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"Phytochemical And Hypoglycemic Evaluation Of *Artemisia Indica* Willd Medicinal Plant"

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Abstract

This study investigates the potential anti-diabetic effects of Artemisia indica, a herb that has been used for therapeutic purposes for centuries. A hydroalcoholic extract of Artemisia indica leaves, which are rich in terpenoids, alkaloids, and flavonoids, was shown to be safe for usage, according to studies that investigated acute toxicity. Experiments using rats that developed diabetes due to streptozotocin revealed that the extract has potent anti-diabetic properties. Treatment groups demonstrated significant improvements in oral glucose tolerance, greater serum insulin levels, and significantly lower fasting blood glucose levels in comparison to the control group, which was comprised of individuals with diabetes. In addition to the detection of increased insulin expression in beta cells, histological examination confirmed that the structure of the pancreatic islets had been conserved. In addition, the extract exhibited a significant level of antioxidant activity, and it also showed a reduction in oxidative stress indicators. The findings of this study indicate that Artemisia indica possesses both antioxidant and antihyperglycemic properties, which makes it a possible therapeutic alternative for the treatment of diabetes. Considering the potential of Artemisia indica as a natural anti-diabetic, there is a need for additional research into the molecular processes and therapeutic applications of this plant.

Keywords: Artemisia indica, Antidibetic, Streptozotocin, Antioxidant, Plant extract.

1. Introduction

The metabolic disease known as diabetes mellitus is characterised by elevated blood glucose levels brought on by insufficient insulin or insulin resistance in target tissues.[1]. Diabetes mellitus was identified as a serious and incurable condition. Hyperglycemia, whether it is acute or chronic, can result in a number of consequences that affect the cardiovascular system. Additionally, hyperglycemia can cause microvascular complications such as nephropathy, neuropathy, and retinopathy [2]. Chronic hyperglycemia is a factor in the development of a number of illnesses and causes disruptions in the metabolism of glucose, lipids, and proteins [3]. Diabetes is becoming more prevalent all over the world, with nations such as India, China, and the United States having more than 30 million people who are diagnosed with the condition [4]. In the treatment of diabetes mellitus, nutritional supplements have been explored for their potential to modify the availability of fatty acids, limit the process of gluconeogenesis [5, 6], and increase lipolysis [5].

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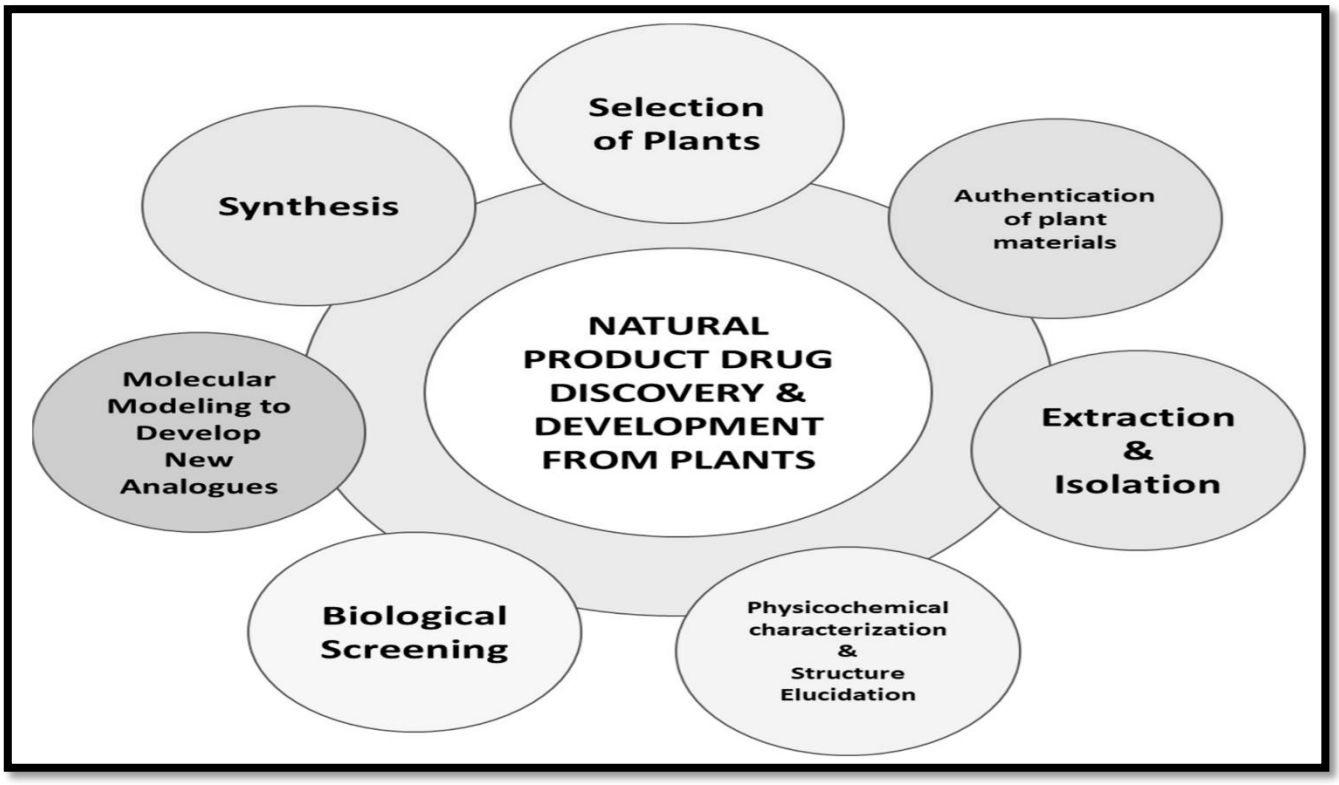

