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ORIGINAL ARTICLE **OPEN ACCESS**

Hypoglycemic Effect of Methanolic Extract of *Callicarpa macrophylla* Fruits on STZ Induced Diabetic Rats

Talha Jawaid[1,*], Mehnaz Kamal[2], Priyanka Maddheshiya[1]
[1]Department of Pharmacology, Hygia Institute of Pharmaceutical Education and Research, Ghaila Road, Lucknow-226002 (U. P.), India.
[2]Department of Pharmaceutical Chemistry, College of Pharmacy for Girls, Prince Sattam Bin Abdulaziz University, P.O. Box- 173, Al-Kharj 11942, Kingdom of Saudi Arabia.
*E-mail: talhajawaid78@gmail.com

ABSTRACT
The present study provides a scientific assessment for the hypoglycemic effect of methanolic extract of Callicarpa macrophylla fruits (MECM) on STZ-induced diabetic rats. Diabetes was induced in Wistar rats by intraperitoneal injection of STZ at a dose of 55 mg/kg body weight for 7 days. Methanolic extract of Callicarpa macrophylla fruits [100 and 200 mg/kg b.w., (p.o.)] was administered to STZ-induced diabetic rats for 14 days. The effect of MECM on blood glucose, serum lipid profile and HbA_{1c} was studied in diabetic rats. Metformin was used as a reference hypoglycemic drug. In OGTT, oral administration of MECM (200 mg/kg b. w., $P < 0.05$) and metformin (11.3 mg/kg b.w., $P < 0.01$) showed significant decrease in the blood glucose levels at 60 min. The administration of MECM (100 and 200 mg/kg b.w.) resulted in a significant dose-dependent decrease ($P < 0.001$) in blood glucose level in STZ-induced Type 2 diabetic rats. Further MECM showed antihyperlipidemic activity as evidenced by significant decrease ($P < 0.001$) in serum TC, TG, LDL-C, VLDL-C levels coupled together with elevation of HDL-C level ($P < 0.001$) in STZ-induced Type 2 diabetic rats. HbA_{1c} significantly decreased with MECM (200 mg/kg b.w. and 100 mg/kg b.w., $P < 0.01$) in STZ-induced diabetic rats. The results suggest that the methanolic extract of Callicarpa macrophylla fruits possess a promising dose-dependent hypoglycemic effect on STZ-induced diabetes.
KEY WORDS: *Hypoglycemic activity, Callicarpa macrophylla fruits, Streptozotocin, serum lipid profile, HbA_{1c}.*

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INTRODUCTION

Diabetes mellitus is complex and diverse group of disorders that disturbs the metabolism of carbohydrates, protein and fat. It results from lack of insulin secretion or reduced sensitivity of the insulin receptors to insulin [1]. Diabetes mellitus is a common irreparable metabolic disorder whose overall incidence continues to increase due to population aging and enhancement in the quality of life [2]. The ailment may result into the development of further anatomic and metabolic disturbances among which is hypercholesterelaemia, lipemia, loss of weight, ketosis, gangrene, arteriosclerosis, pathologic changes in eyes, nephropathy, neuropathy and coma [3]. Diabetes was estimated to influence 177 million people worldwide in 2000 and this figure is projected to amplify to 300 million by 2025 [4]. Statistical projection about India suggests that the number of Diabetic patients will rise from 15 million in 1995 to 57 million in 2025 making it the country with the maximum number of Diabetic in the world [5]. This terrible disease is found in all parts of world and is becoming a serious hazard to mankind. There are a lot of synthetic drugs to control and treat diabetics, but total recovery from diabetes has not been reported yet. Plants are provided with a potential source of hypoglycemic drugs and they are widely used in several conventional system of medicine. The effects of these plants may interrupt the development of diabetic complications and correct the metabolic abnormalities [6].

Callicarpa macrophylla Vahl. (Family: Verbenaceae) is an important less common medicinal plant of the lower warm valleys of the Himalaya and is commonly known as Priyangoo or Daya. It is a perennial, deciduous shrub attaining 2.5 meter in height [7]. Fruits and flowers are useful in rheumatoid arthritis, anorexia, asthma, catarrh, headache, flatulence, colic diarrhoea and dysentery, foul ulcer, skin disease, burning sensation, diabetes, vomiting, excessive sweating, fever and general debility. Leaves are useful in arthralgia and gout. Root is chewed to allay rashes on the tongue [8]. This plant contains diterpenoid

(16α,17-Isopropylideno-3-oxo-phyllocladane) [9], diterpene (Calliterpenone) [10], 3β,16α,17-trihydroxy-phyllocladane [11], flavanoids (β-sitosterol, luteolin, ursolic acid and apigenin) [12], 16,17-dihydroxy-kauranoids [13], fatty acids and other constituents [14].

