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In-vivo Anti-diabetic activity of Polyherbal formulation on Streptozotocin Induced diabetic Wistar Rats

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Abstract

Objective: To investigate the *In-vivo* antidiabetic activity of a polyherbal mixture in streptozotocin-induced diabetic Wistar albino rats. **Methods:** Optimization of Polyherbal formulation combinations was done by testing for oral glucose tolerance test (OGTT) (2 h) in non-diabetic rats and Antidiabetic activity (28 days) in non-diabetic and STZ-induced diabetic Wistar albino rats. Five sets of Wistar albino rats (n = 6) were used in this investigation. Male Wistar rats were given intraperitoneal injections of streptozotocin to induce diabetes. Upon confirmation of diabetes, the animals were given oral treatments for 30 days, consisting of 200 or 400 mg/kg body weight of extracts or distilled water. **Results:** The result of investigation revealed that PHF2 significantly decreases blood glucose level as compared to PHF1 and PHF3 ,no crystals of Uric acid and Calcium oxalte crystals was observed and was further selected for antidiabetic in-vivo activity. The optimized Polyherbal formulation combination (PHF2) administered at a dosage of 400 mg/kg followed by 200 mg/kg shows significant antidiabetic activity. This investigation of the antidiabetic and biochemical effects of polyherbal formulation (PHF) was carried out on diabetic rats induced with streptozotocin (STZ). In the experimental animals, biochemical parameters such as hemoglobin, glycosylated hemoglobin, high-density lipoprotein, low-density lipoprotein, glucose, creatinine, serum cholesterol, serum triglyceride, and so on were also measured. It was concluded that PHF 2 had strong antihyperglycemic effects. Methanol extract of PHF 2 treatment brought the elevated biochemical parameters significantly ($P<0.05$) back to normal in diabetic rats.

Keywords: Diabetes, Biochemical and Hematological parameters Polyherbal formulation, Streptozotocin , Wistar Albino Rats.

Introduction

Globally, the number of people with diabetes has more than doubled during the last 20 years. One of the most concerning aspects associated with this sharp rise is the rise in type 2 diabetes in children, adolescents, and young adults. [1] Uncontrolled diabetes can lead to problems in many different organs. Severe macro vascular complications like heart attacks, strokes, kidney failure, damage to small and large blood vessels, and nerve injury are the most alarming trends[2,3]. Insulin and a number of oral hypoglycemic drugs, including biguanides and sulfonylureas, are currently the available treatments for diabetes mellitus. Although they have some disadvantages, such as side effects and high rates of secondary failure, these drugs are used to treat diabetes mellitus. To meet this need, the diverse traditional plant kingdom offers many promising therapeutic uses. A plethora of natural remedies have been recommended to treat diabetes [4]. The World Health Organization (WHO) defines a medicinal plant as one that "contains substances in one or more of its organs that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." The idea of polyhedral formulation is well documented in ancient literature. Compared to a single plant, the polyherbal formulation has a larger and more comprehensive medicinal potential. The goal of the current study was to evaluate the therapeutic benefits of a plant known to have antidiabetic action in order to create and standardize a polyherbal formulation. [5]

The medicine formulation in Ayurveda is based on two principles:

1. Several herbs are combined to create a single product in polyherbal formulations (PHF). It blends a variety of herbs to achieve therapeutic efficacy.

2. PHF has a synergistic, broad therapeutic index, making it safe at high doses while still being effective at low doses (better risk to benefit ratio) than allopathic hypoglycemic medications, which have a narrow therapeutic range. This treatments are ideal due to their efficacy, safety, affordability, acceptability, and accessibility.[6] Prior studies have demonstrated the presence of antioxidant and antiurolithiatic properties in *S. grandiflora*, along with chemopreventive and anticancer properties, anxiolytic and anticonvulsant effects, hepatoprotective properties, cardioprotective properties, antiulcer properties, antimicrobial properties, analgesic and antipyretic properties, diuretic properties, CNS depressant and laxative hypolipidemic properties, and anthelmintic properties. After a careful analysis of the literature, it was found that there hasn't been much research done on the leaves' potential to prevent diabetes [7-8]. While a number

of pharmacological effects, such as anti-inflammatory, antioxidant, neuroprotective, hyperglycemic, and anticancer, have been demonstrated for the genus *Beta vulgaris* L. Additionally, earlier studies have demonstrated the anticancer *activity of Beta vulgaris* L. against tumor cells, particularly breast cancer cells. Many illnesses, such as leukemia, esophageal cancer, glandular cancer, prostate cancer, and breast cancer, are treated with it in traditional medicine.[9-12]

Material and Method

Collection of Plants

The fresh leaves of *Sesbania Grandiflora* and root of *Beta Vulgaris* was collected from local area of Ale, Junnar ,Pune ,Maharashtra .Taxonomically leaves of Sesbania *Grandiflora* and root of *Beta Vulgaris* was identified and authenticated by Dr.R.K Chaudhary,Scientist, Agharkar Research Institute, Autonomous Body under DST, GOI, Pune. Herbarium specimens have been preserved in the laboratory.

