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Hypoglycemic and Hypolipidaemic Activity of a Polyherbal Formulation in High Fat Fed and Low Dose Streptozotocin Induced Diabetes in Wistar Rats

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Abstract: Increased glucose levels due to abnormalities in insulin production or activity define the metabolic illness group known as diabetes mellitus. Increased glucose levels in diabetes are linked to hyperlipidemia, which may lead to damage to blood vessels, kidneys, liver, and the heart over the long term. Blood glucose and lipid levels were estimated at regular intervals during the polyherbal formulation (PHF) intake and at the end of the study, respectively, proving the hypoglycemic activity and hypolipidemic activity of the PHF, as demonstrated by the results of an *in vivo* study of a polyherbal formulation consisting of seven plant parts.

Keywords: Polyherbal formulation, Diabetes, Low blood sugar, Low cholesterol, Lipid, Hypoglycemic, Hypolipidemic

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Introduction

Hyperglycemia due to abnormalities in insulin action or secretion, or both, characterises the metabolic illnesses known together as diabetes mellitus. Not maintaining appropriate blood glucose levels might have serious consequences over time (Ziegler and Filer, 1996; Berdainer, 2002). Long-term malfunction and failure or damage to organs including the eyes, kidneys, nerves, heart, and blood vessels are related with the hyperglycemia seen in diabetes (Eastman *et*

al., 1992). Incorrect or absent insulin response is the root cause of diabetes. Diabetes mellitus may be broken down into three distinct subtypes. Insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes occurs when the pancreas is unable to generate adequate insulin because of the death of beta cells. An autoimmune reaction is responsible for the death of beta cells. Insulin resistance, a condition in which cells fail to respond normally to insulin, is the root cause of

type II diabetes. Noninsulin-dependent diabetic mellitus (NIDDM) or adult-onset diabetes were older terms for this kind. There is a strong correlation between being overweight and not getting enough exercise. The third most common kind of diabetes is called gestational diabetes, and it manifests itself in healthy pregnant women who suddenly have high blood sugar. According to Hernando *et al.* (2000), it is estimated that by 2030, 25% more individuals will have diabetes mellitus, and by 2045, 51% more people would have the disease. There are around 255 data sources, covering the years 1990 to 2018, from which 138 nations may be inferred. The prevalence of diabetes in persons aged 20-79 was estimated statistically (Saeedi *et al.*, 2019). Increased understanding and novel approaches or improvements in diabetes treatment have not slowed this trend, which is barreling headlong toward its apex. When discussing diabetes, most people think of type II. There may be as many as 91% of individuals with type 2 diabetes in nations with high per capita incomes. To put it simply, diabetes is a major problem for healthcare systems and a barrier to nations' long-term economic growth (Defay *et al.*, 2001).

The high cost of treating diabetic complications, the time off work caused by those complications, and the commitment to long-term care is required to successfully manage the disease. The high price of medications has a significant impact on the economy and families. Around 800 plant species have been identified worldwide as having antidiabetic properties (Gurib-Fakim *et al.*, 2005; Hennebelle *et al.*, 2008; Hussain *et al.*, 2009). Researchers have developed a variety of formulations that aim to combat this by promoting a healthy metabolic rate and stable blood sugar levels (Davidson and Hsia, 2017).

In this study blood glucose and lipid levels were estimated at regular intervals during the polyherbal formulation (PHF) intake.

Materials and Methods

Acute toxicity tests on rats showed that the

polyherbal formulation under investigation was safe up to a dose of 2000 mg/kg body weight. Approximately 180 g of adult Wistar rats were kept in polypropylene cages with a maximum of six individuals per cage, and they were given a 12:12 h light and dark cycle with a constant ambient temperature of 25 ± 1 °C (Gupta and Sharma, 2017). Before the diet alteration, the rats were given commercial rat NPD and *ad libitum* water for a week. After two weeks of this, the rats were given a high-fat diet (HFD) (Srinivasan *et al.*, 2005). On day 15, rats were given an intraperitoneal injection of a newly manufactured low dosage of streptozotocin (35 mg/kg b.w.), dissolved in 0.1M ice cold citrate buffer (pH 4.5). Fasting blood sugar levels were checked and diabetes verified using a glucometer in the experimental rats three days after intraperitoneal injection of STZ (Roch One touch). Fasting blood glucose levels in excess of 250 mg/dl were considered to choose which rats would be involved in the subsequent experiments. Institutional Animal Ethics Committee Guidelines and other ethical standards approved by the Indian Ministry of Social Justice and Empowerment were followed throughout all animal experiments (NCP/IAEC/2018-19/18).

