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Preparation and Evaluation of Polyherbal Extract (*Azadirachta indica, Ocimum sanctum, Clitoria ternatea & Hibiscus rosa sinensis*) for Antidiabetic Activity

Shweta Singh*,[1] and Dr. Varsha Deva[2]

Abstract

Diabetes mellitus is a metabolic disorder which has abnormal higher glucose in the blood. The current study was based on the preparation and evaluation of polyherbal extract (*Azadirachta indica, Ocimum sanctum, Clitoria ternatea & Hibiscus rosa sinensis*) for antidiabetic Activity. The fresh leaves of *Azadirachta Indica, Ocimum Sanctum, Hibiscus rosa sinensis L.,* and fresh flowers of *Clitoria ternatea L.* were collected from the Prayagraj region, UP East, India. There were authenticated by the botanist at Botanical Survey of India, Allahabad, UP with the reference no. 2023-24/534. The dried leaves of *Azadirachta indica*, *Ocimum sanctum* and *Hibiscus rosa sinensis L.* were rendered into fine powder and extracted using hydroalcoholic (distilled water and ethanol; 1:1) solution through cold maceration process. The leaves of different plants were soaked in a beaker containing hydroalcoholic (distilled water and ethanol; 1:1) solution for 15 days with gradual stirrings, separately. Similarly, dried flowers of *Clitoria ternatea L.* was rendered into fine powders then soaked into a beaker containing hydroalcoholic (distilled water and ethanol; 1:1) solution for 15 days with gradual stirrings and thus extracted-out. Acute toxicity study was performed. Streptozotocin (STZ) -induced diabetes model was used and evaluated for various parameters i.e., blood glucose level (glucometer), lipid profile, body weights, food consumption, haematological tests, urine analysis, weight of organs and histopathological studies using liver and pancreas. Results showed that *Azadirachta indica*, *Ocimum sanctum*, *Clitoria ternatea* and *Hibiscus rosa sinensis* have enormous antioxidant and anti-diabetic role as compared with acarbose, ascorbic acid and glibenclamide. It concludes that DM-01, DM-02, DM-03 and DM-04 polyherbal formulations are an effective in the management of diabetes but it's mode of action is still unknown. So further research may carried-out to confirm its mode of action and optimum doses required in management of DM.

Keywords: Diabetes mellitus, *Azadirachta indica*, *Ocimum sanctum, Clitoria ternatea, Hibiscus rosa sinensis,* Anti-diabetic activity

Introduction

Diabetes mellitus is a metabolic disorder which has abnormal higher glucose in the blood. In 1922, Banting et al. extracted insulin from bovine pancreas at University of Toronto, which resulted the development of a viable therapy for diabetes. (Arumugam G. et al. 2013). Symptoms of DM includes (Garcia E. D. et al. 2018) as frequent urination, blurred vision, extreme weakness, fatigue, irritability, extreme hunger, loss of weight and unusual

Affiliation:

[1]Research Scholar, Glocal University Pharmacy College, Glocal University, Mirzapur Pole Saharanpur U.P. 247121

[2]Professor, Glocal University Pharmacy College, Glocal University, Mirzapur Pole Saharanpur U.P. 247121

***Corresponding author:**
Shweta Singh. Research Scholar, Glocal University Pharmacy College, Glocal University, Mirzapur Pole Saharanpur U.P. 247121.

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Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
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thirst. Age and obesity are the most common contributing factors to insulin resistance, which is a complicated syndrome that usually results from a combination of variables (Rajaei E. et al., 2019). New blood glucose-lowering drugs have been developed throughout the past ten years. Additionally, these drugs have a remarkable ability to reduce cardiovascular risk factors. These medications show a notable capacity to lower the incidence of cardio-renal events in both diabetic patients and healthy persons (Heerspink H.J.L. et al. 2020).

The bioactive chemicals quercetin and rutin have the ability to suppress reactive oxygen species (ROS), which are free radicals. These compounds are particularly powerful in neutralizing ROS and have shown promising results in combating cardiovascular and diabetes problems (Ratnaningtyas N.I. et al., 2022). Flavonoids possess high antioxidant properties, which enable them to shield the body from harm caused by reactive oxygen species. They achieve this by donating hydrogen atoms and forming stable flavonoid radicals through binding with free radicals. Consequently, it can hinder the harmful effects of lipid peroxidation on cells and mitigate the development of problems such as diabetes mellitus (Bhat & Bhat, 2021).