However, in spite of the various bioactive phytoconstituents and diverse medicinal activities attributed to this plant, no biochemical studies have been carried out to shed light on the role of this plant in diabetes. In the light of the above, the present study was undertaken to investigate the effect of methanolic extract of *Callicarpa macrophylla* fruits (MECM) on blood glucose in STZ-induced diabetic rat model, serum lipid profile and HbA_{1c} estimation.

MATERIALS AND METHODS

Plant material

Fresh fruits of *Callicarpa macrophylla* were collected from Central Institute of Medicinal and Aromatic Plant, Lucknow, India and its identification and authentication were done from National Botanical Research Institute, Lucknow-226001, India (Ref. No: NBRI/CIF/262/2011). The aerial parts were cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction.

Preparation of extract of *Callicarpa macrophylla* fruits

The methanolic extract of *Callicarpa macrophylla* fruits was prepared by soxhletion. The powdered fruit was defatted with petroleum ether (60-80 °C) in a Soxhlet apparatus for 48 hr. The defatted marc was repeatedly extracted with methanol in a Soxhlet apparatus, for 24 hr. The extract was cooled at room temperature, and evaporated to dryness under reduced pressure in a rotary evaporator. It was stored in refrigerator and kept in desiccators few hours before use [15].

Preliminary phytochemical screening

An attempt was made to observe the presence and absence of diverse phytochemical constituents in MECM, viz., alkaloids (Wagner's test), flavonoids (Shinoda test), tannins (Ferric chloride test), steroids and triterpenes (Liberman-Burchard's test) and saponins (Foam test) according to standard methods [16].

Chemicals

Streptozotocin was purchased from Himedia (Mumbai, India). Triglycerides, total cholesterol, and HDL kits were purchased from Span Diagnostics Pvt. Ltd. (Gujarat, India). All other chemicals used were of analytical grade.

Animals

The experiments were carried out with male Wistar rats weighing 200-250 g obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow, India. Research on experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care (1088/07/CPCSEA). They were kept in polyacrylic cages (22.5 cm^2 × 37.5 cm) and were maintained under standard housing conditions (room temperature, 24 – 27 °C, and humidity, 60 - 65%) with a 12-h light/12-h dark cycle. Food and water were available *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures [Hygia/M.Pharm./17/2011-12].

Induction of experimental diabetes in rats

Streptozotocin induced diabetes

Streptozotocin (STZ) induced hyperglycemia was described as a useful experimental model to study the activity of hypoglycemic agents. Animals were fasted for overnight (deprived of food for 16 hours and allowed free access to water). Diabetes was induced in Wistar rats by intraperitoneal injection of STZ dissolved in freshly prepared 0.1 M sodium citrate buffer (pH 4.5), at a dose of 55 mg/kg body weight in five groups of 6 rats for 7 days. The control rats were received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 7 days time for the development of diabetes, the rats with diabetes having glucosuria and hyperglycemia; blood glucose levels ≥ 300 mg/dl were selected and used for the study [17].

Experimental design

Acute Toxicity Study

The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioural, neurological toxicity and mortality for 14 days [18].

Assessment of oral glucose tolerance test (OGTT)

OGTT of plant extracts was carried out in overnight fasted normal rats, which were equally divided into four groups of six rats each. Group of normal control received only normal saline and diabetic control received only glucose 1.75 gm/kg *p.o*. Standard group received 11.3 mg/kg b.w. of reference drug Metformin suspended in the vehicle, while extract treated group were administered with MECM (200 mg/kg, *p.o.*). Thereafter, following 30 min post extract administration all the animals were fed with glucose (1.75 gm/kg *p.o.*). Blood samples were collected from tail vein prior to dosing and then at 30, 60 and 90 min after glucose administration. The fasting blood glucose level was analyzed by trinder's glucose oxidase method using spectrophotometer [19].

Group	Treatment
Group 1- Normal control	Normal undiseased animals who only received normal saline.
Group 2- Diabetic control	Diseased animals which received glucose 1.75 gm/kg *p.o.*
Group 3- Standard treated	Diseased animals who first received Metformin 11.3 mg/kg and then glucose (2 hrs. later) 1.75 gm/kg, *p.o.*
Group 4- Extract treated	Diabetic rats received MECM 200 mg/kg b.w., *p.o.*

Assessment of hypoglycemic activity of MECM in STZ-induced diabetic rats

The animals were divided into five groups and each group consisted of six rats (*n*=6).