Experimental Design: Rats were divided into 6 groups and treated as follow:

Group I– Control rats; Group II–HFD fed - low dose STZ induced type 2 diabetic rats; Group III– Diabetic rats orally treated with PHF (200 mg/ kg b.w./rat/day); Group IV–Diabetic rats orally treated with PHF (400 mg/ kg b.w./rat/day); Group V– Diabetic rats orally treated with PHF (600 mg/ kg b.w./rat/day), and Group VI – Diabetic rats orally treated with a standard drug, metformin (50mg/kg b.w./rat/day) in aqueous solution.

The blood was drawn on 1st, 18th, 32nd and 46th day for analysis. After the stipulated time period of study, the rats were given anaesthesia (80 mg/kg body weight intraperitoneally of ketamine) and sacrificed via cervical decapitation. The rats fasted overnight, and then their blood (in

some samples anticoagulant was used) was drawn to separate plasma and serum.

Plant materials:

This PHF consists of seven different herbs: *Marsilea quadrifolia* leaves, *Aegle marmelos* leaves, *Senna auriculata* leaves, *Curcuma longa* rhizome, *Terminalia chebula* pericarp, *Terminalia bellerica* pericarp, and *Phyllanthus emblica* pericarp. Antibacterial, diabetic, hepato-protective, antihepatotoxic, uterine, and gastrointestinal stimulating effects were discovered in these plant sections. These plants were cited from Vaidya Ratnam K.S. Murugesha Mudaliar's "Gunapadam" (Siddha Materia Medica), originally published in 1936. These flowers, leaves, and stems were all gathered from the fields and the local market in the district of Thiruvallur. Plant experts from D.G. Vaishnav College, Chennai verified the identity of these plants.

Formulation preparation:

Formulation for the aforementioned mixture was achieved by grinding all of the materials to a powder, passing the powder through a 100# screen, and then mixing the powders together in the appropriate quantities. 10 g of the formulation were cooked in 500 ml of water for around an hour. Under decreased pressure, the decoction or filtrate was evaporated to dryness in a rotatory evaporator at temperatures between 50 and 55 °C (Ramamoorthy *et al.*, 2017). Formulation preparation resulted in a yield of 5.3 g. Extracts that had evaporated were collected and placed in airtight containers for later use.

LABINDIA Clinical Chemistry Analyzer was used for all biochemical measurements. Serum glucose levels were calculated using the enzyme technique GOD-POD (Lab India Optima 1). The Millipore Sigma Roche kit was used to determine the amount of glycogen in the liver. We used Biorad D10 to check for glycosylated Hb. The amount of insulin produced was determined using the Elisa technique using a Roche e411. Serum samples were analysed for their lipid

profiles using Lab India Optima 1. Triglycerides, Cholesterol and HDL were analysed by cholesterol oxidase/peroxidase method (Lab India Optima 1). LDL Cholesterol was calculated using the Friedewald's formula and was expressed as mg/dl:

$$\text{LDL cholesterol} = \text{Total cholesterol} - (\text{Triglycerides} / 5) + \text{HDL cholesterol}.$$

Analyses of Data:

SPSS, version 2.0, was used for the statistical analysis. One-way ANOVA and then Tukey's test were used to analyse the data. Mean±standard deviations are shown, and there were a total of six rats in each group. Statistical significance was assumed at the 0.05 level.

