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Phytochemical screening and anti-hyperglycemic effect of *Jatropha multifida* L. ethanol extract and its fraction on a high-fat diet and Streptozotocin-induced diabetic rats

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Jatropha multifida L. is a plant traditionally recognized for its medicinal properties. This plant has been reported to possess anti-microbial, anti-oxidative, wound healing and anti-inflammatory properties. The study aimed to evaluate the anti-diabetic potentials of *J. multifida*. The study deals with the extraction of the leaves of the plant by using 50% ethanol followed by fractionation of the ethanol extract obtained. Various physicochemical parameters and phytochemical analyses were done by TLC and HPTLC. Phytochemical analysis of the extract and its fraction indicated high concentrations of flavonoids, phenolic acid and saponins. The effects of the 50% ethanol extract of *J. multifida* leaves, and its hexane fraction were investigated in high-fat diet and Streptozotocin-induced diabetic rats. At the end of the treatment, the reduction in blood glucose level in rats treated with ethanol extract at a dose of 400 mg/kg was found to be 117.5±1.7 and hexane fraction at 200 mg/kg was found to be 105±2.5. A significant improvement in lipid profile and liver abnormalities of the rats was observed. The result of the present study is also justified by histopathological examinations of the pancreas and liver. Therefore, the current study proved that both the ethanolic extract of *J. multifida* and its hexane fraction possess bioactive constituents that could be responsible for the anti-diabetic properties. Out of the two, maximum improvement was seen using hexane fraction at 200 mg/kg. This may be due to the active phytoconstituents isolated from the fraction of the 50% ethanolic extract of the leaves.

Keywords: Blood sugar level, Diabetes mellitus, High fat diet, *Jatropha multifida*, Streptozotocin

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Introduction

Diabetes is a metabolic condition that has detrimental effects on one's health. Insulin is indicated for type I diabetes and type II diabetic patients, along with certain oral medications[1-3]. However, risks of upper respiratory tract infections have been reported with medications such as repaglinide and sulfonyl ureas[4]. Therefore, research into safer and more powerful anti-diabetic drugs is required. Treatment of diabetes has been demonstrated to be successful with medications made from natural ingredients[5,6].

Jatropha multifida L., often designated as "coral bush", is a species of *Jatropha*, belonging to the family Euphorbiaceae, a rapidly rising shrub or tiny shrub/tree upto a height of 1.30 m. It is an endogenous plant to Tropical Americas but is now extensively grown for its attractive plants and flora in Tropical to sub-tropical areas across the world[7]. It is easily disseminated by seeds or cuttings[8]. It is often called the "Penicillin, Iodine and Betadin plant"[9]. In African continental, this plant is called "MIODINE" meaning "tree of iodine" due to its antiseptic properties.

J. multifida contains polyphenols, flavonoids, tannins, terpenoids, alkaloids and saponins that possess anti-microbial, anti-oxidative, wound healing and anti-inflammatory properties[10-13]. Phenolic acids such as vanillic[14], *cis* and *trans* ferulic[14], *p*- OH benzoic acid[14], phloretic acids and glycoflavones[14] and flavonoids such asvitexin and isovitexin have been reported in this plant.

An imbalance between the organism's anti-oxidant potential and oxygen-derived radicals results in oxidative stress; as a result, proteins, lipids, and nucleic acids suffer oxidative damage. Diabetes mellitus, atherosclerosis, inflammatory disorders, hypertension, cancer and heart disease are only a few

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ailments oxidative stress causes over time[15,16]. Diabetes has been linked in numerous studies to increase free radical generation and decrease anti-oxidant capacity[17]. As a result, it is logical to suppose that anti-oxidants can significantly contribute to reducing diabetes symptoms[18].

Rats fed with a High fat diet (HFD) followed by a low dose of Streptozotocin (STZ) induced intra-peritoneally develop obesity, insulin resistance and hyperinsulinemia. These together bring the onset of type II diabetes. HFD causes obesity, resulting in high levels of triglycerides, which causes an increase of fatty acids availability and oxidation, resulting in hyperinsulinemia by reducing glucose uptake or utilisation in skeletal muscle[19,20]. Leaves of *J. multifida* are known to contain flavonoids and phenolic acids as phytoconstituents, which are believed to lower blood glucose levels through the following ways: by inhibiting the activities of carbohydrate-hydrolysing enzymes, by possessing inhibitory potential towards α-glucosidase and as an anti-oxidant preventing the malfunction of the pancreatic beta cell due to oxidative stress[21].

Materials and Methods

Plant Sample Collection and Identification

Fresh leaves of *J. multifida* were collected from the United Institute of Pharmacy, Prayagraj, Uttar Pradesh, in November 2021. They were botanically identified by the Botanical Survey of India, Prayagraj (Approval No.-BSI/CRC/2021-22/435) by Dr. Arti Garg, Scientist-E and Head of The Botanical Survey of India, Prayagraj, Uttar Pradesh, in December 2021. Aerial parts of the plant are shown in Fig. 1.