Group	Treatment
Group 1- Normal control	Normal rats + vehicle
Group 2- Diabetic control	Streptozotocin (55 mg/kg b.w., *i.p.*) + vehicle
Group 3- Extract treated	Streptozotocin (55 mg/kg b.w., *i.p.*) + MECM (100 mg/kg b.w., *p.o.*) for 14 days
Group 4- Extract treated	Streptozotocin (55 mg/kg b.w., *i.p.*) + MECM (200 mg/kg b.w., *p.o.*) for 14 days
Group 5- Standard treated	Streptozotocin (55 mg/kg b.w., *i.p.*) + Metformin (11.5 mg/kg, *p.o.*)

After the induction of diabetes treatment was continued for 14 days. Blood glucose level of extract treated groups, normal control, diabetic control and standard treated groups were taken on day 0, 7th and 14th day of post treatment. On the 14th day of treatment, serum lipid profile and HbA_{1c} was performed.

Biochemical estimations

Blood glucose estimation

Blood samples were collected from the tail vein in anesthetized rats using ether solution inhalation. Blood glucose levels were determined by the glucose oxidase method using a reflective glucometer [20].

Serum lipid profile estimation

After 14 days of treatment, rats were anesthetized by ether, their blood was collected from heart in ordinary tubes and were allowed to clot, and then the clotted blood were centrifuged at 2500 *g* for 5 min to separate the serum. Serum TC, TG and HDL-C was estimated by using commercially available kits (Span Diagnostics, Gujarat, India). VLDL-C and LDL-C were calculated by Friedewald's formula [21,22].

VLDL = Triglycerides / 5

LDL = Total cholesterol – (HDL-C + VLDL-C)

HbA_{1c} estimation

After collecting blood samples in heparinised tubes, centrifugation was performed at 1000 *g* for 15 min. to remove the buffy coat. The packed cells obtained at the bottom were washed thrice with phosphate buffer saline (0.9% NaCl in 0.01 M phosphate buffer, *p*H 7.4). A known amount of erythrocytes was lysed with hypotonic phosphate buffer. The hemolysate was obtained after removing the cell debris by centrifugation at 3000 g for 15 min and used for determination. [24] To the erythrocytes (0.5 ml) from whole blood, 0.125 ml of distilled water and 0.125 ml of carbon tetrachloride was added, mixed well and centrifuged. The supernatant was separated and its haemoglobin concentration was adjusted to 10% with distilled water. To 2 ml of hemosylate, 1ml of 0.3 N oxalic acid was added in stoppered test tubes and heated at 100 ºC in a water bath for 60 min. After cooling the contents, 1 ml of 40% trichloroacetic acid was added, shaken well and centrifuged.

To 2 ml of supernatant pipette out into another set of test tubes, 0.5 ml of 0.05 M thiobarbituric acid was added and incubated at 37 ºC for 40 min. A blank with 2 ml of distilled water was treated similarly. The resulting yellowish colour was seen in a spectrophotometer at 443 nm. Concentration of HbA_{1c} was calculated on the assumption that 1% HbA_{1c} corresponds to an absorbance of 0.029 at 443 nm [23].

Statistical analysis

The experimental results were expressed as mean ± S.E.M. and were statistically analyzed using one-way ANOVA followed by Dunnet's multiple test. *P* values < 0.001 were considered statistically significant.

RESULTS

Effect of MECM (200 mg/kg b.w.) on oral glucose tolerance test (OGTT) in rats

In the OGTT, the highest increase in blood glucose was observed in both treated and untreated rats after 30 min of oral administration of glucose. At 60 min, oral administration of MECM (200 mg/kg b. w., $P < 0.05$) and metformin (11.3 mg/kg b.w., $P < 0.01$) showed significant decrease in the blood glucose levels respectively (Table I).

Table I: Hypoglycemic effect of MECM (200 mg/kg b.w.) on OGTT in rats.

Groups	Dose	Blood glucose level (mg/dl)			
		0 minute	30 minute	60 minute	90 minute
Group 1- Normal control	-	71.25 ± 0.23	72.5 ± 0.32	73 ± 0.35	73.75 ± 0.42
Group 2- (Standard treated)	11.3 mg/kg	71.75 ± 0.42	105 ± 0.64	65.75 ± 1.71**	65.75 ± 1.04***
Group 3- Extract treated	200 mg/kg	73.25 ± 0.55	113.75 ± 1.26	71.75 ± 0.42*	68 ± 1.10**

Each value is mean ± S.E.M of 6 rats in each group. *P < 0.05, **P < 0.01 and ***P < 0.001 in comparison to normal control.