Fig.1 Flow diagram showing the natural product drug discovery &development from plants

2. Natural plants and its Pharmacological activities

When opposed to synthetic substances, natural goods, particularly plants, have garnered interest their ability to reduce the risk of developing diabetes. These natural products have lower toxicity and fewer unwanted effects [7, 8]. It has been reported by the World Health Organization (WHO) that approximately seventy percent of diabetic patients rely on plants as primary sources of antidiabetic compounds in order to fulfill their medical requirements [9]. "Mugwort" is the popular name for Artemisia indica, which is a perennial shrub that belongs to the Astereaceae family and can grow to heights of two to eight meters. The chilly temperate zone of Asia and the northern regions of India the places where it can be found. Artemisia indica has been shown to possess a wide range of pharmacological activities, which include antiplasmodial effects [10], antispasmodic and bronchodilator properties [11], antihypertensive effects [12], antiallergic activity [13], hepatoprotective effects [14], antibacterial properties [15], and antinociceptive effects [16]. Each of these pharmacological activities has been documented. Artemisia indica Linn is a species both highly polymorphic and broadly distributed, and it displays a substantial amount of variation within its populations. As a consequence of this, a number of intraspecific taxa have been recognized and identified within this species. It is a plant species that is widely spread and has a geographical range that extends from North Africa and Europe to Caucasia, Turkestan, Siberia, Iran, Afghanistan, Pakistan, and India. A large variety of active phytochemical compounds can be found in Artemisia indica Linn. These constituents include alkaloids, glycosides, flavonoids, terpenoids, phenols, steroids, tannins, and saponins. It has also been brought to North America, where it has become a naturalized species [17]. Artemisia indica, especially, is regarded for its antibacterial, anthelmintic, expectorant, and antispasmodic qualities. It has been found that many portions of the plant, including the aerial parts, can be exploited for a variety of therapeutic application [18]. As an illustration, an infusion of Artemisia indica is utilized for the treatment of infections that affect the central nervous system as well as for the purpose of enhancing hunger. The juice of the plant is applied to ease symptoms such as diarrhea, gastrointestinal pain, and inflammation. Additionally, the juice can be used as an eye wash to alleviate pain, burning sensation, and inflammation due to conjunctivitis. When it comes to traditional medicine, the numerous applications of Artemisia species illustrate both the medical significance of these plants as well as the cultural significance of these plants in a variety of communities. In addition, the tomentum, which is the fine, soft hair of the plant, is used as moxa, which is a traditional kind of heat therapy [19]. Both antidiabetic and antioxidant activity were investigated in this study. The streptozocin-induced diabetic model and the DPPH assay were utilized to evaluate the respective properties of the compounds.