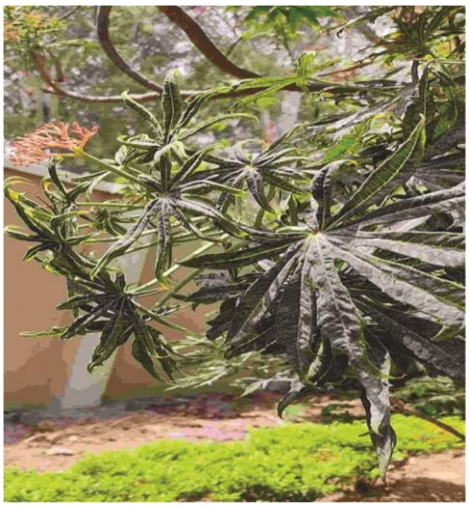

Fig. 1 — Aerial parts of *Jatropha multifida*.

Animals

Adult Wistar rats (200-250 g) were taken from CPCSEA registered Laboratory Animal Supplier m/s Chakraborty Enterprises Kolkata (Registration No.-1443/PO/Bt/S/11/CPCSEA). All rats were shifted to a quarantine area for acclimatisation to the animal house for two weeks before the experiment. The experimental protocol was approved by the IAEC committee with Approval no.UIP/IAEC/Nov.-2021/04.

Extraction and Fractionation of the plant leaves

The fresh leaves were thoroughly washed with tap water and air dried under shade at room temperature for 7 days[22] and pulverised into a coarse powder. About 500 g of the leaves were defatted using 3 L of Petroleum ether through hot percolation, and the marc obtained was dried in air and then extracted with 50% aqueous ethanol in the Soxhlet apparatus. The obtained filtrate was stored in an airtight container for future use. The filtrate obtained was successively fractionated using a separating funnel with the following solvents in order of increasing polarity viz. n-hexane, chloroform, ethyl acetate, butanol and methanol. These extracts were further subjected to phytochemical testing.

Assessment of physicochemical parameters

The variables under study are loss on drying, alcohol and water-soluble extractive values, total ash, and acid-insoluble ash. The quality and purity of crude drugs are assessed using ash values. It suggests the existence of different contaminants, including oxalates, carbonates, and silicates. Silica is the primary component of the acid-insoluble ash, which is a sign of contamination with earthy material. Drugs should contain a minimum amount of moisture to prevent the growth of yeast, bacteria, or fungi while being stored. Thus, the compositions of these phytoconstituents indicate whether the crude drug is exhausted or not[23]. These physicochemical parameters were evaluated as per Khandelwal[24].

Assessment of Loss On Drying (LOD)

The crude drug was powdered, placed into a weighed porcelain dish, and dried in an oven at 100-105°C until the two following weighing did not vary by more than 0.5 mg. It was cooled in desiccators for 15 min and then weighed. The loss in weight is normally taken as moisture. The results were expressed in %. The formula for the assessment of LOD is given below:

$$\text{Moisture content } \% = \frac{(X - Y)}{W} \times 100$$

where X = weight of the empty dish, Y = weight of dish + sample before drying, and Z = weight of dish + sample after drying.

Assessment of Extractive Value

Determination of Alcohol-Soluble Extractives by cold maceration (ASEV)

The plant leaves were powdered, and 4 g of it was taken in a bottle and transferred into a 250 mL conical flask. The bottle was washed using 100 mL of 90% alcohol, and the washings were poured into the conical flask. It was macerated for 24 h, filtered, and 25 mL of the filtrate was taken to a weighed porcelain dish. It was concentrated in a steam bath, and then oven dried at 105°C for six hours. Cooled in a desiccator for 30 min and weighed. % w/w was known with reference to the air-dried drug. The formula for the determination of ASEV is given in the equation below:

$$\% \text{ ASEV} = \text{weight of the residue / weight of drug} \times 100$$

Water-soluble extractives by cold maceration (WSEV)

Coarsely powdered plant leaves were transferred into a 250 mL conical flask. The weighing bottle was washed using chloroform water, and the washings were poured into the conical flask. Shaking frequently, it was macerated for 24 h. Filtered and 25 mL of the filtrate was taken to a weighed porcelain dish. It was evaporated in a steam bath followed by oven drying for 6 h at 105°C. Kept for cooling in desiccators for thirty minutes and then weighed. % w/w was estimated. The formula for determination of WSEV is given in the equation below:

$$\% \text{ WSEV} = \text{weight of the residue / weight of drug} \times 100$$

Ash value

Two grams of powdered leaves were taken into a weighed thin porcelain dish and ignited in a muffle furnace for 3 h 600-700°C until all the carbons burnt off. It was kept for cooling in desiccators. The ash was weighed, and the % of total ash was determined using the formula given below:

$$Total\ ash\ value = 100\frac{Z - X}{Y}\%$$

where, X = weight of the empty dish, Y = weight of taken drug, and Z = weight of the dish + ash (after complete incineration).