Neem belongs to the family Meliaceae. It is extensively grown in tropical and semitropical regions such as Bangladesh, Nepal, India, and Pakistan. This tree can reach a height of 20 to 23 meters and has a straight trunk that is 4 to 5 feet broad. It grows quickly. Each leaf has five to fifteen leaflets and is complex and imparipinnate. When it ripens, its greenish drupes become golden yellow (Alzohairy Mohammad A., 2016). The Quercetin & ß-sitosterol, flavonoids were refined from fresh neem leaves, exhibited antibacterial and antifungal effects (Govindachari et al. 1998), while seeds contain important elements i.e., gedunin & azadirachtin (Hossain et al. 2011).

Tulsi is an upright, aromatic plant with many branches. 30 to 60 cm tall, fragrant plant. The oval leaves have a basic green or purple color, a gently serrated or dented edge, and a blade length of 5 cm. The blooms have a short, hairy stalk and are violet. The plant produces reddish-yellow seeds and little fruit. It has a harsh and caustic taste (Prajapati et al. 2003). The two kinds of flavonoids that were isolated from the aqueous leaf extract were orientin and vicenin. The extract of Tulsi leaves also contains other substances such as orientin, luteolin, apigenin-7-O-glucuronide, molludistin, ursolic acid, and luteolin-7-Oglucuronide. Numerous monoterpenes and sesquiterpenes. Zn, Mn, and Na ions are present in plant extracts along with tannins, camphor, and other flavonoids such as luteolin, orientin, vicenin, and triterpene; urolic acid (Singh S. et al. 1996).

Clitoria ternatea is an annual twining plant that grows in Madagascar, China, the Philippines, and India. It is a member of the Fabaceae family (Pulok et al. 2008). In the past, the root was used to induce abortions, and the paste is frequently used to treat mucous issues, sore throats, and stomach inflammations. Nootropic, anticonvulsant, anti-inflammatory, and other properties have been demonstrated for C. ternatea (Jain N. et al. 2003; Parimala Devi B. et al. 2003). It increases acetylcholine levels in rats and enhances memory (Taranalli et al. 2003).

Hibiscus rosa-sinensis plant is actually a perennial shrub with a taproot. The leaf sizes range from 3.5 to 12 centimetres in length and 2 to 5.5 centimetres in width. The leaves are either oblong or oval in shape. The leaves are whole at their stalks and coarsely serrated at their tips. Flavor is sticky and thick. Flowers have an actinomorphic shape, are pedicellate, have five meristematic parts, and are fully formed. The Corolla has five red petals and a diameter of around 3 inches; it is widely accessible in many climates where it may thrive. Most hibiscus rosa sinensis plants sold as ornamentals are actually hybrids. Eight or more species from the east coast of Africa and islands in the Indian and Pacific oceans are thought to have hybridised to produce the current diverse range of cultivars (Goutam M. et al. 2018). The three primary parts of stems are teraxyl acetate, beta-sitosterol, and malvalic acid. There are a lot of beneficial substances in the root, such as glycosides, fixed oils, lipids, proteins, amino acids, and mucilage.

Materials and Methods

Experimental requirements

Fresh leaves of *Azadirachta Indica, Ocimum Sanctum,* and *Hibiscus rosa sinensis L.,* Fresh flowers of *Clitoria ternatea L.* Wistar rats, diethyl ether, Glibenclamide, streptozotocin, D-glucose, hematoxylin, eosin stains, ethanol, distilled water, Soxhlet apparatus.

Collection and authentication of plants materials

The fresh leaves of *Azadirachta Indica, Ocimum Sanctum, Hibiscus rosa sinensis L.,* and fresh flowers of *Clitoria ternatea L.* were collected from the Prayagraj region, UP East, India. There were authenticated by the botanist at Botanical Survey of India, Allahabad, UP with the reference no. 2023-24/534. The plant materials were washed, shade dried and crushed into coarse powders.