3. Material and methods[20]

Collection and authentication of plant

Plants belonging to the Artemisia indica species were gathered from the upper region of the Dehradun district. The taxonomist Professor Pramod Mishra, who is also the Head of the Department of Agriculture at Mangalayatan University in Aligarh, was the one who recognized the plant. The herbarium of the Department of Pharmacy at Mangalayatan University in Aligarh received a voucher sample of the plant, which was assigned the voucher number 202200106.

Preparation of plant extract of Artemisia indica[21]

The plant's new aerial components were allowed to air dry at room temperature in the shade for a duration of three weeks. During this time, the material was continuously repositioned in order to prevent the formation of fungi. After the plant had been dried, the aerial parts were crushed into a coarse powder. After that, a hot extraction was performed using methanol, chloroform, ethyl acetate, n-butanol, and n-hexane as solvents. The Soxhlet apparatus was used to perform the extraction. A rotary evaporator operating at 45^0C (Heidolph Laborata 4000 efficient Germany) was used to evaporate the solvent after the extraction process was complete.

Fig. 2 shows the wild Artemisia indica plant and powder extract.

4. Phytochemical screening of Artemisia indica[22]

Numerous phytochemical experiments were performed on the Artemisia indica extract, including ones in methanolic, chloroform, ethyl acetate, n-butanol, and n-hexanol. These tests were performed to assess the presence of triterpenoids, alkaloids, glycosides, saponins, and tannins, among other active components.

Test animals

Throughout the several trials, adult Sprague Dawley rats weighing between 150 and 200 grammes were employed. The university maintained the animal house facilities where the rats were kept in addition to having unlimited access to regular food and fresh water.

Acute oral toxicity study[23]

According to the standards established by the Organisation for Economic Co-operation and Development (OECD 423, 2001), the determination of acute oral toxicity was carried out. In a nutshell, rats were given extracts of varying quantities on an oral basis, and they were regularly observed for any signs of behavioural abnormalities or mortality over the course of the subsequent fourteen days. The LD50 value of extracts was calculated based on the results of this experiment.

5. Experimental design for Antidiabetic activity [24]

The rats were divided into seven groups at random, each with eight rats, after they had fasted for twelve hours the night before. Individuals in the first group were given normal saline and functioned as the normal control group, which did not have diabetes. The second group, which was used as a control for diabetes, received a 5% concentration of Tween-80 suspension. Oral administration of the standard drug glibenclamide at a dose of 500 µg/kg was done to the third group. The fourth group received 200 mg/kg orally delivered doses of the methanolic extract of Artemisia indica, whereas the fifth group received 400 mg/kg of the same extract. The Artemisia indica chloroform, ethyl acetate, n-butanol, and n-hexane extracts were given orally in doses of 200 mg/kg to the sixth, seventh, eighth, and ninth groups, respectively.
The medication, which included fractions and crude extract, was administered daily at nine o'clock in the morning for fifteen days. On the first, fourth, seventh, tenth, and fifteenth days of treatment, the patient's body weight and blood glucose levels were measured.

6. Antidiabetic activity

Model of streptozocin-induced hyperglycemia
The administration of a single intra-peritoneal (i.p.) injection of streptozotocin (STZ) at a dose of 50 mg/kg, which was then reconstituted in normal saline (0.9%), was used to induce hyperglycemia in Sprague Dawley rats. Before receiving the STZ injection, the rats were required to abstain from food for a full 24 hours. After a period of seventy-two hours had passed since the administration of STZ, blood samples were taken from the veins in the rats' tails. The glucose levels in the blood were determined with the assistance of one-touch Glucometer strips and an SD glucometer manufactured in Germany. Researchers determined that rats with fasting blood glucose levels that were more than 300 mg/dl were diagnosed with diabetes and were chosen for further participation in the study.