Acid insoluble ash value

The ash from the above method was taken into a 100 mL beaker and washed with 25 mL dil. hydrochloric acid. Boiled for 5 min and made to run through an 'ash less' filter paper. Washed the residue left twice with heated water. The weight of the ignited crucible was taken. Kept for cooling in a desiccator and weighed. The following calculations were done. The formula for determining the Acid insoluble ash is given in Eq. (1.5).

$$Acid - insoluble\ ash\ value = 100 \times \frac{A}{Y}\%$$

where A = weight of the residue and Y = weight of the drug taken (In the assessment of ash value).

Analysis of Fluorescence

This analysis test was performed in the UV chamber using different reagents at wavelengths 254 nm, 366 nm and visible light to find out the transparency of the crude powdered drug.

Detection of Phytochemicals

The extract and fractions of *J. multifida* leaves were subjected to various phytochemical screening to find different phytoconstituents[24,25].

Thin Layer Chromatography(TLC)

TLC is a very commonly used method for separating non-volatile mixtures based on the principle of separation through adsorption[26]. The mobile phase of chloroform: methanol (2:4) was used to check the efficiency of different constituents present in the samples. Two mL of the 50% ethanol extract and various fractions of *J. multifida* leaves were dissolved in their respective solvents of extraction and fractionation. R_f values were calculated after spotting the sample on a stationary phase under UV light. Table 1 shows the R_f value of the various extracts. TLC of all the fractionated extract and ethanol extract was performed in which ethanol

Table 1 — R_f value of *J. multifida* ethanol extract and its entire fractions

Solvent	R_f value					
Chloroform: Methanol (2:4)	Ethanolic extract	n-hexane fraction	Ethyl acetate fraction	Chloroform fraction	Butanol fraction	Methanol fraction
	0.8	0.88	0.75	0.8	0.6	0.58

extract and hexane fraction showed better separation of phytoconstituents, and these two were selected for further analysis.

HPTLC of the hexane extract

2 g of *J. multifida* hexane fraction was utilised for HPTLC evaluation. The plates were prepared in Chloroform: Methanol (2:4). Prepared plates were then observed under the colourimetry light after derivatisation with observation at 254 nm, 366 nm and visible light in UV light. The plates were scanned densitometrically at wavelength 366 nm, and the profile was recorded. Also, the values and the relative percentage of area in each peak in the applied extract were calculated.

Selection of the solvent system

The process of the selection of solvent system was on the spots detected in TLC. HPTLC was performed using mobile phase chloroform: methanol (2:4) at 366 nm.

Test solution

The extract dissolved in the ratio of 10 mg/mL in hexane was used for HPTLC.

Quantification and documentation

Densitometry is an *in situ* evaluation of 200-700 nm and fluorescent reducing light. The scanner changes the spots onto the layer into a chromatogram, which consists of the peak alike in presence to that HPTLC chromatogram. The portion of the scanned picture on the plotter graph is correlated to retention factor parameters of the spots onto the layer, and the height of the peak or area is related to the concentration of the substances on the spot[27].

Pharmacological Studies

Oral Acute Toxicity Test (LD_{50})

This study was conducted in animals according to OECD guidelines 423[28]. No toxicity or death was recorded. This estimates the safe use of leaves of *J. multifida*.

Development of HFD

The diet was freshly prepared in the laboratory every 3rd day. Fat-enriched diets have been popularly used to model obesity and dyslipidemia. A diet rich in fats easily induces type II diabetes. HFD brings about hyper-insulinemia, resistance to insulin and intolerance of glucose[29]. The Food Analysis & Research Laboratory, University of Allahabad tested the high-fat diet. The protein, fat and total sugar in the diet were 7.38, 37.17, and 15.20 g/100g. A high-fat diet was given for 12 days for the induction of type II diabetes with the help of low-dose streptozotocin, and HFD was continued till the end of the experiment. A list of ingredients required to make HFD are given in Table 2.

Induction of Diabetes mellitus

On the 13th day, diabetes was induced by intraperitoneal injection of streptozotocin dissolved in citrate buffer at a low dose (40 mg/kg) followed by 10% fructose solution given orally for two hours. On the 16th day, fasting BGL was measured using One Touch Glucometer. Rats with BGL above 200 mg/dL were used for the study.

Dose Preparation

Suspension of *J. multifida* ethanolic extract (JMEE) and *J. multifida* hexane fraction (JMHF) were prepared by triturating the extracts with 2% gum acacia in a mortar pestle separately. JMEE was given at 200 and 400 mg/Kg body weight, while JMHF was given at doses of 100 and 200 mg/Kg.

Treatment protocol

The diabetic rats were randomly divided into seven groups (n=6/groups). The experimental protocol is given in Table 3. The experimental protocol has been followed as per Magalhães[30].

