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Original Article

Antidiabetic Activity of Polyherbal Formulation in Streptozotocin – Nicotinamide Induced Diabetic Wistar Rats

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ABSTRACT

Camellia sinensis and Macrotyloma uniflorum are well-known plants available throughout India and they are commonly used for the treatment of various diseases including diabetes mellitus. The antidiabetic activity of the individual plant is well known, but the synergistic are unclear. The polyherbal formulation contains the methanolic extracts of *Camellia sinensis and Macrotyloma uniflorum* in the ratio of 1:1. The acute toxicity studies of the polyherbal formulation did not show any toxic symptoms in doses up to 2000 mg/kg over 14 days. The oral antidiabetic activity of the polyherbal (200 and 400 mg/kg) was screened against streptozotocin (50 mg/kg; i.p.) + nicotinamide (120 mg/kg; i.p.) induced diabetes mellitus in rats. The drug was administered for 21 consecutive days, and the effect of polyherbal formulation on blood glucose levels was studied at regular intervals. At the end of the study, Polyherbal formulation showed significant antidiabetic activity at 200 and 400 mg/kg, respectively, and this effect was comparable with that of glibenclamide.

Keywords: *Camellia sinensis, Macrotyloma uniflorum,* streptozotocin-nicotinamide and glibenclamide, antidiabetic activity.

INTRODUCTION

Plants are very useful to mankind. Many of them are used exclusively for medicinal purposes. According to the World Health Organization (WHO), "a medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." Such plants are in great demand by pharmaceutical companies for their active ingredients.[1,2] Diabetes mellitus is one of the most common disorders affecting almost 6% of the world population and the dynamics of the diabetes are changing rapidly in low- to middle-income countries.[3]

Diabetes is a metabolic disorder characterized by increased fasting and postprandial blood sugar levels. The prevalence of diabetes is likely to be increased by 35% [4]. It may be projected from15million in 1995 to 57 million in 2025 [5]. Globally, diabetes is oneof the six major causes of death and also causing various systemic complications. Diabetes mellitus is treated by hormone therapy (insulin) or by administering glucose-lowering agents such as alpha-glucosidase inhibitors, sulfonylureas, biguanides, and thiazolidinediones. Development of an adverse event is one of the complications in the treatment of any systemic disorder; hence, many of the research institutes and pharmaceutical companies are involved in drug development to find the molecules with good therapeutic potential and less adverse events.[6] In the USA, 10-25% of patients experience an adverse drug reaction and these adverse drug reactions are responsible for 3.4-7.0% of hospital admissions.[7]

In traditional systems of medicine, many plants have been documented to be useful for the treatment of various systemic disorders. Many of the traditional/indigenous systems of medicine are effective than the modern system of medicine, but they suffer from lack of complete standardization which is one of the important challenges faced by the traditional systemof medicine. The concept of polyhedral formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. Hence, the present study was planned to evaluate a polyherbal formulation using a plant having known antidiabetic activity and its therapeutic effects in rodents.

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MATERIALS AND METHODS

Plant material: Dried leave of *Camellia sinesis* and legumes of *Macrotyloma uniflorum* was procured from Madhavachetti botanical garden, Thirupathi and was authenticated by Dr. K. Madhavachetti, Assistant Professor in Department of Botany at Sri Venkateswara University, Tirupathi.

Plant extracts preparation: The dried leaves are made into powder and then gone for the Maceration with sufficient quantity of methanol for 7 days. During maceration, it was shaked twice daily. On 7th day it was filtered and the filtrate was concentrated. The remaining solvent was evaporated by heating on a water bath (50^{o}C) to get methanolic extract and the extract was stored in desiccator.

EXPERIMENTAL ANIMALS

Albino mice of either sex weighing between 16 - 25 g were procured from **albino research and training institute** for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry conditions.

Room temperature - 26 ± 2^{0} C
Relative humidity - 45 - 55%
Light/ dark cycle - 12 : 12 h

The animals were fed with a standard diet purchased from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli, Maharashtra, India. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed as per the guidelines of CPCSEA and Institutional Animal Ethical Committee (IAEC). CPCSEA Approval Number: 1722/RO/ERe/S/13/CPCSEA.