Extraction process

The dried leaves of *Azadirachta indica*, *Ocimum sanctum* and *Hibiscus rosa sinensis L.* were rendered into fine powder and extracted using hydroalcoholic (distilled water and ethanol; 1:1) solution through cold maceration process. The leaves of different plants were soaked in a beaker containing hydroalcoholic (distilled water and ethanol; 1:1) solution for 15 days with gradual stirrings, separately. Similarly,

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
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dried flowers of *Clitoria ternatea L.* was rendered into fine powders then soaked into a beaker containing hydroalcoholic (distilled water and ethanol; 1:1) solution for 15 days with gradual stirrings. Each beaker was mounted with aluminium foil. After the due time, each beaker's aluminium foil was removed and filtered using the cotton plug and finally with Whatman filter paper. The obtained slurry was made concentrated through evaporation using rotatory evaporator. Thus, the herbal extract was found in powder form and weight to calculate the % yield. All the extracts were kept in desiccator to keep the extract moisture free (Khan M.A. *et al.* 2020).

Acute toxicity studies

The study of acute toxicity studies was done according to the OECD 420 standards. Standard lab conditions were used to take care of Wistar rats that weight between 160 and 200 grams. Six animals were used in each group, and each got a single dose of poly herbal drug body weight. Before giving the drug, the animals must fast all night. After the polyherbal drug dosing, the recipient was not eaten for 3-4 hours. Each animal was observed at least once within the first thirty minutes after receiving a dose, multiple times within the first twenty-four hours (with a focus on the first four hours), and then regularly for fourteen days. During daily cage-side observations, alterations in the epidermis, fur, pupils, and nose mucous membranes, as well as the rate of breathing, circulation, and autonomic systems were observed (No OT. 423, 2002).

Group design

Rats were comprised of 7 groups of animals (n = 6) as follows:

Group 1: Non-diabetic, normal control animals- Vehicle-treated (0.5% CMC is used as a vehicle to ensure a uniform dispersal of plant extract material during oral administration)

Group 2: Diabetic animals - Untreated or non-intervened diabetic control

Group 3: Diabetic animals - Administered daily once with 2mg/kg Glibenclamide (standard anti-diabetic pharmaceutical)

Group 4: Diabetic animals - DM1 Administered daily once

Group 5: Diabetic animals - DM2 Administered daily once

Group 6: Diabetic animals - DM3 Administered daily once

Group 7: Diabetic animals - DM4 Administered daily once

Streptozotocin (STZ) -induced diabetes model

The pre-clinical in vivo efficacy screening of the polyherbal extracts was performed using a streptozotocin (STZ)- induced diabetes model in Wistar rats over 28 days. Briefly, diabetic symptoms were induced in acclimatized Wistar rats (170-200 g) by intra- peritoneal administration of STZ at 45mg/kg. The Wistar rats that developed diabetic symptoms (blood glucose level at fasting >250mg/dl) were included in the study and divided into specific groups. (All herbal extracts were administered once daily by oral gavage at three different doses.

Evaluation of anti-diabetic activity

Oral glucose tolerance test

The animals were divided into five groups of six rats each and kept on a fast for eighteen hours with unrestricted access to water. Oral gavage of D-glucose solution (2g/kg) was administered to all animals. 2nd, 3rd and 4th groups were received polyherbal extract by mouth, 30 minutes before the glucose load. Control animals were received vehicles. Under ether anaesthesia, blood was drawn from each animal's retro-orbital plexus at 0, 30, 90, and 120 min following the glucose challenge. Positive control glibenclamide (2mg/kg) was given to the fifth group. The OPTIMA S Semi Biochemistry analyser (LAB INDIA) and GOD-POD kit was utilized in the assessment of blood glucose levels. (Sumalatha G. *et al.,* 2010).

Blood sugar level

Seven blood glucose readings are taken at 0, 5, 10, and 15 days following the initiation of medication therapy. Dr. Morepen blood glucometer is used to estimate blood glucose levels after a blood sample is taken from the puncturing tail vein. This process is genuine and simple.

Lipid profile

The rats were compassionately put to death with an overdose of ether at the end of the experiment. After the rat was sacrificed, blood samples were taken straight from the heart using the heart puncture procedure. The collected blood samples were then transferred into plain tubes for subsequent biochemical analyses, such as the estimation of lipid profiles (HDL, LDL, TG, and TC) (Tiwari D.D. *et al.* 2024).

Body Weights

The weight of each rat was documented upon their allocation to respective groups, at the initiation of therapy, on a weekly basis afterwards, and at the time of necropsy. The weights of rats in the reversal group were documented on a weekly basis throughout the post treatment period and also at the time of necropsy.