Analysis of Blood sugar level

Blood samples were collected at 16th, 23rd, 30th, 37th, and 39th day intervals. On the 39thday, a blood sample was collected through the retro bulbar route in

Table 2 —List of ingredients used to make HFD

Ingredients	Quantity (g)
Crushed normal diet	300
Vegetable ghee	450
Sugar	150

Table 3 — Experimental design and procedure

Groups	Diet	STZ Induced	Treatments
Group I (Normal Control)	ND	No STZ	NS
Group II (Diabetic Control)	HFD	STZ	NS
Group III (Standard drug treated)	HFD	STZ	Metformin (100 mg/kg)
Group IV (Ethanolic extract- Low dose)	HFD	STZ	JMEE (200 mg/kg)
Group V (Ethanolic extract- High dose)	HFD	STZ	JMEE (400 mg/kg)
Group VI (Hexane fraction- Low dose)	HFD	STZ	JMHF (100 mg/kg)
Group VII (Hexane fraction- High dose)	HFD	STZ	JMHF (200 mg/kg)

the blood collecting tube. Various biochemical parameters such as lipid profile and liver function test were performed at United Diagnostic, Prayagraj, Uttar Pradesh.

Body weight measurement

Body weight measurement was taken at 1st, 13th, 16th, 23rd, 30th, 37th and 39th day using weighing balance.

Statistical analysis

Data obtained were analysed using Two Way ANOVA (Version 9.3.1) software and expressed as mean±SD followed by Dunnet's *t*-test. Differences between means were regarded as statistically significant below $P < 0.05$.

Results and Discussion

Percentage yield of extract and its various fractions

The extract obtained through ethanol extraction and its fractionated extracts were evaluated for percentage yield. The percentage yield of the ethanolic extract and its various fractions are mentioned in Table 4.

Table 4 — Percentage yield of extract and its various fractions

Extracts	Percentage yield (%)
Ethanolic extract	11.00
Hexane fraction	39.40
Chloroform fraction	6.00
Ethyl acetate fraction	1.80
Butanol fraction	1.60
Methanol fraction	1.50

Table 5 — Physicochemical parameters of *J. multifida* Linn. leaves

Parameters	% w/w
LOD	0.2
Total ash value	12.5
Acid-insoluble ash value	3.0
Water soluble extractive value	25
Alcohol soluble extractive value	21

Physicochemical parameters

Different physicochemical parameters of crude powder of *J. multifida* leaves are given in Table 5.

Analysis of Fluorescence

This analysis test was performed in the UV chamber using different reagents at wavelengths 254 nm, 366 nm and visible light. The results of the crude powder of *J. multifida* leaves are shown in Table 6.

Phytochemical tests

Phytochemical screening of the extract and fractions of *J. multifida* showed the presence of various chemical constituents. Saponins, tannins, phenolic acids, flavonoids, and alkaloids are in large amounts. Table 7 represents the result of various phytochemical tests performed.

Thin Layer Chromatography

TLC of the ethanol extract and its fractions were performed in which JMEE and JMHF showed better separation of phytoconstituents, so these two were selected for further analysis.

HPTLC Analysis

The result showed peak, start R_f, max R_f, end R_f value, max % value, start height, maximum height, and end height were given in Table 8. In JMHF, the

Table 6 — Fluorescence analysis of *J. multifida* leaves

Reagents	UV light (254 nm)	UV light (366 nm)	Visible light
Iodine	Greenish	Slightly greenish black	Greenish
Methanol	Greenish	Brown	Greenish
Sulphuric acid	Black	Black	Dark brown
Nitric acid	Greenish	Black	Brown
Picric acid	Greenish	Dark green	Greenish
Glacial acetic acid	Greenish	Black	Brown
Hydrochloric acid	Greenish	Brown	Greenish
Sodium hydroxide	Greenish	Slightly brown	Greenish

Table 7 — Phytochemical analysis of *J. multifida* leaves extract

Tests performed		Ethanolic extract	HF	CF	EAF	MF
Alkaloid	Mayer's test	++	+	+	-	-
	Dragendorff's test	+	+	+	++	+
Saponins	Foam test	++	++	++	+	+
Flavonoids	Sulphuric acid	+	++	+	++	+
	Lead acetate test	+	+	+	+	_
	NaOH	++	++	+		+
Phenols	$FeCl_3$	+	++	+	+	+
	Acetic acid sol.	++	++	++	+	+
	Dilute HNO_3	+	+	+	+	+
Steroids	Salkowski test	+	+	+	+	+
Tannins	5% $FeCl_3$	+	+	+	+	+

Table 8 — HPTLC of *J. multifida* leaf extract gives number of peaks at 365 nm

Peak	R_f			Height			Max %	Area	Area %
	Start R_f	Max R_f	End R_f	Start Height	Max Height	End Height			
1	0.22	0.28	0.34	7.7	42.6	20.3	15.32	2194.7	12.98
2	0.38	0.39	0.45	16.5	18.3	2.5	6.6	531.6	3.14
3	0.58	0.65	0.71	7.9	66.2	6.7	23.79	3425.4	20.26
4	0.74	0.87	0.95	9.5	151.2	3.4	54.33	10755.8	63.62