Acute oral toxicity: Acute oral toxicity of the polyherbal formulation was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. The principle involves a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Healthy Wistar rats (3 animals/dose) of either sex were used for the experiment. Overnight fasted rats were orally fed with the plant extracts and polyherbal formulation in increasing dose levels of 5, 50, 300, and 2000 mg/kg body weight, respectively. The animals were observed for their behavioural (alertness, restlessness, irritability, and fearfulness), neurological (spontaneous activity, reactivity, touch response, pain response, and gait), and autonomic (defecation and urination) profiles continuously for 24 h. After a period of 24 h, the animals were observed for 14 days for mortality.[8]

EXPERIMENTAL DESIGN

Antidiabetic effect of herbal formulation in streptozotocin- and nicotimanide-induced diabetic rats: The male Wistar rats were divided into five different groups of six animals each as follows.

Group I: Normal control
Group II: Diabetic control
Group III: Diabetic rats treated with polyherbal preparation (200 mg/kg)
Group IV: Diabetic rats treated with polyherbal preparation (400 mg/kg)
Group V: Diabetic rats treated with glibenclamide (10 mg/kg).

Diabetes was induced in overnight-fasted rats by administering single intraperitoneal (i.p.) injection of freshly prepared streptozotocin (STZ) 50 mg/kg b.w. followed by 120 mg/kg of nicotimanide (NIC) in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg b.wt.[9] Diabetes was confirmed in the STZ + NIC treated rats by measuring fasting blood glucose levels after 48 h of induction. After 24 h of STZ + NIC injection, the rats were given 5% w/v of glucose solution (2 ml/kg b.w.) to prevent hypoglycemic mortality. Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics and they were divided randomly into four different groups. The standard (glibenclamide) and herbal formulation were suspended in 1% w/v carboxymethyl cellulose (CMC) and administered once daily through oral gavage for 21 consecutive days. The blood samples were collected on 1 , 7 , 14 , and 21 days of the treatment, through the retro-orbital plexus puncture blood was collected in sodium ethylenediaminetetraacetic acid (EDTA) tubes for biochemical analysis of blood glucose and lipid profile[10]. Weekly body weight variations were monitored for all the experimental animals.

Biochemical Analysis: The whole blood sample was used for the estimation of glucose .The plasma sample was used for the estimation of biochemical markers such as total serum cholesterol, serum triglyceride, high density lipoprotein (HDL)-cholesterol, The biochemical markers were measured using Biochemistry Analyser and the LAB-KITS enzymatic kits.

***Estimation of glucose*:** A drop of the whole blood sample was used for measuring glucose using One-Touch H orizon glucometer, with gluco strips.

Statistical analysis: All the data were expressed as mean ± SEM. Statistical significance between the

groups were tested using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test P less than 0.5 was considered significant.

RESULTS

Effect of Methanolic Polyherbal Extract (MPHE) On Glucose Levels In Streptozocin-Nicotinamide Induced Diabetic Rats: Two different doses of the Methanolic Polyherbal Extract (MPHE 200 and MPHE 400) blood glucose level were studied in two different groups of animals. Both groups showed a extremely significant decrease of blood glucose level on Streptozocin-Nicotinamide induced diabetic rats when compared to control group. The initial readings of blood glucose level of MPHE 200 and MPHE 400 were 302.40±7.748 and 299.83±3.436 respectively. After the trial period both doses produced consistent reduction in the blood glucose levels after 7 days (241.12±4.62, 239.05±2.21) and marked reduction in 21 days (121.36±3.18 and 106.07±2.16). However MPHE 400 has shown maximum effect than MPHE 200. In standard group initial blood glucose level was 303.38±6.739 and the post test was 102.77±5.12 which showed that the standard drug produced maximum hypoglycemic effect and the statistical analysis was extremely significant and slightly higher than that of trial drug groups. Untreated diabetic rat group showed increase in blood glucose level throughout the entire study period. Initially blood glucose level of untreated diabetic control group was 299.23±11.38 and after 21 days of trial period the blood glucose level was increased to 331.16±2.12. The results were summarized in Table No.1.

Table 1: Changes in fasting blood glucose levels of control and experimental animals

S. NO	GROUPS	Glucose levels (mg/dl)			
		1st day	7th day	14th day	21st day
1	Normal control	98.54±3.164	98.21±2.12	97.40±1.14	97.52±2.12
2	Diabetic control	299.23±11.38	310.29±2.10	320.15±1.10	331.16±2.12
3	Standard	303.38±6.739	250.12±6.12	173.34±4.16	102.77±5.12
4	MPHE(200mg/kg)	302.40±7.748	241.12±4.62	182.22±3.32	121.36±3.18
5	MPHE(400mg/kg)	299.83±3.436	239.05±2.21	173.42±3.12	106.07±2.16