Food Consumption pattern

The researchers documented the amount of food ingested by the rats in each cage on the day treatment began, and continued to do so on a weekly basis. The quantification of food consumption per rat was determined by measuring the quantity of food provided to each cage and the remaining

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
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amount after a 96-hour period, in conjunction with the number of rats that survived within each respective cage. The weekly food consumption of the rats in the reversal group was documented throughout the post-treatment period (Wanjiku N K *et al.* 2017).

Haematological Estimations

The following haematological parameters were estimated with their units of measurement.

- Haemoglobin (Hb) (g/dl)
- Packed cell volume (%)
- Total red cell count
- Total white cell count
- Total Platelets Count
- Mean corpuscular volume (fl)
- Mean corpuscular haemoglobin (pg)
- Mean corpuscular haemoglobin concentration (g/dl)
- Clotting time measurement
- Reticulocytes count
- Differential WBC counts
- Neutrophils %
- Lymphocytes %
- Eosinophils %
- Monocytes %

Urine Analysis

Every individual rat was accommodated in the designated cage. Urine samples were obtained throughout a 4-hour time frame. If the urine sample did not give sufficient volume for thorough analysis, the rat was administered 1-2 cc of drinking water via oral gavage. Subsequently, the rat was placed in the urine collection cage for further housing. During this era, provisions of food and water were not made available. Termination Studies: The necropsy examination refers to the postmortem examination of an animal's body in order to determine the cause of death and after undergoing a treatment period of 28 days and/or a reversal period of 14 days, any rats that survived were euthanized via exsanguination under carbon dioxide anaesthesia. Subsequently, a thorough necropsy was performed on each rat. The post-treatment necropsy was conducted in a staggered manner, taking place on either day 29 or day 30.

Weight of organs

After being sacrificed, all of the mice from the various groups are cut. Organs such the brain, kidney, and lever were weighed independently to verify the effect on other organs as well.

Histopathological examination

The tissues underwent microscopic inspection via a conventional histopathology method. At the conclusion of the investigation, the animals were euthanized and subjected to dissection to remove the necessary organs, including the liver, pancreas, kidney, and heart. These organs were subsequently preserved by immersion in a 10% formalin solution. The tissue sections, measuring 5-6µm in thickness, prepared by cutting and subsequently stained using haematoxylin and eosin stains. The study's last day involved the animals' sacrifice, the dissection of necessary organs such the liver and pancreas, and their fixation in 10% formalin. tissues examined under a microscope using a typical histological technique. Hematoxylin and eosin stains were applied to 5–6 µm tissue sections after they were sliced. The tissue pieces were rehydrated by exposing them to alcohol concentrations ranging from 100% to 30%. Hematoxylin was then used to stain the sections. The sections were then stained with eosin after being dried using progressively higher alcohol concentrations. The sections were then put on the slide for microscopic inspection after being treated with DPX (diphenylxylene). These tissues were sectioned at five micrometers, embedded in paraffin wax, and stained with hematoxylin and eosin. All animals from the control and high dose level groups were killed at termination after a thorough histological analysis of the designated list of tissues, which included all macroscopically aberrant tissues. Additional tissues, of animals from other groups, which exhibited gross pathological changes at necropsy, were also subjected to histopathological evaluation. (Harini, V.S *et al.* 2024).

Results and Discussion

Percentage yield

The percentage yield of dried leaves of *Azadirachta indica*, *Ocimum sanctum*, flowers of *Clitoria ternatea* and leaves of *Hibiscus rosa sinensis* were found as 47.26%, 52.64%, 49.34% and 54.12%, respectively when extracted using hydro-alcoholic solvent.

Standardization of herbal extracts

The floral crude extract, DW10, did not have a significant impact on the blood glucose levels of fasted normoglycemic mice in terms of its hypoglycemic action. It was discovered that the extract's effect was dependent on the dosage. The crude extract exhibited a delayed although notable reduction in blood sugar levels, indicating that the extract's ability to lower blood sugar increased over time. The maximum effect was observed at the 6th hour. This suggests that the active components in the extract require sufficient time to reach a sufficient concentration at the intended location, as a comparable trend was observed with other plants that exhibit anti-diabetic properties (Kim J S *et al.* 2000).