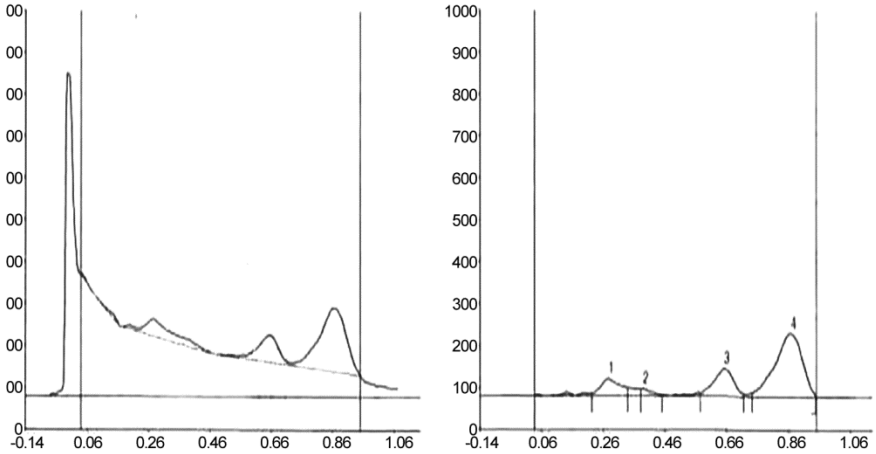

Fig. 2 — HPTLC spectra of JMHF.

maximum % area 63.62% was enclosed by peak No. 4 (R_f value 0.74). This HPTLC method was used for the determination and the quality estimation of formulation, which consists of *J. multifida* leaves. HPTLC spectra of *J. multifida* are shown in Fig. 2.

Acute Toxicity Test

It was performed according to OECD guideline 423. Animals were orally administered with JMEE and hexane fraction (JMHF) of the *J. multifida* leaves at various doses of 5, 50, 300, 2000 and 5000 mg/Kg, separately. No death or toxicity was recorded for both JMEE and JMHF.

Effect of the JMEE and JMHF on glucose level of glycemic rats

The effect of ethanol extract and its hexane fraction on the BGL of glycemic rats is presented in Fig. 3. Therapeutic intervention of diabetes is aimed at reducing or avoiding elevations in the glucose level of blood using hypoglycemic agents or insulin. The ethanol extract and its hexane fraction showed a significant decrease in BGL. BGL in the active fraction of *J. multifida* ethanolic extract was observed to reduce effectively as the study duration increased. At the same time, the untreated glycemic rats showed a geometric increase in BGL with an increase in study duration. Table 9 shows the result of the treatment of various doses of the extract.

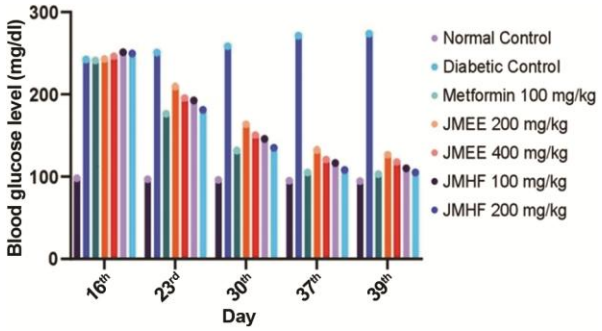

Fig. 3 — Graph representing blood glucose level in diabetes induced experimental animals.

Effect of the JMEE and JMHF on body weight

HFD and STZ-induced diabetes significantly reduce the body weight of the diabetic untreated rats as the study duration increases compared to diabetic and normal control rats (Table 10). Diabetes is accompanied by increased lipolysis, gluconeogenesis, and glycogenolysis, and all of these biochemical activities result in muscle wasting and loss of tissue protein. *J. multifida* prevents such changes by restoring the body weight of diabetic-treated rats. Findings showed that the oral administration of Metformin (100 mg/Kg), JMEE (200 and 400 mg/Kg), and JMHF (100 and 200 mg/Kg) to diabetic rats is improved. Treatment with ethanol extract in rats has shown prominent results in restoring body

Table 9 — Effects of JMEE and JMHF on blood glucose level of blood in HFD and STZ-induced diabetic rats