Results

Blood glucose:

On day 18 (caused by streptozotocin medication), group I had a glucose level within the normal range, whereas the other groups exhibited a larger range. To determine the efficacy of the new PHF extract, the difference in blood glucose levels between the 18th and 46th days was used for statistical analysis. Both Group I (where glucose levels were within normal range) and Group II (where glucose levels remained elevated) showed no statistically significant differences during the course of the trial. This showed that both Group I and Group II had stable glucose levels within the outlined time period (18th day and 46th day). The sequence of groups IV and V combined, followed by group VI (standard metformin treatment), and finally group III showed a statistically significant difference in the rate at which blood glucose levels dropped during the set time (Table 1). Figure 1 shows a graphical depiction of the glucose concentration. This demonstrated that PHF at both 400 mg/kg (group IV) and 600 mg/kg (group V) had the same capacity of lowering blood glucose levels and was found to be superior even to that of standard metformin treated (group VI) and 200 mg/kg PHF treated (group III), demonstrating its superiority in

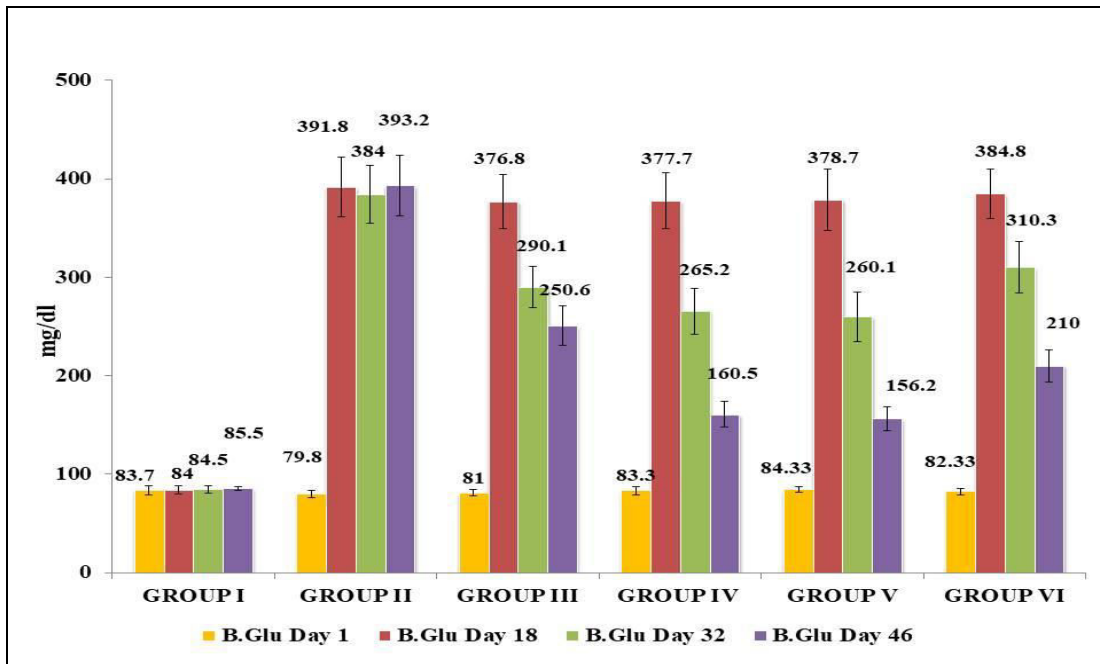


Fig. 1: Blood glucose levels at regular interval of time to assess the efficiency of the PHF extract among the various study groups.

Table 1: Effect of PHF extract on the blood glucose concentrations studied at different time intervals

Groups	1 st day	18 th day	32 nd day	46 th day
Group I	83.66 ± 4.5	84 ± 4.42	84.5 ± 4.1	85.5 ± 1.64
Group II	79.83 ± 3.9	391.83 ± 30.12	384 ± 29.5	393.16 ± 30.6
Group III	81 ± 3.46	376.83 ± 27.16*	290.1 ± 21.34	250.6 ± 20.16*
Group IV	83.3 ± 4.26	377.66 ± 28.33*	265.2 ± 23.44	160.5 ± 13.12*
Group V	84.33 ± 2.46	378.66 ± 31.2*	260.1 ± 25.14	156.17 ± 12.1*
Group VI	82.33 ± 3.1	384.83 ± 25.1*	310.3 ± 26.14	210 ± 16.2*