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
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Standardization of herbal extracts

Table 1: Organoleptic characteristics

Hydro-alcoholic Leaves Extract	Organoleptic characteristics		
	Appearance	Color	Odor
Azadirachta indica	Powder	Dark green	Characteristics
Ocimum sanctum	Powder	Dark green	Characteristics
Clitoria ternatea	Powder	Dark green	Characteristics
Hibiscus rosa sinensis	Powder	Dark green	Characteristics

Acute toxicity studies

Table 2. Behavioral Observation of polyherbal formulations (DM-01, DM-02, DM-03, DM-04) in acute toxicity studies

Gross activity	Behavioral observation of polyherbal formulations (DM-01, DM-02, DM-03, DM-04) in acute toxicity studies					
	Epidermis	Fur	Pupils	Breathing	Circulation	Mortality
30 min	_	_	_	+	+	_
1hr	_	_	_	+	+	_
2hr	_	_	_	+	+	_
3hr	_	_	_	+	+	_
4hr	_	_	_	+	+	_
24hr	_	_	_	+	+	_
Day 2	_	_	_	+	+	_
Day 3	_	_	_	+	+	_
Day 4	_	_	_	+	+	_
Day 5	_	_	_	+	+	_
Day 6	_	_	_	+	+	_
Day 7	_	_	_	+	+	_
Day 8	_	_	_	+	+	_
Day 9	_	_	_	+	+	_
Day 10	_	_	_	+	+	_
Day 11	_	_	_	+	+	_
Day 12	_	_	_	+	+	_
Day 13	_	_	_	+	+	_
Day 14	_	_	_	+	+	_

Table 3: Oral glucose tolerance test of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Blood sugar level (mg/dl) in OGTT			
	0 min	30 min	90 min	120 min
Normal saline	82.54±0.23	85.30±0.64	79.34±0.16	88.45±0.11
STZ (45mg/kg, i. p.)	79.26±0.54	128.23±0.10	176.63±0.21	227.56±0.23
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	84.17±0.75	92.17±0.34	104.16±0.29	109.34±0.20
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	86.61±0.35	123.22±0.24	149.21±0.56	126.19±0.56
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	83.56±0.23	114.17±0.39	139.34±0.15	121.34±0.19
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	92.34±0.11	110.34±0.14	128.45±0.26	114.12±0.65
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	78.16±0.40	121.53±0.12	146.31±0.10	123.68±0.10

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Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
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Estimation of blood sugar level

Table 4: Estimation of blood sugar level of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Blood sugar level (mg/dl)			
	0 day	5 days	10 days	15 days
Normal saline	82.54±0.23	85.30±0.64	79.34±0.16	88.45±0.11
STZ (45mg/kg, i. p.)	79.26±0.54	108.34±0.21	159.43±0.15	193.24±0.23
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	84.17±0.75	89.45±0.36	93.25±0.44	95.43±0.23
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	86.61±0.35	108.34±0.16	124.56±0.19	118.30±0.11
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	83.56±0.23	102.35±0.12	119.26±0.45	110.12±0.56
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	92.34±0.11	105.21±0.54	116.29±0.67	107.52±0.14
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	78.16±0.40	98.15±0.64	127.45±0.39	115.10±0.67

Estimation of lipid profile

Table 5: Estimation of serum Triglyceride of control, disease control and polyherbal formulations treated Wistar rats

	TG (mg/dl)				
Treatment	Day 1	Day 3	Day 5	Day 10	Day 15
Normal saline	134.14±0.67	142.10±0.45	139.56±0.18	145.72±0.53	136.15±0.68
STZ (45mg/kg, i. p.)	157.55±0.25	172.45±0.10	184.58±0.19	217.16±0.27	243.29±0.34
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	146.18±0.72	152.67±0.26	159.67±0.15	164.45±0.11	167.45±0.23
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	144.45±0.28	162.13±0.62	169.24±0.71	176.67±0.20	187.34±0.14
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	143.27±0.14	159.46±0.17	167.14±0.75	174.13±0.58	183.56±0.20
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	138.23±0.67	156.17±0.56	166.45±0.15	171.40±0.11	179.16±0.54
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	142.78±0.12	164.67±0.25	172.11±0.57	181.35±0.18	189.45±0.71

Estimation of total cholesterol

Table 6: Estimation of total cholesterol level of control, disease control and polyherbal formulations treated Wistar rats