Treatment	16th day (mg/dL)	23th day (mg/dL)	30th day (mg/dL)	37th day (mg/dL)	39th day (mg/dL)
Normal control	98.1±3.6	96.8±2.4	96±2.7	95±2.8	94.5±3
Diabetic control	242±3.3	250.6±3.2	258.3±3.1	271±2.3	273.5±2.2
Metformin 100 mg/kg	241.1±2.4	176.3±3.2[a,f]	131.6±3.9	105±2.5	103.1±2.8[f]
JMEE 200 mg/kg	242.8±2.7	209±4.4	163.6±3	132.1±4	126.5±1.7
JMEE 400 mg/kg	246±3.2	195.3±1.9[a]	150.3±2.3[a,b]	120.5±1.7[b]	117.5±1.7[a,b]
JMHF 100 mg/kg	251.1±2.5	192.5±1.7[a]	145.8±2.6[a]	116.5±1.7	110.1±3.1[a]
JMHF 200 mg/kg	249.6±2.1[b]	181±2.3[b]	135±2.8[a,b]	108±3.5[a,b]	105±2.5[b]

Mean±SD (n=6) Statistical significance in comparison to group II is [a]*P* <0.0001 and group III is [b]*P* <0.0001 while [f]*P* <0.0001 as compared to normal control.

Table 10 — Effects of JMEE and JMHF on body weight of rats

Groups	16th day	23rd day	30th day	37th day	39th day
Normal Control	147.6±4.6	163.1±3.4	179.5±3.3	192.5±3.2	195.6±3.2
Diabetic Control	125.5±2.6	115.3±2.5	109±2.1	96.5±1.7	95.5±2.5
Metformin 100 mg/kg	120±3.2	142.1±3.4[b]	147.6±3.7[b,f]	153.1±3.8[a,f]	155±2.6[b,f]
JMEE 200 mg/kg	130±1.2	132±2.1[c]	138.8±1.7	142.3±2.3	143±2.5[c]
JMEE 400 mg/kg	133.5±2.2[a]	140.5±2.9	146±2.3	151.3±2.4[b]	152.8±2.1
JMHF 100 mg/kg	130.5±4	144.3±3.3	149±3.8	156±4.7	157±3.6
JMHF 200 mg/kg	132.3±2.8[b]	151±3.4[c]	154±5.1[b]	161±3.4[d]	162±5.6[b,d]

Mean±SD (standard deviation) for 6 experimental animals in each group. Statistical significance in comparison to Diabetic control ([a]*P* <0.001) and ([b]*P* <0.0001) and [c]*P* <0.0001 and [d]*P* <0.01 as compared to Metformin while [f]*P* <0.0001 as compared to normal control.

Table 11 — Effects of JMEE and JMHF on lipid profile after 39 days

Groups	Triglyceride	Total Cholesterol	HDL	LDL	VLDL
Normal control	74.1±5.6	79.5±3.4	26.5±2.8	38.1±5.5	14.8±1.1
Diabetic control	146.6 ±5	154.3±1.8	14.8±2.1	113.1±3.2	29.3±1
Metformin 100 mg/kg	91.3±5.4[a,f]	93.2±1.9[f]	19.1±2.2[a,h]	55.81±3[f]	18.2±1[f]
JMEE 200 mg/kg	121.3±5.5[a,d]	125.1±1.3[d]	17.6±1.4[b,f]	85.7±4.8[a,d]	24.2±1.1[b,e]
JMEE 400 mg/kg	100.6±5[a,d]	92.8±3.3[a]	20.1±1.6[a]	52.3±3.3[a]	20.1±1[a,g]
JMHF 100 mg/kg	98.5±4.7[a,d]	95±3.3[a,f]	18.6±2.7[c]	56.6±3.7[a,f]	19.7±0.95[a]
JMHF 200 mg/kg	95.1±4.8[a,f]	91.6±2.1[a]	21.3±2.1[a]	52.9±5.5[a]	19±0.97[a]

The data represent as mean±SD of 6 rats in each group [f]*P* <0.0001 and [g]*P* <0.05 and [h]*P* <0.01 as compared to normal control and [a]*P* <0.0001, [b]*P* <0.05 and [c]*P* <0.001 in comparison to diabetic control group. While, [d]*P* <0.0001 and [e]*P* <0.05 as compared to Metformin.

weight, but the effects were more pronounced with JMHF. This improvement may be due to the control of hyperglycemic conditions.

Effect of the JMEE and JMHF on the lipid profile of glycemic rats

Diabetes is associated with a rise in cholesterol levels due to insulin deficiency. Insulin causes activation of triglyceride hydrolysing lipoprotein lipase[31]. Due to insulin deficiency, there is a build-up of cholesterol[32]. The active fraction of JMEE lowered the elevated TG, VLDL, TC, and LDL levels in diabetic animals to nearly normal levels as that of the standard drug treated (Table 11). An increase in HDL cholesterol level was a desirable result obtained.