Extraction and Lyophilization

The collected plant material were shade dried, coarsely powdered using mixer grinder and passed through 100 number sieve and stored in an airtight container.100 grams of each powder were extracted with methanol by Soxhlet Extraction procedure till complete powder color disappears .The extract were concentrated under vacuum using rotary evaporator at 40°C.The concentrated extract was freeze dried at -20°C for 12h then lyophilized using lyophilizer .These lyophilized extract was stored in air tight container and kept in desiccators for further study.

Chemicals: Streptozotocin was procured from Sigma Chemical Laboratories, Shree Chemicals, Pune. Glibenclamide Tablet (5mg) was purchased from Aventis Pharma, Citrate Buffer, Glucose was purchased from Scientific Chemicals, Mumbai.

Animals

Adult male Wistar rats (180-250 g) were procured from Lachmi Biofarm Pvt.Ltd,,Pune ,Maharashtra India. The animals were housed in large, spacious polyacrylic cages at an ambient room temperature with 12-h light/12-h dark cycle. Rats had free access to water and rodent pellets diet (Nutrivate Pvt. Ltd, Bangalore, India). The study was approved by the Institute

Animal Ethics Committee of the Vishal Institute of Pharmaceutical Education and Research Ale with Reg. No. 1409/PO/RE/S/11/IAEC/2020-2021/07/01 were used for the study and all the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Ministry of Environment and Forests, Government of India.

Development of Polyherbal formulation [13,14]

The lyophilized powder of methanolic extract was evaluated for antihyperglycemic potential using the OGTT model in Wistar rats for a single dose of 1000 mg/Kg. As a result, by altering the ratios, several extract combinations were developed for the formulation design .The three distinct batches of polyherbal formulation, as listed in Table No. 1 below, contain methanolic extracts of leaves of *Sesbania Grandiflora* and root of *Beta Vulgaris* in varying ratios. According to WHO guidelines for herbal medicine quality control, batches were tested for quality. The batch optimized after OGTT was chosen for further *in vivo* activity on diabetes.

Table no:1 Polyherbal Formulation design

Formulation	Drug combination	Ratio
PHF 1	MEBG+MESV	2:1
PHF2	MEBG+MESV	1:1
PHF3	MEBG+MESV	1:2

PHF: Polyherbal Formulation, MESG: Methanolic extract of *Sesbania Grandiflora* leaves, MEBV- Methanolic extract of *Beta Vulgaris* Root.

Optimization of the formulation as per Oral Glucose Tolerance Test Model

The OGTT study was conducted on overnight fasted glucose (2g/kg)induced hypoglycemic normal rats. The rats were divided into five groups (n=6)

Group I-Normal Group-0.5 % w/v carboxymethylcellulose (CMC) solution pretreated rats.

Group II-Glucose Load- Glibenclamide 5mg/kg

Group III-Single dose levels (1000 mg/kg per oral) serving as bPHF1-Animal treated with combination of MESG and MEBV with ratio 2:1

Group IV-Single dose levels (1000 mg/kg per oral) serving as PHF2-Animal treated with combination of equal amount of extracts of MESG and MEBV i.e with ratio 1:1

Group V-Single dose levels (1000 mg/kg per oral) serving as PHF2-Animal treated with combination of equal amount of extracts of MESG and MEBV i.e with ratio 1:2

The blood sample was withdrawn from the tail vein before and 0,30,60,90,120 min after glucose administration .The serum glucose level was estimated within 30 mins of withdrawal of the blood sample. Also the urine samples collected was microscopically examined for presence of crystals of Uric Acid and Calcium Oxalate.

***In-vivo* Antidiabetic Effect of Polyherbal Formulation in Streptozotocin Induced Diabetic Rats[13-17]**

Administration of Glibenclamide (GLB) and Streptozotocin (STZ)

A single intraperitoneal (i.p.) dose of freshly prepared Streptozotocin (STZ) 45 mg/kg in 0.1 M citrate buffer (pH 4.5) was given to overnight-fasted Wistar Albino rats to induce diabetes. In order to prevent hypoglycemia-related death, the rats were given 5 % w/v glucose solution and given access to a standard diet after receiving STZ for 24 hours. The animals treated with STZ were found to have diabetes when their fasting blood glucose levels were measured 48 hours after induction. The standard dosage of Glibenclamide was given orally once a day for 30 days in a suspension of 0.5% w/w distilled water. **[13-15]**

Administration of Polyherbal Formulation

PHF2 extract was suspended in 5 ml of sterile water and administered orally for 30 days, while the control group received water as a vehicle. After 4 hours of Polyherbal formulation administration, the rats were allowed free access to food (standard rodent pellet).