Assessment of Liver Function and Serum Lipids
After the antidiabetic test was completed on the fifteenth day, pentobarbital sodium (35 mg/kg) was given to every animal. It became necessary to puncture the heart in order to get blood samples for further study on biochemical markers. In order to separate the serum, the blood samples that were taken were spun at 1500 × g for a duration of 10 minutes. A spectrophotometer from Perkin Elmer, Germany, was used to detect the levels of serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (ALP), and serum glutamic pyruvic transaminase (SGPT).
The study was performed with a kit from Bioneed (Germany) using the conventional IFCC kinetic method. Using a UV spectrophotometer, the CHOD-PAP and GPO-PAP techniques, which are part of a Human (Germany) kit, were used to measure serum creatinine, triglycerides (TG), low-density lipoproteins (LDL), high-density lipoprotein (HDL), and total cholesterol (TC).

7. Antioxidant Activity DPPH Assay[25]

Various extracted fractions (EF) were tested for antioxidant activity using the DPPH technique. The DPPH(2,2-Diphenyl-1-picrylhydrazyl) radical scavenging capability was used to measure the antioxidant activity. Soluble in 2 millilitres of methanol was 1 millilitre of each EF. After incubating the solution for 24 hours, we made sure that all of the active components in the extract were released. The mixed solutions were spun in a centrifuge to separate the solid proteins. 1.5 ml of the supernatants (10, 20, 30, and 40 µl) from each suspension were combined with a 0.1 mM DPPH solution in methanol. The total volume of the methanol solution is 2 millilitres. After being shaken, the combinations were left to stand at room temperature in a dark spot for half an hour. The reference solution consisted of 1 milligramme of ascorbic acid in 2 millilitres of methanol. Using ultraviolet (UV) spectroscopy, we found that the DPPH radical had an absorbance of 517 nm. To calculate the percentage of radical scavenging activity against DPPH, the following formula was used:

% DPPH radical scavenging activity= [(ABSconc. – ABStest)/ABSconc.] ×100Where: ABSconc. = absorbance of control reaction
ABSpre = absorbance of test sample

Statistical analysis
Biochemical markers, including body weight, blood glucose levels, and standard error of the mean (SEM) were presented as means plus or minus the SEM. Students' t-test was used to compare the two sets of data. A one-way analysis of variance (ANOVA) with Dunnett's post hoc multiple comparison test was employed when comparing more than two groups. At $p < 0.05$, statistical significance was determined.

8. Result and Discussion

Percentage yield of plant extracts

The obtained percentage yield of different plant extracts is summarized in Table 1.

Table 1: Percentage yield of different plant extracts

Plant extracts	Weight	Percentage yield (%)
Crude methanolic extract	500 gm	92.33
n-hexane fraction	100 gm	11.5
Chloroform fraction	240 gm	44.33
Ethyl acetate fraction	90 gm	10.22
n-butanol fraction	150gm	15.3
Aqueous fraction	180 gm	30.56

Preliminary phytochemical analysis

As shown in Table 2, the results showed that the methanolic, chloroform, ethyl acetate, n-butanol, n-hexane, and aqueous extract of Artemisia indica included alkaloids, flavonoids, glycosides, terpenoids, saponins, and tannins.

Table 2: Initial phytochemical examination of several Artemisia indica extracts

Plant extracts	Flavonoids	Glycosides	Alkaloids	Saponins	Tannins	Terpenoids
Crude methanolic extract	++	++	++	++	++	++
n-hexane fraction	++	_	++	++	++	++
Chloroform fraction	++	++	_	++	_	++
Ethylacetate fraction	++	++	_	++	++	_
n-butanol fraction	_	++	_	++	_	_
Aqueous fraction	_	++	_	++	_	_

++ (Positive): Presence of phytochemical constituents

- (Negative): Absence of phytochemical constituents Acute oral toxicity study

Acute oral toxicity studies provide an LD50 value for the plant extracts of 2000 mg/kg. The methanolic extract was used in all following tests at 200 mg/kg and 400 mg/kg, in addition to 200 mg/kg doses of chloroform, ethyl acetate, n-butanol, and n-hexane.