Liver function test

An increase in the level of liver enzymes (ALP, SGOT, SGPT and bilirubin) in diabetic rats is due to liver damage due to inflammation in liver cells by oxidative stress, cytokines release, IL-1β or other inflammatory hormones. Liver abnormalities in diabetic patients can cause a risk of exposure to hepatitis B, hepatitis C and other chronic liver diseases[33,34]. However, in Metformin-treated rats, the activities of these enzymes somewhat improved. In JMEE and JMHF-treated groups, the level of these enzymes was decreased. This shows that *J. multifida* leaves improve diabetic complications. It is probably due to the active principle(s) might be one or more flavonoids, terpenoids or phenolic acids[35]. These phytoconstituents are believed to protect β cells of the pancreas from oxidative stress by their anti-oxidant properties[36-39]. Serum ALP, SGOT, SGPT and bilirubin of the hyperglycemic rats were increased significantly relative to their respective normal

Table 12 — Effects of JMEE and JMHF on Liver function test

Groups	ALP	SGOT	SGPT	Bilirubin
Normal control	155.8±3	66.5±1.3	55±3.3	0.75±0.3
Diabetic control	265.5±3.3	125.1±2	107.1±1.7	2.13±0.15
Metformin 100mg/kg	174.8±2.8[f]	59.5±1.3[c]	64.1±2.6[c,f]	0.78±0.3
JMEE 200mg/kg	234.3±4	119.1±2.2[b]	92.5±1.6[a,b]	2.1±0.02
JMEE 400 mg/kg	216.6±3.3[a,f]	110.5±1.3[b]	83.3±2.4[b,c]	2±0.6
JMHF 100 mg/kg	207.6±2.1[c]	104.1±3.2[f]	79±1.2[c]	1.8±0.19
JMHF 200 mg/kg	193.8±3.4[a,c]	96.1±2.1[c]	71.1±3.9[c]	1.4±0.2

Mean±SD (n=6) [a]*P* <0.0001 compared to Metformin, [b]*P* <0.001 compared to diabetic control and [c]*P* <0.0001 in comparison to diabetic control group while [f]*P* <0.0001 as compared to normal control.

control. These, however, decreased significantly upon treatment with JMEE at 400 mg/kg 216.6±3.3, 110.5±1.3, 83.3±2.4 and 2±0.6 (Table 12). While these were more significantly reduced in JMHF at 200 mg/kg.

Histopathological assessment of pancreas and liver

Histopathological examination of the pancreas and liver was performed by their thin sections. The sections were stained with haematoxylin /eosin dye using a routine protocol and examined at 40X using a microscope.

Histopathology of pancreas

Group I pancreas shows that islets are normal with usual epithelial cells, acni of serous and free of fibrosis. Examination of Group II in Fig. 4 shows the distorted architecture of the pancreas with disorganised islets of Langerhans. Damage to necrosis of β-cells may be due to streptozotocin. Demolition of acni of serous epithelial cells lined by oval cells with moderate cytoplasm and lymphocytic inflammation is seen. The minutely examined pancreas of Group III shows some protection from diabetic conditions. Investigation of Group IV shows a demolition structure with scattered lymphocytes. Examination of Group V shows inflammation with similarity to normal architecture. Minutely Examination of Group VI shows mild demolition in cells of acinar, and there is no evidence of inflammation and recovery seen in lymphocytes. In contrast, the section of Group VII revealed a normal architecture with cellular regeneration and no inflammation identical to that of a pancreas treated with Metformin.

Histopathology of liver

Investigation of the liver section in Group Ib in Fig. 5 shows normal architecture of hepatocytes with moderate cytoplasm and von kupffer cells lining the sinusoidal spaces, and portal triads appeared normal. Microscopically investigation of Group IIb shows disrupted architecture with swelling of hepatic

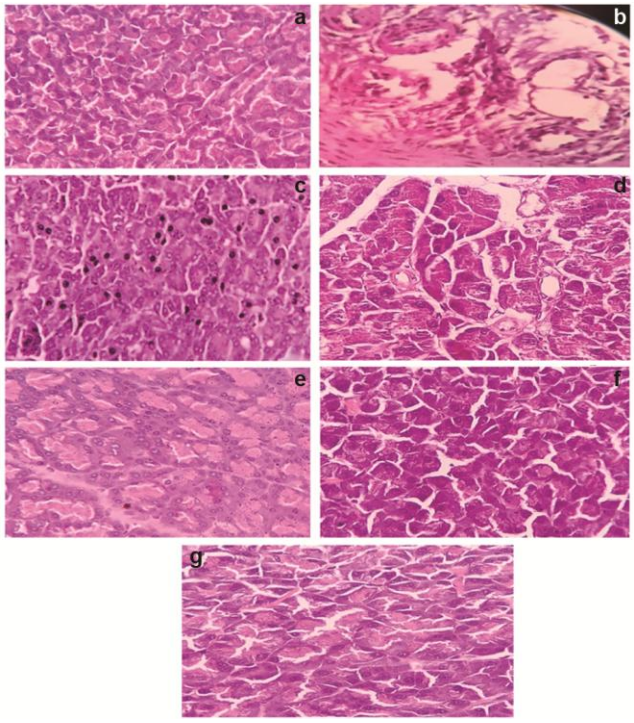

Fig. 4 — Histopathology of Pancreas. a) Group I Normal control; b) Group II Diabetic control; c) Group III Metformin 100 mg/kg; d) Group IV JMEE 200 mg/kg; e) Group V JMEE 400 mg/kg; e) Group VI JMHF 100 mg/kg; and f) Group VII JMHF 200 mg/kg.