Effect of MECM (100 and 200 mg/kg b.w.) on blood glucose level in STZ-induced diabetic rats

The effect of MECM on the blood glucose levels of diabetic rats is given in Table 2. STZ-induced diabetic rats showed significant increase ($P < 0.001$) in the levels of blood glucose when compared to the vehicle control. Oral administration of MECM at 100 and 200 mg/kg b. w. showed significant decrease ($P < 0.001$) in the blood glucose levels on 7th and 14th day, but not on day 1 (Table II). Standard drug metformin showed significant decrease ($P < 0.001$) in the blood glucose level. On 14th day, MECM at the dose of 200 mg/kg body weight in diabetic rats was more effective than 100 mg/kg, indicating high efficacy at higher dose.

Table II: Hypoglycemic effect of MECM (100 and 200 mg/kg b.w.) on blood glucose level in STZ-induced diabetic rats.

Groups	Dose	Blood glucose level (mg/dl)		
		1st Day	7th Day	14th Day
Group 1- Normal control	-	76 ± 0.61	105.4 ± 0.81	99.4 ± 0.68
Group 2- Diabetic control	150 mg/kg	350 ± 2.75***	373.2 ± 1.69***	379 ± 1.98***
Group 3- Extract treated	100 mg/kg	352.6 ± 4.13	271.6 ± 4.6	148.2 ± 1.59
Group 4- Extract treated	200 mg/kg	352.2 ± 3.36	235 ± 1.89	123.6 ± 0.7
Group 5- Standard treated	11.3 mg/kg	364.8 ± 4.44	196.6 ± 1.1	109.6 ± 0.74

Each value is mean ± S.E.M of 6 rats in each group. ***P < 0.001 compared with control, $P < 0.001$ compared with diabetic control, $P < 0.001$ compared with 100 mg/kg MECM.

Effect of MECM (100 and 200 mg/kg b.w.) on serum lipid profile in STZ-induced diabetic rats

MECM at a dose of 100 mg/kg b.w. and 200 mg/kg b.w., significantly decreases ($P < 0.001$) TC, TG, LDL-C and VLDL-C. Metformin 11.3 mg/kg, b.w. significantly decreases ($P < 0.001$) TC, TG, LDL-C and VLDL-C. HDL-C increased significantly ($P < 0.001$) in diabetic rats treated with MECM 100 mg/kg b.w., 200 mg/kg b.w., as well as in metformin 11.3 mg/kg, b.w. (Table III).

Table III: Antihyperlipidemic effect of MECM (100 and 200 mg/kg b.w.) on total cholesterol, triglyceride, HDL-C, LDL-C and VLDL-C levels in STZ-induced diabetic rats.

Lipid level	Control	Diabetic control	Diabetic + MECM (100 mg/kg b.w.)	Diabetic + MECM (200 mg/kg b.w.)	Diabetic + Metformin (11.3 mg/kg b.w.)
TC (mg/dl)	81.53 ± 6.43	114.35 ± 1.65*	99.99 ± 0.51***	95.44 ± 0.45***	90.25 ± 0.43***
TG (mg/dl)	80.86 ± 0.74	133.32 ± 1.41***	107.41 ± 1.16	95.96 ± 1.01	88.88 ± 0.88
HDL-C (mg/dl)	45.36 ± 0.346	26.66 ± 0.530***	32.01 ± 0.21	37.96 ± 0.29	41.09 ± 0.31
LDL-C (mg/dl)	20.00 ± 0.22	61.03 ± 4.15	46.5 ± 0.54	38.29 ± 0.19	31.38 ± 0.13
VLDL-C (mg/dl)	16.17 ± 0.15	26.66 ± 0.28	21.48 ± 0.23	19.19 ± 0.20	17.78 ± 0.18

Each value is mean ± SEM of 6 rats in each group. ***P < 0.001 compared with control, P < 0.001 compared with diabetic control, and P < 0.05 compared with 100 mg/kg MECM.

Effect of MECM (100 and 200 mg/kg b.w.) on the level of plasma HbA_{1c} in STZ-induced diabetic rats

The effect of MECM on the level of plasma HbA_{1c} in STZ-induced diabetic rats is given in Table IV. STZ-induced diabetic rats showed significant increase (P < 0.001) in the level of plasma HbA_{1c} when compared to the vehicle control. Oral administration of MECM at 100 and 200 mg/kg b. w. (P < 0.01) and standard drug metformin showed significant decrease (P < 0.001) in plasma HbA_{1c} level when compared with diabetic control. MECM at the dose of 200 mg/kg b.w. in STZ-induced diabetic rats was more effective than 100 mg/kg b.w., indicating high efficacy at higher dose.