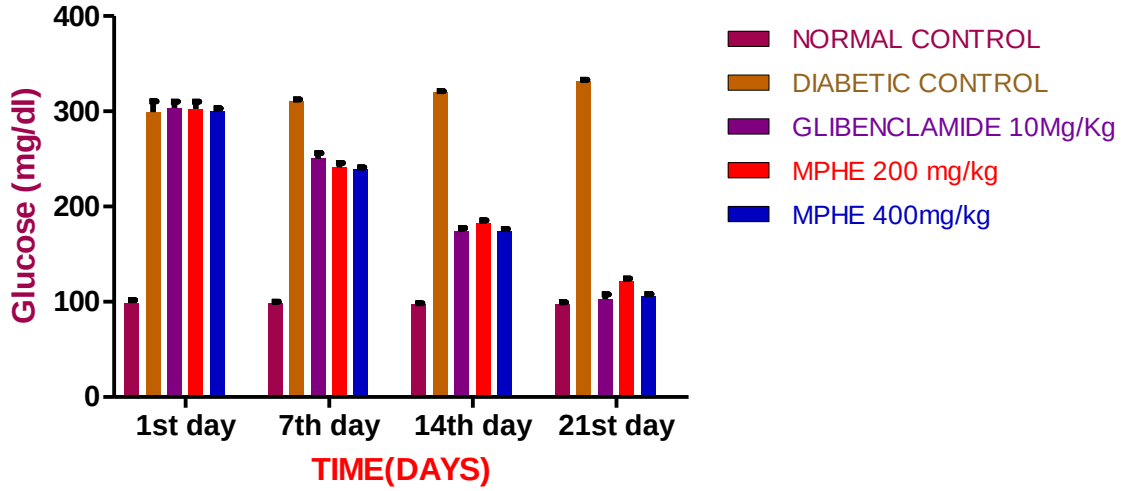

Fig.No.1: Effect of methanolic polyherbal extract (MPHE) on glucose levels in Streptozotocin-Nicotinamide induced diabetic rats.

Effect Of Methanolic Polyherbal Extract (MPHE) On TC, TG & HDL In Streptozocin-Nicotinamide Induced Diabetic Rats: In Table No.2 & Table No.3, the Total cholesterol (TC) and Triglyceride levels (TG) were summarized, the TC and TG levels was decreased at dose of MPHE 200 from 154.01±0.10 to 102.07±0.12 and MPHE 400 from 156.07±0.08 to 89.04±1.2 after 21 days. The triglyceride levels was decreased at dose of MPHE 200 from 182.4±2.2 to 106.4±2.2 and for MPHE 400 the TG levels decreased from 179.04±0.13 to 91.06±1.2 respectively after 21 days. In Table No.4 the HDL levels for the two extract treated groups were increased than diabetic control and values were 31.08±1.2 to 41.02±1.4 for MPHE 200 and MPHE 400 the levels increased from 32.10±1.8 to

43.12±2.8 after 21 days. When compared between the two different doses of extract [MPHE 200 and MPHE 400], MPHE 400 more decrease in TG and TC levels and more increase in HDL level than the MPHE 200 group.

Table 2**:** Changes in total cholesterol levels of control and experimental animals.

S. NO	GROUPS	Total Cholestrol (mg/dl)			
		1st day	7th day	14th day	21st day
1	Normal control	82.54±0.13	81.95±0.12	82.01±0.14	82.05±0.16
2	Diabetic control	152.10±0.14	153.03±0.14	168.02±0.13	158.07±0.16
3	Standard	156.09±0.45	130.06±0.15	110.08±0.19	85.06±0.15
4	MPHE(200mg/kg)	154.01±0.10	135.07±0.14	119.06±0.13	102.07±0.12
5	MPHE(400mg/kg)	156.07±0.08	131.06±0.13	109.09±0.18	89.04±1.2