Antihyperglycemic Effect of *Artemisia indica*

Table No.3 shows the effects on blood glucose levels of glibenclamide, the crude methanolic extract of Artemisia indica, and other fractions on rats with normal and diabetic blood sugar levels.

Table 3: Effects on the blood glucose levels of STZ-induced diabetic rats after daily oral treatment of Artemisia indica extracts and glibenclamide

S. No.	Groups	Dose (mg/kg)	1st day	4th day	7th day	10th day	15th day
1	Diabetic control	0.5 ml	371.3±37	384.7±20	400.8±50	406±34	416.7±50
2	Normal control saline	0.5 ml	98 ±22	82.3±9	82.3±6	81.3±7	81.5 ±5
3	Gliben clamide	0.4 ml	360.8±33	320.7±45**	280.5±50**	250.8±53**	200.2±40**
4	Crude methanolic Ext	200	377.7±18	376.3±32	345.7±20**	300.5±12**	250.3±12**
5	Crude methanolic Ext	400	450.7±53	37.2±9	300.8±10**	320.8±7**	310.6±20**
6	Chloroform fraction	200	480±52	240.8±48**	252.1±26**	230.2±14**	240.8±23**
7	Ethylacetate fraction	200	450±38	430±23	407.3 ±31	480.3±34	410.3±42
8	n-butanol fraction	200	372±32	376.5±48	393.8±30	397±18	390.2±48
9	n-hexane fraction	200	375±45	378±45	386±28	389±28	376±44

Each group has eight values, which are expressed as mean ± SEM. When comparing the diabetic control group at the same time, the results showed **$p < 0.01$ (one-way ANOVA followed by Dunnett's multiple comparison test).

Artemisia indica's Effects on Body Weight in Diabetic Rats

Table 4 summarises the changes in body weight of the experimental and control rats that were given glibenclamide and the extracts.

Table 4: Effects of various extracts of *Artemisia indica* on body weight inSTZ -induceddiabetic rats.

S.No.	Treatments	Dose(mg/kg)	Change in body weight (g) at days					% Changein body weight
			0	4	7	10	15	
1	[a]Normal (Control)	0. 5 ml	143±0.5	145±9	155±0.9	160±0.9	170±3	
2	[b]Diabetic (Control)	0.5 ml	150± 7	148±5*	146±5**	143±3*	145±4***	-11.5
3	[c]Glibenclamide	0.6	165±4	159±3*	162±6*	165±4**	170±5**	+ 10.8
4	[c]Crude methanolic Ext	200	162±3	165±5*	157±7*	150±5**	164±4**	+8.8
5	[c]Crude methanolic Ext	400	164±4	165±6*	158±4*	152±3**	165±6**	+8.1
6	[c]Chloroform fraction	200	161±5	164±5*	156±3*	162±4**	167±5**	+10.6
7	[c]Ethylacetate fraction	200	163±7	157±4	142±2	139±4	135±4	-11.7
8	cn-butanol fraction	200	164±5	159±3	145±4	140±3	134±4	-13.0
9	cn-hexane fraction	200	165±6	158±2	146±5	142±4	133±5	-14.0

The mean ± SEM is used to express the values. Every figure represents the average of eight animals. *p<0.05, **p̂0.01, ***p<0.001; Student t-test comparison of a (normal control) to b (diabetes control) *p<0.05, **p<0.01; one-way ANOVA with Dunnett's posthoc multiple comparison test between b (diabetic control) and c (Glibenclamide and extracts treated groups).

$$\% \text{ Change in B.W} = \frac{\text{Initial weight (g)- Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

Artemisia indica's Effects on the Renal and Lipid Profile in STZ-Induced Diabetic Rats

Table 5: Antihyperlipidemic effect *Artemisia indica* extracts and variousfractions in STZ-induced diabetic rats.