cells, fatty degeneration and patchy necrosis of the hepatocytes. In experimental group IIIb, histopathological investigation shows normal globular architecture with mild disarrangement and inflammation at the site of the portal trial. Group IVb shows dilation of sinusoids with mild disarray at the portal trial site. Group Vb sections resemble normal hepatocytes as that of Group III, and cytoplasmic mild changes were seen. While examination of Group VIb, there is a low change in liver fat with gentle inflammation. Hepatocytes appeared normal. An examination of Group VIIb shows the absence of any degenerated apoptotic cells. Active and healthy

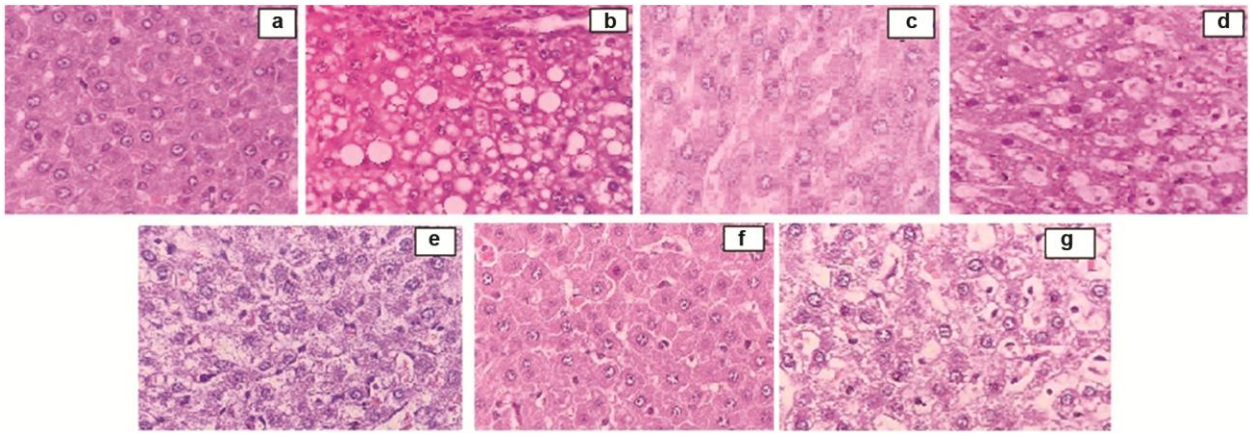

Fig. 5 — Histopathology of Liver. a) Normal control; b) Diabetic control; c) Metformin treated group; d) JMEE (200 mg/kg); e) JMEE (400 mg/kg); e) JMHF (100 mg/kg); and f) JMHF (200 mg/kg).

hepatocytes with active vesicular nuclei and moderate cytoplasm were seen.

Normally, insulin causes activation of the triglyceride, hydrolysing lipoprotein lipase. However, in a diabetic state, there is no activation of lipoprotein lipase due to a deficiency of insulin, causing a build-up of lipids in diabetic people, including cholesterol and triglycerides, resulting in hypertriglyceridemia, and dyslipidemia, as per Taskinen and Nikkilä[38]. In the present study, diabetic rats manifested hypertriglyceridemia, hypercholesterolemia. However, treatment with both JMEE and JMHF significantly decreased the level of cholesterol and triglyceride. It is probable that the active principle(s) might be one or more flavonoids, terpenoids or phenolic acids, as per Parmar and Ghosh[36]. These phytoconstituents are believed to protect β cells of the pancreas from oxidative stress by their anti-oxidant properties, as per Donath *et al*.[38] and Herna´ndez *et al*.[39].

From the observations, we conclude that the ethanol extract and its fraction showed anti-hyperglycemic activity in Wistar rats. However, JMHF was more effective at low doses in effectively lowering the glucose level of blood in comparison to ethanol extract. Rats treated with the standard drug show much-improved health conditions. This is consistent with the research conducted by Nanda *et al*.[40], which shows improvement in body weight and reduction in blood glucose level with standard drug and combined extracts of *Plumeria alba* Linn. The desired activity may be produced via peripheral glucose utilisation, enhanced insulin secretion or inhibition of enzymes that break down carbohydrates[41].

Conclusion

Leaves of *J. multifida* have shown to be a potential agent for the treatment of diabetes mellitus, restoration of body weight and improvement of the lipid profile of diabetic-treated rats. Thus, it has a protective role against complications associated with diabetes, which may be due to the presence of flavonoids, saponin or phenolic acids. Additional research is being conducted to identify and characterize the active ingredient and better understand the mechanism underlying the anti-diabetic action.

Conflicts of interest

The authors declare no conflict of interest.

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