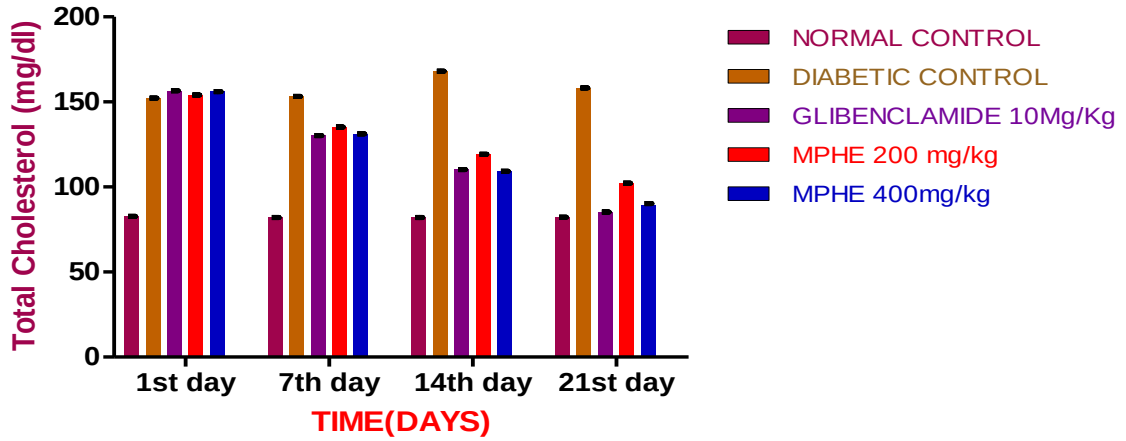

Fig.No.2: Effect of methanolic polyherbal extract (MPHE) on Total Cholesterol levels in Streptozotocin-Nicotinamide induced diabetic rats.

Table 3: Changes in Triglyceride levels of control and experimental animals

S. NO	GROUPS	Triglyceride (mg/dl)			
		1st day	7th day	14th day	21st day
1	Normal control	89.2±1.6	88.9±2.6	89.1±1.4	86.3±2.2
2	Diabetic control	182.1±2.4	194.4±1.2	189.7±2.2	176.9±2.4
3	Standard	184.2±2.1	130.2±2.4	105.3±2.6	89.04±0.12
4	MPHE(200mg/kg)	182.4±2.2	150.4±1.1	120.4±2.1	106.4±2.2
5	MPHE(400mg/kg)	179.04±0.13	139.2±1.3	112.45±1.1	91.06±1.2

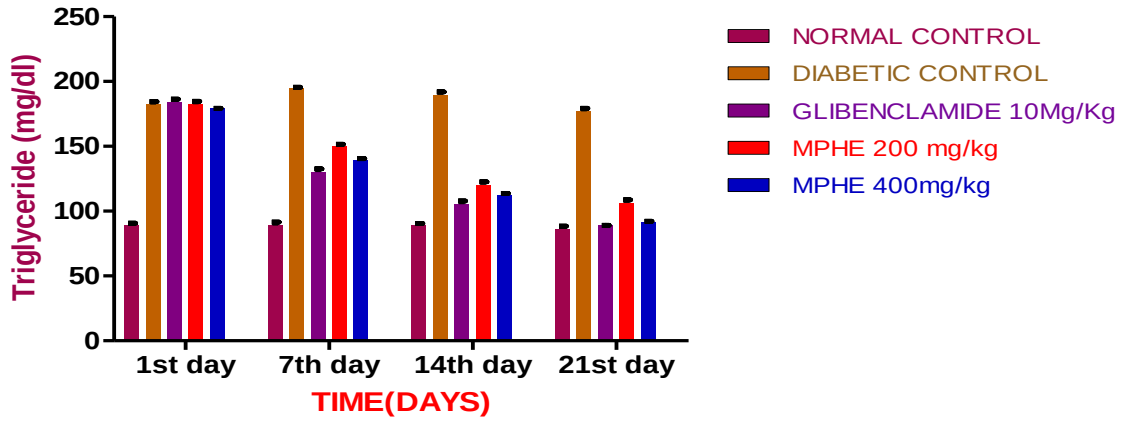

Fig.No.3: Effect of methanolic polyherbal extract (MPHE) on Triglyceride levels in Streptozotocin-Nicotinamide induced diabetic rats.

Table 4: Changes in HDL levels of control and experimental animals

S. NO	GROUPS	HDL (mg/dl)			
		1st day	7th day	14th day	21st day
1	Normal control	43.23 ±1.2	42.76±2.1	44.54±2.2	43.09±2.4
2	Diabetic control	32.06±1.4	29.02±1.3	26.01±1.1	24.10±1.4
3	Standard	31.03±1.1	36.03±1.6	41.05±1.1	45.03±2.2
4	MPHE(200mg/kg)	31.08±1.2	33.04±1.2	38.04±1.8	41.02±1.4
5	MPHE(400mg/kg)	32.10±1.8	35.04±2.0	41.05±1.8	43.12±2.8