	TC (mg/dl) level				
Treatment	Day 1	Day 3	Day 5	Day 10	Day 15
Normal saline	49.65±0.21	51.37±0.10	54.13±0.56	52.32±0.43	56.46±0.19
STZ (45mg/kg, i. p.)	66.12±0.45	77.24±0.68	89.23±0.74	97.45±0.13	112.64±0.24
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	51.67±0.23	56.23±0.89	63.31±0.84	69.25±0.76	73.51±0.85
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	58.23±0.57	64.87±0.24	72.64±0.14	81.62±0.40	92.13±0.65
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	56.78±0.32	62.68±0.20	68.76±0.23	78.62±0.16	89.75±0.10
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	54.67±0.23	61.89±0.23	67.25±0.85	77.35±0.60	86.67±0.21
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	59.23±0.62	66.11±0.46	73.34±0.54	84.26±0.72	91.25±0.49

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Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
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Body weight- determination

Table 7. Body weight determination of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Body weight (g)	
	Before	After
Normal saline	180	195
STZ (45mg/kg, i. p.)	200	245
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	170	182
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	200	218
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	200	216
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	200	215
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	200	220

Food consumption pattern

Table 8: Food consumption (g/day) pattern of control, disease control and polyherbal formulations treated Wistar rats

	Food consumption (g/day)				
Treatment	**Day 1**	**Day 3**	**Day 5**	**Day 10**	**Day 15**
Normal saline	19.27±0.56	17.20±0.18	20.13±0.27	21.56±0.11	18.12±0.16
STZ (45mg/kg, i. p.)	32.16±0.23	37.24±0.28	41.19±0.34	43.10±0.28	46.16±0.49
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	21.18±0.58	25.20±0.56	28.11±0.46	31.76±0.29	33.14±0.62
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	27.48±0.13	30.24±0.67	32.19±0.65	35.26±0.19	37.64±0.23
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	25.17±0.48	29.24±0.67	32.20±0.64	34.27±0.62	36.19±0.57
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	24.67±0.23	27.89±0.23	29.25±0.85	32.35±0.60	34.67±0.21
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	26.23±0.62	28.11±0.46	31.34±0.54	34.26±0.72	38.25±0.49

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
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Urine analysis

Table 9. Urine analysis of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Urine volume (ml/ 5 hr)
Normal saline	1.43±1.17
STZ (45mg/kg, i. p.)	2.63±1.47
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	1.56±1.32
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	1.78±1.32
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	1.73±1.20
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	1.69±1.34
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	1.82±1.59

Weight of organs

Table 10. Weight of organs of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Weight of organs (g)		
	Spleen (g)	Kidney (g)	Liver (g)
Normal saline	0.63±0.49	0.72±0.14	7.43±0.56
STZ (45mg/kg, i. p.)	0.52±0.74	0.61±0.46	6.25±0.49
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	0.65±0.40	0.79±0.12	7.36±0.20
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	0.58±0.23	0.68±0.19	6.83±0.35
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	0.60±0.17	0.70±0.45	6.94±0.10
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	0.62±0.40	0.73±0.78	7.26±0.45
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	0.57±0.49	0.66±0.20	6.83±0.32

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
DOI:10.26502/fjppr.0125

Haematological Parameters

Table 11. Estimation of haemoglobin (g/dl) of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Haemoglobin (g/dl)
Normal saline	12.46±0.17
STZ (45mg/kg, i. p.)	8.12±0.35
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	15.29±0.14
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	13.64±0.29
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	12.31±0.56
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	14.45±0.10
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	13.20±0.14

Table 12. Estimation of RBC (10^6/mm^3) of control, disease control and polyherbal formulations treated Wistar rats

Treatment	RBC (10^6/mm^3)
Normal saline	9.14±0.63
STZ (45mg/kg, i. p.)	4.27±0.16
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	8.56±0.20
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	7.56±0.20
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	6.74±0.23
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	7.84±0.17
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	7.20±0.53

Table 13. Estimation of WBC (10^6/mm^3) of control, disease control and polyherbal formulations treated Wistar rats

Treatment	WBC (10^3/mm^3)
Normal saline	14.57±0.20
STZ (45mg/kg, i. p.)	5.43±0.62
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	13.79±0.67
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	12.83±0.18
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	11.74±0.52
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	13.25±0.17
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	12.39±0.24