Table IV: Effect of MECM (100 and 200 mg/kg b.w.) on the plasma level of HbA_{1c} in STZ-induced diabetic rats.

Groups	**Dose**	**Level of HbA_{1c} (%)**
Group 1- Normal control	-	4.84 ± 0.12
Group 2- Diabetic control	55 mg/kg	11.90 ± 0.23***
Group 3- Extract treated	100 mg/kg	8.04 ± 0.17
Group 4- Extract treated	200 mg/kg	6.34 ± 0. 37
Group 5- Standard treated	11.3 mg/kg	5.29 ± 0.11

Each value is mean ± S.E.M of 6 rats in each group. ***P < 0.001 compared with control, P < 0.001 compared with diabetic control, and P < 0.01 compared with diabetic control.

DISCUSSION

Callicarpa macrophylla (Common name Priyangu) has a long history of traditional use in Southeast Asia, southeast America, Australia, and Central America. More recently, a range of commercial formulation containing Priyangu are gaining popularity as dietary supplements, with claims of hypoglycaemic activity. The main objective of this study was to evaluate hypoglycemic effect of the fruits extract.

Preliminary phytochemical screening revealed that MECM showed positive response to flavonoids, tannins, saponins, steroids and triterpenes.

Significant decrease in glucose level in normal animals can be attributed to the inhibition of α-glucosidase enzymes which reduce intestinal absorption of glucose [24] or may be due to a stimulating effect on the remainder β-cells or improvement in insulin action at the tissue level.

Streptozotocin has been observed to cause a massive reduction of the *β*-cells of the islets of Langerhans and induce hyperglycemia [25]. STZ-induced diabetic rats showed significant increase (P < 0.001) in the levels of blood glucose when compared to the vehicle control. Oral administration of MECM at 100 and 200 mg/kg b. w. showed significant decrease (P < 0.001) in the blood glucose levels on 7th and 14th day, but not on day 1. Standard drug metformin showed significant decrease (P < 0.001) in the blood glucose level. On 14th day, MECM at the dose of 200 mg/kg body weight in diabetic rats was more effective than 100 mg/kg, indicating more efficacy at higher dose.

A marked increased in serum concentration of TC, TG, LDL-C, VLDL-C and decreased HDL-C was observed in diabetic rats than normal control group which is often linked with hyperlipidaemia. Hyperlipidemia certainly contributes to most important risk factor for cardiovascular diseases [26-27]. During diabetic state, insulin deficiency contributes to derangements of various regulatory and metabolic mechanisms in body. At normal state insulin activates the lipolytic hormones action on the peripheral fat depots which hydrolyses TG and prevents mobilization of free fatty acids [28]. However, insulin deficiency inactivates the lipoprotein lipase which promotes liver conversion of free fatty acids into phospholipids and cholesterol and finally discharged into blood, resulted into elevated serum phospholipid level [29].

Our result showed significant decrease (P < 0.001) in serum TC, TG, LDL-C, VLDL-C levels coupled together with elevation of HDL-C level (P < 0.001) in STZ-induced diabetic rats on oral administration of MECM (100 and 200 mg/kg b.w.) after 14 days repeatedly. This implies that plant may possess insulin-like activity which would be helpful to reduce the incidence of lipid born complications. The significant control on serum lipids may prevent from simultaneous coexistence of hypercholesterolemia and hypertriglyceridemia and also reduce the cardiovascular risk factors [30].

The increased level of glycosylated hemoglobin (HbA_{1c}) is directly proportional to the decreased level of total hemoglobin in diabetic control experimental rats. Glycosylated hemoglobin (HbA_{1c}) is used as most reliable marker and standard diagnosis practices for estimating the degree of protein glycation during diabetes mellitus [31]. Protein glycation is a non-enzymatic reaction between excess glucose present in the blood and free amino groups on the globin component of hemoglobin. Measurement of HbA_{1c} level provides information of long-term glycemic status and to correlate with various complications related to DM. On oral administration of MECM significantly decreased (P < 0.001) the HbA_{1c} level possibly due to normoglycemic control mechanisms in experimental rats which also reflect the decreased protein

glycation condensation reactions [32]. MECM at the dose of 200 mg/kg b.w. in diabetic rats was more effective than 100 mg/kg b.w., indicating high efficacy at higher dose.

CONCLUSION

The results suggest that the methanolic extract of *Callicarpa macrophylla* fruit possess a promising dose-dependent hypoglycemic effect on STZ-induced diabetes.

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CONFLICT OF INTEREST: Nil

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