* indicates the statistical significance $p < 0.05$

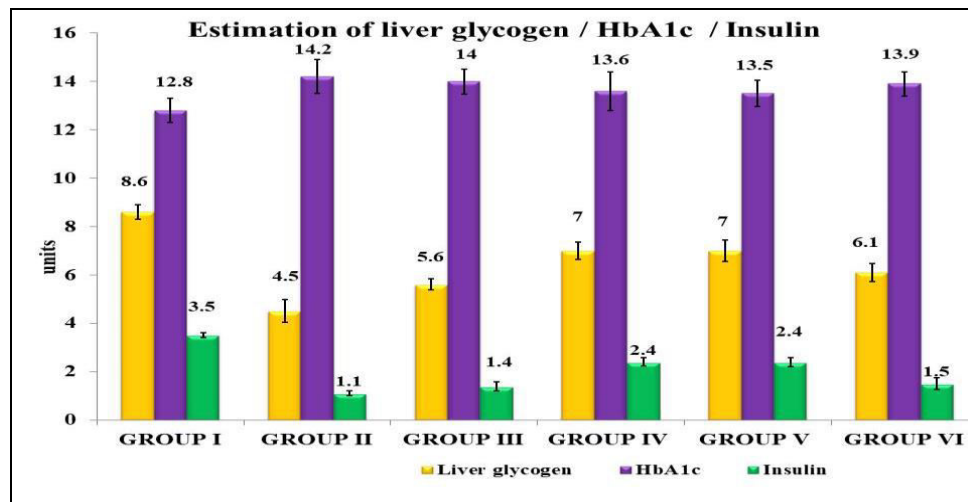


Fig. 2: Impact of PHF on trends of liver glycogen, HbA1c and insulin in the controls against the various groups. Liver glycogen was expressed in the units of mg/dl of tissue. Insulin was expressed in IU/l.

Table 2: Effect of PHF extract on the liver glycogen, HbA1c and Insulin concentrations

S. No	Category of groups	Liver glycogen (mg/g of tissue)	HbA1c (%)	Insulin (IU)
1	Group 1	8.55 ± 0.3	12.81 ± 0.5	3.46 ± 0.1
2	Group 2	4.51 ± 0.47	14.23 ± 0.7	1.06 ± 0.1
3	Group 3	5.58 ± 0.23	14 ± 0.51	1.43 ± 0.18
4	Group 4	7 ± 0.35	13.6 ± 0.8	2.43 ± 0.17
5	Group 5	7.01 ± 0.45	13.54 ± 0.55	2.41 ± 0.18
6	Group 6	6.11 ± 0.38	13.9 ± 0.5	1.53 ± 0.24

reversing the higher blood glucose level to maintain normoglycemic state in rats.

Liver Glycogen:

When compared to the other groups, group II had the lowest rate of liver glycogen degradation prevention efficiency, followed by groups IV, V, and VI, and finally group III. With regards to group VI evaluation, it is determined that group V is the best option. PHF, namely those in groups V, IV, and III (Table 2), have been shown to be beneficial in the destruction of liver glycogen stores, making the PHF an antidiabetic medicine that helps keep blood sugar levels stable (Fig. 2)

Analyzing the Role of Glycosylated Hb:

In terms of HbA1c, the mean values of groups I, II, and VI were not significantly different from the mean values of the remaining study groups; however, it was recorded that only groups V and IV showed better potential than group III in lowering glucose levels, which brought the glycemic control nearly to the levels of the control group I and resulted in a decrease in the HbA1c value. Doses of 600 mg/kg and 400 mg/kg of the PHF extract compound may be used as an effective antidiabetic drug (Table 2, Fig. 2).