S. No.	Treatment	Dose (mg/k g)	Total cholesterol(mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	[a]Normal control	0.4 ml	120 ± 9.12	125±8.80	34±2.2	81± 2.5
2	[b]Diabetic Control	0.4 ml	185.3±4.4**	169.0±7.9**	30.2±2.4*	177.4±8.9**
3	[c]Glibenclamide	0.5	137.7±5.3**	128.3±6.5**	39.1±2.3**	90.3±3.5***
4	[c]Crude methanolic Ext	200	144.2±4.3**	138.5±4.7**	37.5±3.1**	95.6±3.2***
5	[c]Crude methanolic Ext	400	140.5±7.5**	136.8±4.5**	35.6±5.5**	93.5±3.6***
6	[c]Chloroform fraction	200	134.4±2.7**	136.2±8.1**	38.2±1.5**	81.3±4.0***
7	[c]Ethyl acetate fraction	200	168.2±3.6*	165.8±9.0	36.1±2.5*	151.8±8.0*
8	[c]*n*-butanol fraction	200	167.9±9.8*	146.8±3.2*	36.2±2*	149.8±7.7*
9	[c]*n*-hexane fraction	200	166.9±8.5*	145.4±2.*	37.2±3*	148.8±5.5*

t-test (*p < 0.05, **p < 0.01) and Dunnett's posthoc multiple comparison test (* p < 0.05, ** p < 0.01, ***p < 0.001) between the diabetes control and c(Glibenclamide/extracts) treated groups using one way ANOVA.

The values are the mean ± SEM of eight animals. Using student data, comparisons between normal control and diabetes control were done.

The impact of Artemisia indica on liver function in diabetic rats induced by STZ

Table No. 6: Artemisia indica extracts' effects on the serum liver profile of rats given STZ-induced diabetes.

S. No.	Treatment	Dose (mg/kg)	GPT)IU	GOT)IU	(ALP) IU	Serum Creatinine (mg/dl)
1	[a]Normal control	0.4 ml	15±4.4	18±3.5	180±20.5	0.52±0.2
2	[b]Diabetic control	0.4 ml	52.2±11.8	41.4±2.1**	290.8±16.3**	2.5±0.2**
3	[c]Glibenclamide	0.5	17.6±4.5**	17.5±2.6**	195.9±5.3**	0.5±0.1** *
4	[c]Crude methanolic Ext	200	22.4±9.0* *	21.9±4.9**	203.2±14.52**	0.78±0.1** *
5	[c]Crude methanolic Ext	400	19.4±9.0* *	18.9±4.9 **	199.2±14.52 **	0.6±0.2** *
6	[c]Chloroform fraction	200	18.8±1.5* *	19.9±4.1 **	183.6±12.5* *	0.8±0.1** *
7	cEthyl acetate fraction	200	50.5±3.5	39.5±3.8	281.3±31.2	2.2±0.2
8	cn-butanol fraction	200	48.8±4.0	37.0±3.9	272.5±10.3	2.3±0.3
9	cn-hexane fraction	200	47.8±5.0	38.0±4.5	270.5±11.5	2.1±0.4

The values represent the mean ± standard error of eight animals. The student t-test was used to compare the bdiabetic control group to the anormal control group (** $p < 0.01$), and one way ANOVA was used to compare the bdiabetic control group to the cGlibenclamide/extract treated groups (* $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$).