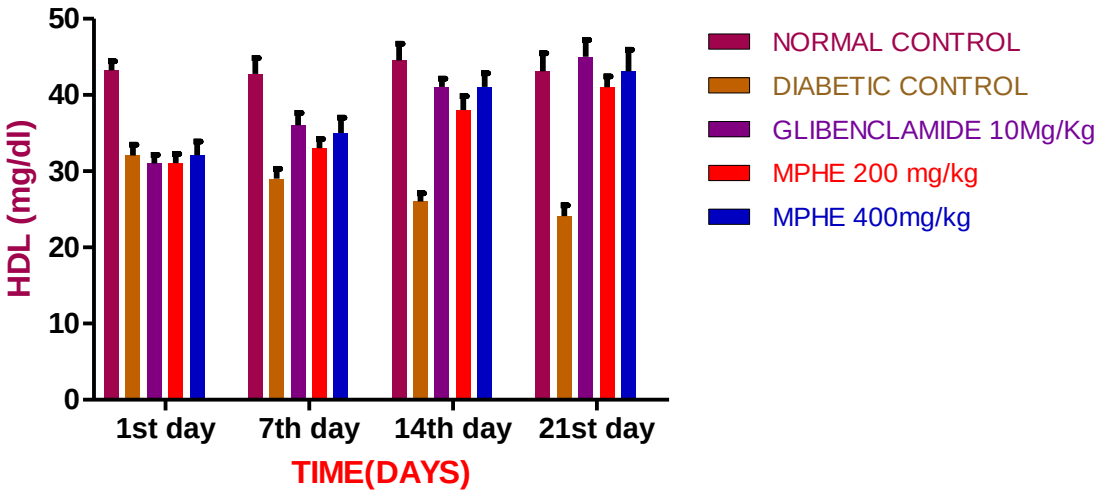

Fig.No.3: Effect of methanolic polyherbal extract (MPHE) on HDL levels in Streptozotocin-Nicotinamide induced diabetic rats.

DISCUSSION

In the present study, streptozotocin- nocotinamide was chosen to induce diabetes in rats because of its lower toxicity and higher beta-cell specificity relative to other diabetogens[11]. Findings of the present investigation revealed that STZ e induced diabetes resulted in a significant increase in serum glucose level and significant decrease in serum insulin level. This diabetogenic effect could be due to the destructive effect streptozotocin on pancreatic islets. The mechanism of decreased insulin secretion could be attributed to the resultant hyperglycemia that induced abnormalities in insulin action and secretion [12]. Hyperglycemia is also associated with the consequences of hyperinsulinemia, insulin resistance, and glucose intolerance in diabetes [13]. In the present study the reduction in glucose levels may be due to increase in plasma insulin levels or enhanced transport of blood glucose in the peripheral tissue.[14] Our study gives evidence that the polyherbal formulation increases the plasma insulin levels and has shows potential antidiabetic activity. The diabetic hyperglycemia induced by streptozocin and nicotinamide causes elevation of plasma levels of TC, TG, and decreased plasma levels of HDL which are considered as significant markers of liver dysfunction. The polyherbal formulation treated animals reversed the effect of STZ and NIC on the liver markers. This may be due to the hepatoprotective mechanism of the individual herbs present in the polyherbal formulation.[15]

In our study, there was a significant elevation in blood glucose level in diabetic control group as compared with normal animals. The Polyherbal extract treated group exhibited significant reduction of fasting plasma glucose levels as compared to the diabetic control group.

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. A marked increase in total cholesterol and decrease in HDL cholesterol have been observed in diabetic control rats. Insulin deficiency results in failure to activate lipoprotein lipase thereby causing hypertriglyceridemia. There was a significant control of the levels of serum lipids in Poly herbal extract treated diabetic rats. In diabetes, LDL carries cholesterol to the peripheral tissues where it is deposited, whereas HDL transports cholesterol from peripheral tissues to the liver and thus aids its excretion. Hence increase in LDL is atherogenic. In our present study, there was a significant decrease in TG, and total cholesterol levels, whereas there was a significant increase in the HDL level.

CONCULSION

The present study involves Polyherbal formulation screening of leaves *Camellia sinensis and Macrotyloma uniflorum* for anti-diabetic activity on Streptozocin and nicotinamide induced diabetic Wister rats using glibenclamide as standard. The *in vivo* study demonstrated significant hypoglycemic activity at 200 and 400 mg/kg, respectively, and this effect was analogous with that of glibenclamide. The blood glucose levels and lipid profile shown to be decreased in poly herbal extract treated diabetic animals. The findings of the present investigation suggest that Polyherbal extract has potential for its evaluation as a protective agent against toxicity induced by Streptozotocin. Evaluation of poly herbal extract for its principal mechanism(s) will be a fascinating topic and requires additional study.

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