Insulin:

A larger mean difference was found between group I and the other groups, with the exception of group V ($p < 0.0009$), group IV ($p < 0.0008$), and group VI ($p < 0.0002$), which revealed the least significant values in the order of efficient capacity for insulin secretion. Thus, the insulin secretions of rats in groups V, IV, and VI were higher than those of control rats. Group II was found to have

very low serum insulin secretion, and compared to the other groups, group IV had the highest levels of circulating insulin ($p < 0.0001$), followed by groups V ($p < 0.0003$), VI ($p < 0.0352$), and III ($p < 0.0358$).

PHF extracts are helpful in halting the breakdown of liver glycogen, keeping glucose levels within normal range, and preserving HbA1c levels by stimulating insulin production.

Lipid Profile:

Total Cholesterol (TC) levels were highest in group II rats and lowest in group I rats ($p < 0.0001$). Compared to rats in Group I, rats in Groups V ($p < 0.03$) and IV ($p < 0.016$) and VI ($p < 0.004$) had significantly better control over the mean concentration of TC, whereas rats in Group II ($p < 0.0001$) exhibited the greatest significant difference, followed by rats in Group III ($p < 0.0005$). This indicated that subgroups V, IV, and VI were the most effective in preserving TC within the normal range. Table 3 displays the results of a similar analysis, this time comparing groups II and VI to the other groups separately. The median TG levels of rats in group VI were similar to those of rats in groups IV and V, but lower than those of rats in group III. Thus, groups IV and V had more of an effect on TG reduction than did the control group and conventional metformin. (Table 3)

Comparing group I with the other groups revealed that groups V, IV, and VI had the smallest individual differences in High Density Lipoproteins (HDL). Rats in groups V, IV, and VI showed larger importance than rats in Group III, whereas rats in Group VI showed lower

Table 3: Effect of PHF extract on Total Cholesterol, Triglycerides, HDL and LDL levels

S. No	Category of groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	Group 1	127.2 ± 2.63	121.8 ± 2.5	38.2 ± 0.8	74.8 ± 2.9
2	Group 2	257 ± 12.8	179.7 ± 6.4	26.8 ± 1.5	158.8 ± 8.9
3	Group 3	214.5 ± 14.1	165.5 ± 6.3	29.8 ± 1.0	137.8 ± 6.6
4	Group 4	145 ± 7.2	140.8 ± 5.3	31.8 ± 1.7	96.7 ± 3.6
5	Group 5	141.8 ± 7.3	136.7 ± 3.3	33.2 ± 1.5	97.2 ± 4.4
6	Group 6	155.2 ± 7.4	144.2 ± 5.0	27.5 ± 1.6	132.5 ± 4.5

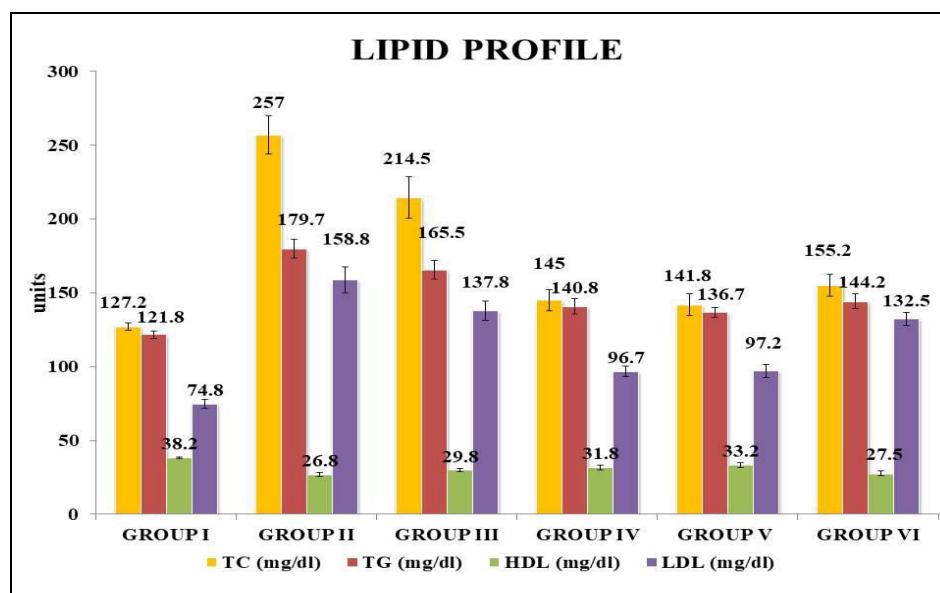


Fig. 3: The lipid profiles of the controls were evaluated against the other groups for their significant association.