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
DOI:10.26502/fjppr.0125

Table 14. Packed cell volume (PCV) (%), Platelet count (10^3/mm^3) of control, disease control and polyherbal formulations treated Wistar rats

Treatment	PCV (%)	Platelet count (10^3/mm^3)
Normal saline	54.37±0.16	1284
STZ (45mg/kg, i. p.)	34.58±0.27	576
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	52.14±0.63	1106
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	45.72±0.28	924
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	46.29±0.11	974
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	49.10±0.56	1032
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	44.12±0.45	968

Table 15. Estimation of MCV (fl) and MCH (pg) of control, disease control and polyherbal formulations treated Wistar rats

Treatment	MCV (fl)	MCH (pg)
Normal saline	64.10±0.53	20.58±1.24
STZ (45mg/kg, i. p.)	43.27±0.14	13.45±1.19
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	61.42±0.20	18.26±1.43
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	54.34±0.59	16.45±1.32
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	55.92±0.45	16.23±1.18
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	58.41±0.56	17.12±1.49
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	53.21±0.37	15.39±1.13

Table 16. Estimation of MCHC (g/dl) and Clotting time (CT) of control, disease control and polyherbal formulations treated Wistar rats

Treatment	MCHC (g/dl)	CT (sec)
Normal saline	33.27±0.63	230.34±1.56
STZ (45mg/kg, i. p.)	24.17±0.45	110.45±1.32
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	32.54±0.23	220.23±1.45
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	30.21±0.49	200.56±1.17
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	29.73±0.10	210.34±1.26
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	31.56±0.21	215.23±1.54
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	30.62±0.11	210.41±1.68

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
DOI:10.26502/fjppr.0125

Table 17. Estimation of RT (%) and Neutrophils (%) of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Reticulocytes count (RT)	Neutrophils (%)
Normal saline	2.94±0.14	24.67±0.19
STZ (45mg/kg, i. p.)	1.06±0.27	17.34±0.56
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	2.73±0.60	22.82±0.15
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	2.39±0.53	19.37±0.64
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	2.47±0.69	19.84±0.39
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	2.51±0.18	20.34±0.11
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	2.24±0.71	18.64±0.37

Table 18. Estimation of Lymphocytes (%) and Monocytes (%) of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Lymphocytes %	Monocytes %
Normal saline	78.94±0.14	3.25±0.53
STZ (45mg/kg, i. p.)	59.06±0.27	0.29±0.37
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	75.73±0.60	2.91±0.34
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	71.39±0.53	2.47±0.16
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	70.47±0.69	2.31±0.57
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	72.51±0.18	2.73±0.29
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	70.24±0.71	2.64±0.30

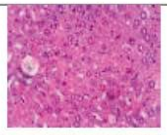
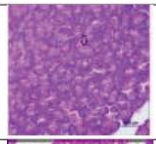
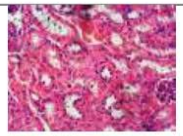
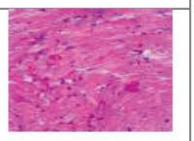
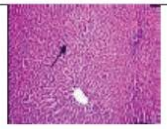
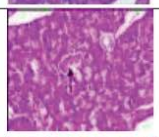
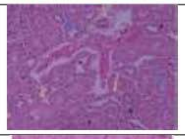
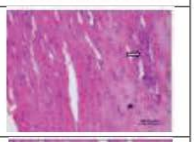
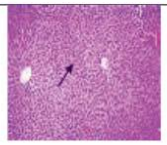
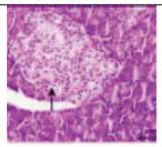
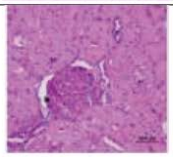
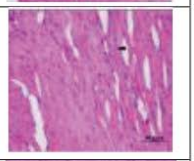
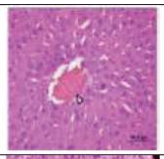
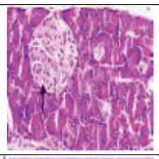
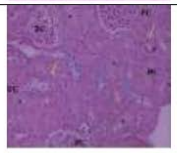
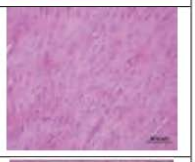
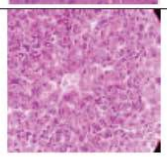
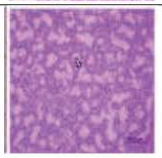
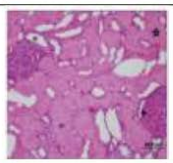
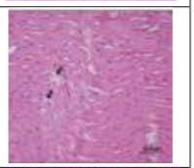
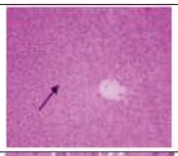
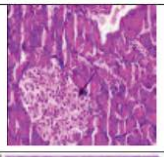
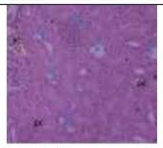
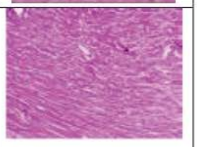
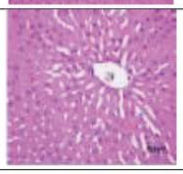
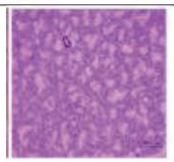
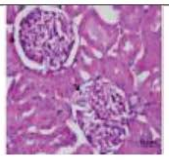
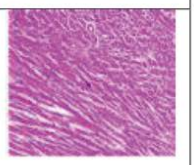
Table 19. Estimation of Eosinophils (E) % of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Eosinophils (E) %
Normal saline	1.82±0.64
STZ (45mg/kg, i. p.)	0.37±0.46
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)	1.63±0.31
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)	1.47±0.56
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)	1.51±0.23
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)	1.56±0.18
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)	1.46±0.23

