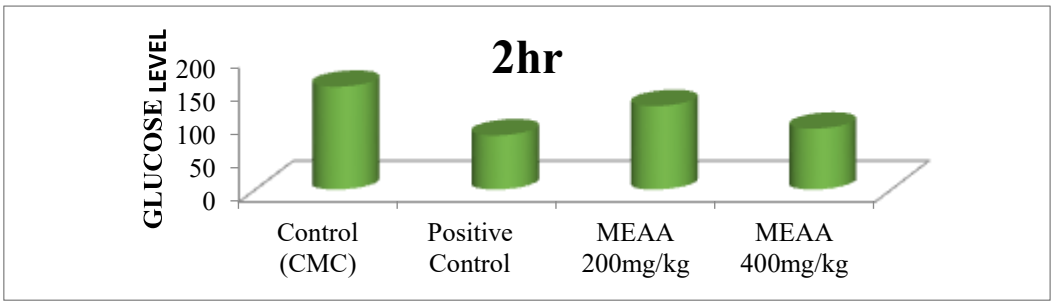
Blood Glucose Level (mg/dl) 0.5hr



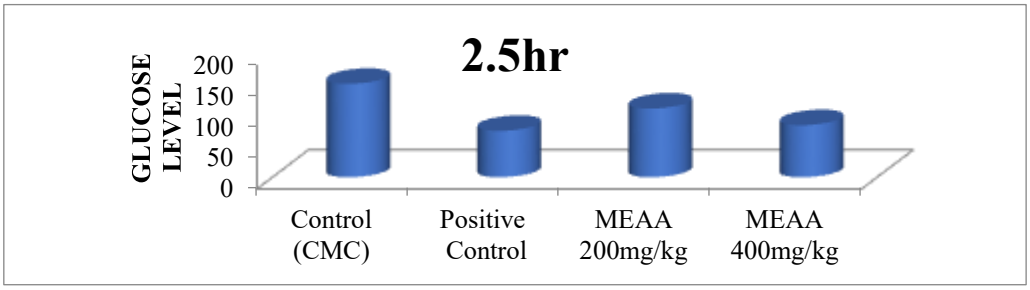
Blood Glucose Level (mg/dl) 1hr



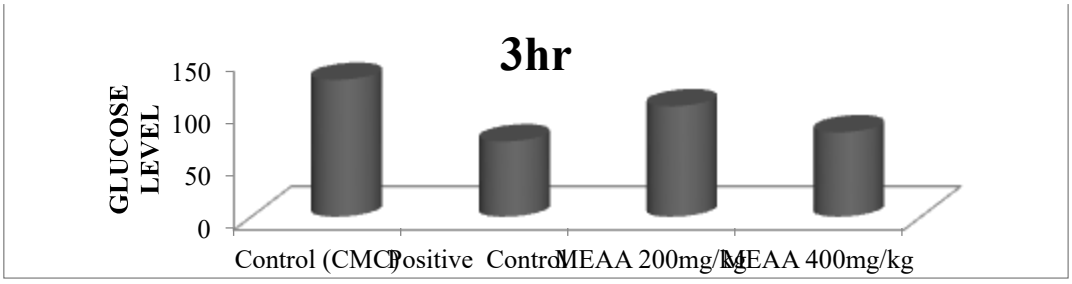
Blood Glucose Level (mg/dl) 1.5hr



Blood Glucose Level (mg/dl) 2hr



Blood Glucose Level (mg/dl) 2.5h



Blood Glucose Level (mg/dl) 3hr

Oral Glucose Tolerance Test (OGTT) results have been expressed in Table. Half an hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased. The blood glucose

levels were significantly decreased for, methanolic extract of *Artemisia afra* 200 & 400 mg/kg when compared to control and positive control at 1 hour and each and every ½ hour blood glucose levels(200 mg/kg were changes in the dose dependent manner extract treated group of animals compared to control and positive control but 400mg/kg produced the equipotent activity.

DISCUSSION: *In-vitro* study is on the principle of Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen are considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes. Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller. Sequential extraction was done according to increasing polarity order. Each extract was tested for α-amylase inhibition to get the extraction with minimum IC_{50} value. As per the above mechanism all the extract has concentration dependent affinity towards the inhibition of α-amylase. Finally, acarbose extract was observed as more active extract.

In this present study acute toxicity study was carried out in Mice. The procedure was followed by using OECD 423 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Average two to three steps may be necessary. The method used to define doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). Observe for signs for toxicity and were noted for 14 days. The onset of toxicity and signs of toxicity were also noted. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study. The principle involved in the Alloxan induced diabetes mellitus in Mice, Alloxan, a cytotoxic; diabetes induced chemical but wide variety of animal species by damaging the insulin secreting cells of the pancreas.

Literature sources indicate that the Alloxan induced Mice are hyperglycemic. The treatment of lower doses of Alloxan (100mg/kg b.w.) produced partial destruction of pancreatic β-cells even though the animals became permanently diabetic.

Thus, these animals have surviving βcells and regeneration is possible. It is well known that the sulfonylureas act by directly stimulating the β-cells of the islets of Langerhans to release more insulin and these compounds are active in mild Alloxan induced diabetes. *Invivo* anti diabetic screening was performed for the confirmation of above mechanism of action was undergone the Methanolic extract of *Artemisia afra* biological system (Which was already resulted for α-amylase inhibitory activity.

At the end of the Methanolic extract of *Artemisia afra* (200 mg/kg p.o, 400 mg/kg p.o.) showed statistically significant decrease in blood glucose levels. So, the Methanolic extract of *Artemisia afra* showed antidiabetic activity. This work will be useful for further diabetes mellitus and its related diseases research worker to develop new entity for the treatment of diabetes mellitus.

CONCLUSION: This study revealed that the crude extract and solvent fractions of *Artemisia afra* showed significant lowering of blood glucose level on diabetic, Hypoglycemic and oral glucose loaded mice and not permitted bodyweight loss of diabetic. The results also verified that inhibition of intestinal αamylase by the extracts may contribute to the antihyperglycemic activity. The results give scientific support for the use of the plant in folk medicine for the management of diabetes and its associated complications. *Artemisia afra* would be promising for further clinical studies in the management of DM. Further studies to find out the mechanism of this plant for its antidiabetic effect and there is a need for bioactivity guided investigation to isolate the lead compound responsible for the antidiabetic activity.

The present study suggested that the isolation of active constituents from Methanolic extract of *Artemisia afra* and characterize the compounds by using preliminary phytochemical studies.

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