significance with V, IV, and III. This demonstrated the efficacy of groups V and IV in keeping HDL levels high in the blood. Low Density Lipoprotein (LDL) levels in the blood were found to be highest in group II rats and lowest in group I rats. The mean LDL level in Group V rats was 97.2 ± 4.4 mg/dl, which is higher than the reported value of 74.8 mg/dl in the control group but still not statistically significant when compared to the other experimental groups. Rats in Group IV had a value of 96.7 ± 3.6 mg/dl, which was statistically significant although not quite as low as the control group ($p < 0.0012$). PHF extract at doses of 400 mg/kg and 600 mg/kg was shown to decrease total cholesterol, triglycerides, and low-density lipoprotein (LDL) and raise high-

density lipoprotein (HDL) concentration in the blood. Therefore, PHF extract has antihyperlipidemic capabilities and has a beneficial effect on lipid profiles.

Discussion

PHF extracts have been reported to possess antimicrobial, antioxidant, antidiabetic (Pari *et al.*, 2002; Rai *et al.*, 2010), radioprotective, anticancer, antifertility (Shahedur Rahman *et al.*, 2014), hepatoprotective (Shamim and Qureshi, 2009), cardioprotective, cytoprotective, renoprotective, and anti-inflammatory properties.

Animals given polyherbal formulations at 400 mg/kg and 600 mg/kg had significantly lower blood glucose levels compared to rats given

regular metformin. HbA1c levels were found to be normal, and the research also noted no deterioration of liver glycogen or insulin depletion. The study showed that doses of the polyherbal compound at 600 mg/kg and 400 mg/kg are superior in converting hyperglycemia to normoglycemia. Possible explanations for the decreased glucose concentration in the treatment group of our research include increased plasma insulin levels and enhanced peripheral tissue blood glucose transport (Wilcox, 2005). As a result, it was concluded that the synthetic polyherbal substance was effective in treating diabetes.

High blood sugar has been shown to cause harm to blood vessel walls and heart control nerves over time. Heart disease is an additional risk factor for people with diabetes. Damage to artery walls is caused by the increased pressure of blood flow that occurs with hypertension. Combining risk factors for cardiovascular disease like hypertension and diabetes is very dangerous. Too much LDL in the circulation might cause plaque to build up on already weak arterial walls. Hardening of the arteries has been linked to having high triglycerides, low HDL, and either high or low LDL. When administered at doses of 400 mg/kg and 600 mg/kg, a polyherbal formulation was shown to significantly lower total cholesterol, triglyceride, and low-density lipoprotein levels in the blood while simultaneously raising high-density lipoprotein levels. As a result, it was determined that the new polyherbal formulations were hypolipidemic.

Increased lipid levels contribute to the accumulation of extra fat in the liver in people with diabetes. Because of its central role in this process, the liver is particularly vulnerable to insulin resistance when lipids accumulate inside its cells. This, in turn, may result in increased blood glucose levels and, ultimately, the development of type 2 diabetes. For this reason, the current research aimed to determine how the PHF affects glucose and lipid levels in the blood.

Conclusion

This investigation in HFD low dosage STZ induced diabetic rats demonstrated the hypoglycemic impact and hypolipidemic effect of the polyherbal formulation. This demonstrated the PHF's therapeutic potential as a preventive agent against the progression of atherosclerosis and its associated, likely cardiovascular consequences in diabetes mellitus. Acute toxicity tests on the PHF showed that it was safe to consume; as a result, it is worth stressing that the PHF has significant potential in bettering the health of the diabetic and reducing the principal complication of diabetes-- an increase in lipids. Therefore, biochemical studies may be conducted to identify the substance with hypoglycemic and hypolipidemic effects and verify and clarify its mode of action.

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