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
DOI:10.26502/fjppr.0125

Histopathological examination

Table 20. Photographs of liver, pancreas, kidney and heart of control, disease control and polyherbal formulations treated Wistar rats

Treatment	Liver	Pancreas	Kidney	Heart
Normal saline				
STZ (45mg/kg, i. p.)				
STZ (45mg/kg, i. p.) + Glibenclamide (2mg/kg)				
STZ (45mg/kg, i. p.) + DM-01 (200mg/kg, p. o.)				
STZ (45mg/kg, i. p.) + DM-02 (200mg/kg, p. o.)				
STZ (45mg/kg, i. p.) + DM-03 (200mg/kg, p. o.)				
STZ (45mg/kg, i. p.) + DM-04 (200mg/kg, p. o.)				

The plant extract exhibited a relatively delayed beginning of hypoglycemic activity compared to the usual medication. The crude extract may possess an insulinomimetic action or elicit insulin production from β-cells. Flavonoids and Tannins, which are compounds derived from medicinal plants, have been found to enhance the release of insulin. Ultimately, the antioxidant and antidiabetic response of the dried leaves of *Azadirachta indica*, *Ocimum sanctum*, flowers of *Clitoria ternatea* and leaves of *Hibiscus rosa sinensis* may be attributed to lower and sustain the cellular oxidation processes and its ability to enhance the responsiveness of tyrosine kinase to insulin. In order to confirm the mechanism of action, it requires molecular investigations to determine the specific receptor subtypes these polyherbal formulations (DM-01, DM-02, DM-03, DM-04) target and to explore methods for enhancing its binding efficiency.

The leaves of *Azadirachta indica*, *Ocimum sanctum*, flowers of *Clitoria ternatea* and leaves of *Hibiscus rosa sinensis* are versatile plant species which have been utilized in the management of various ailments since ancient era. These are rich sources of chemical compounds that are effective against a wide range of illnesses. Studies have demonstrated that these crude plant extracts are a reliable source of bioactive compounds. Therefore, if the plants examined here are subjected to additional research, they could be a source of useful drugs. Study showed that *Azadirachta indica*, *Ocimum sanctum*, *Clitoria ternatea* and *Hibiscus rosa sinensis* have enormous antioxidant and anti-diabetic role as compared with acarbose, ascorbic acid and glibenclamide.

Singh S and Dr. Deva V., J Pharm Pharmacol Res 2026
DOI:10.26502/fjppr.0125

Conclusion

The research suggests that DM-01, DM-02, DM-03 and DM-04 polyherbal formulations are an effective in the management of diabetes but it's mode of action is still unknown. So further research may carried-out to confirm its mode of action and optimum doses required in management of DM. Furthermore, the current study proposes the identification and isolation of the key chemical component that played a crucial role in this particular healing process. Subsequently, a suitable dosage form will be formulated to enhance patient adherence and the extent to which the drug is absorbed and available for use in the body.

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