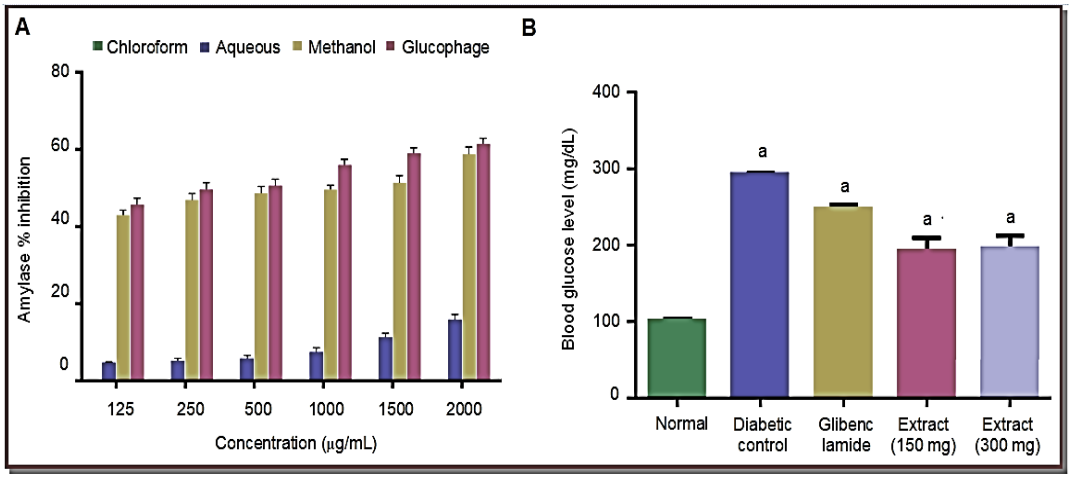

Fig.3 Bar graph showing the antidiabetic response of different concentration of Extracts

Antioxidant activity of *Artemisia indica*

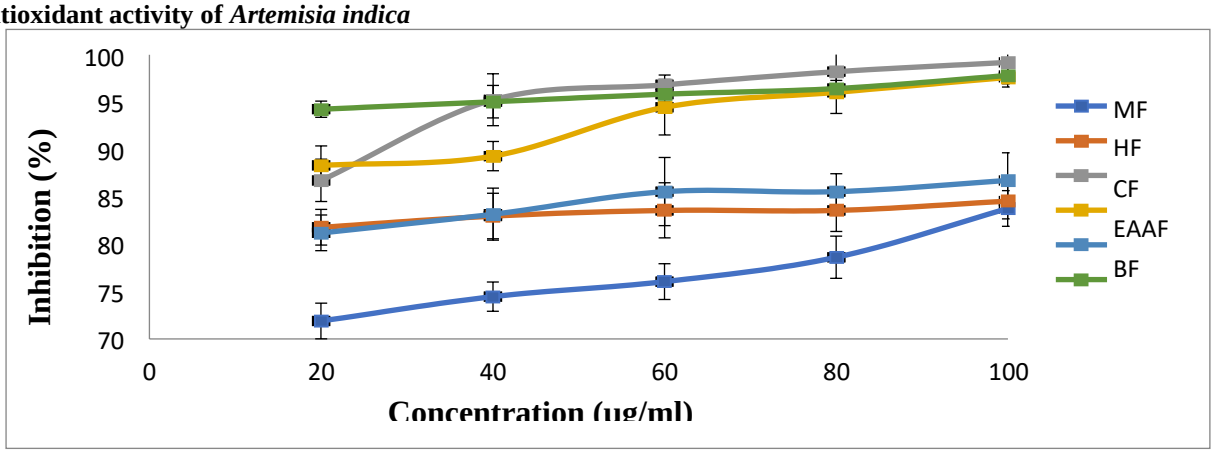

Fig.4: Percentage inhibition of DPPH. MF-Methanolic Fraction, HF-n- Hexane Fraction, CF-Chloform Fraction, EAA-Ethyl Acetate Fraction, BF-n- Butanol Fraction, AF-Aqueous Fraction

9. Conclusion

The information and discussion above indicate that there is significant evidence of the antidiabetic and antioxidant properties of the methanolic, chloroform, ethyl acetate, n-hexane, and n-butanol extracts of Artemisia indica. As demonstrated in STZ-induced diabetic rats by improvements in body weight, lipid profiles, and decreases in blood creatinine, SGPT, SGOT, and ALP levels, these extracts have been shown to have good effects on body weight and show promise in treating the state of diabetes mellitus for diabetic rats. Additionally, these extracts have a substantial capacity to scavenge radicals identified as DPPH, which stands for 2,2-diphenyl-1-picrylhydrazyl. It may be concluded that Artemisia indica has the potential to be used in the therapy of oxidative stress. For the treatment of diabetes mellitus, Artemisia indica has been used traditionally, and these data lend support to that practice.
In spite of this, additional study is required in order to separate and purify the bioactive chemicals that are present in the plant extracts. These research would make it possible to have a deeper comprehension of the molecular pathways that are responsible for the impacts that have been observed. Following the identification and characterization of the individual active compounds, the possible therapeutic targets of these compounds can be explored, which will ultimately lead to the creation of a treatment that is more targeted and successful against diabetes.

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Conflict of Interests
The authors have no conflict of interests.

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