Experimental Design

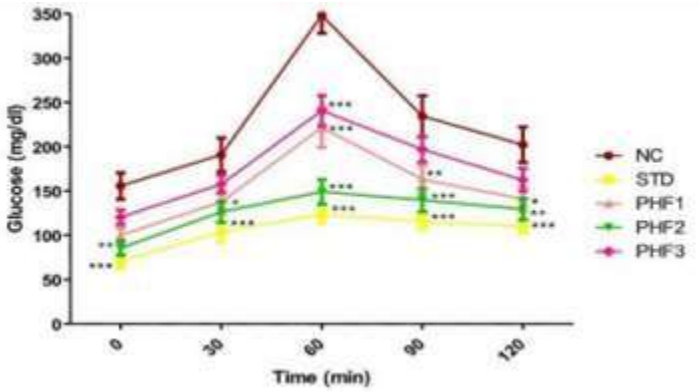
After 48 hours of induction, fasting blood glucose levels were measured to confirm the presence of diabetes in the STZ-treated animals. Wistar albino rats were randomly assigned to Group II–Group V after their blood glucose levels exceeded 200 mg/dl, which was considered the threshold for diabetes

Table no: 2 Experimental Design of Antidiabetic Polyherbal Formulation

Group	Codes	Route and Dose of drug
Group I	Normal control(NC)	Orally with vehicle (1 ml/kg BW)
Group II	Diabetic Control(DC)	Orally with STZ (45mg/kg BW)
Group III	Test solution (F 200)	Orally with vehicle (200 mg/kg BW)
Group IV	Test solution (F 400)	Orally with vehicle (400 mg/kg BW)
Group V	Standard control(STD)	Orally with Glibenclamide (5 mg/kg BW)

Diabetes was produced in overnight starved rats with a single intraperitoneal (i.p.) injection of freshly prepared Streptozotocin (STZ) 45 mg/kg b.w., in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg b.wt. Diabetes was confirmed in STZ rats after 48 hours of induction by assessing fasting blood glucose levels. To prevent hypoglycemia mortality, the rats were administered 5% w/v glucose solution (2 ml/kg b.w.) after STZ injection. Diabetic rats had fasting blood glucose levels of greater than 200 mg/dl and were randomly assigned to one of four groups. The standard (Glibenclamide) and Polyherbal formulation were suspended in 1% w/v carboxymethyl cellulose (CMC) and given orally once daily for 21 days. Blood samples were taken by pricking the tail vein of rats on the first, seventh, fourteenth, and twenty-first days of therapy and were immediately utilized to estimate blood glucose with a Glucometer. All of the experimental animals' weekly body weight fluctuations were tracked.[16,17,18] At the conclusion of the examination, blood was collected from all of the experimental animals through retro-orbital plexus puncture for further biochemical studies.

Result



Effect of PHF 1, PHF 2, PHF 3 extract on blood glucose level (mg/dl) in experimental group of rats receiving an oral glucose load. Values are Expressed As Mean ± SEM (n=6) analyzed by two-way Anova***represent significance At $p < 0.001$

***Invivo* Antidiabetic Effect of Polyherbal Formulation on Biochemical parameters in Streptozotocin Induced Diabetic Rats**

Biochemical Parameters

Blood Glucose (mg/dl)

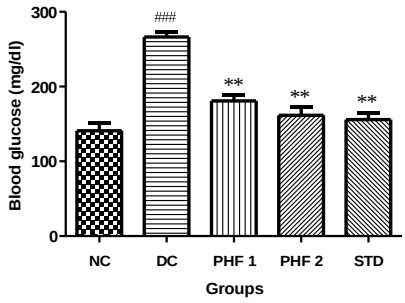


Figure 1: Effect of PHF 200 and 400 on Blood glucose level (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increase in blood glucose level when compared with DC rats. However, the treatment of rats with PHF (200 and

400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) decrease in blood glucose level when compared with DC rats.

Serum Creatinine (mg/dl)

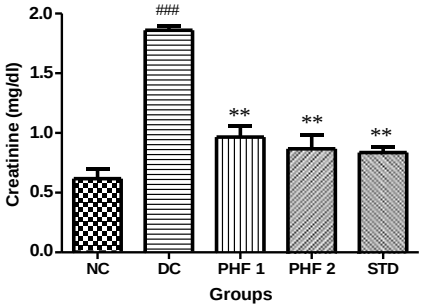


Figure 2 : Effect of PHF 200 and 400 on creatinine level (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increase in creatinine level when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.001$) decrease in creatinine level when compared with DC rats.

Serum Protein (g/dl)

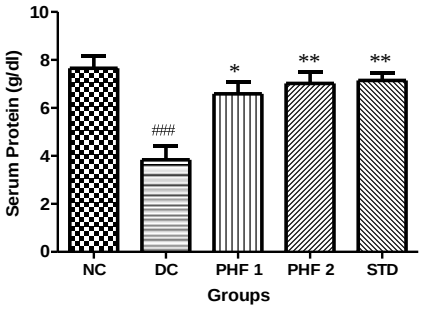


Figure 3: Effect of PHF 200 and 400 on serum proteins level (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) decrement in protein levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) increment in protein levels when compared with DC rats.

Alanine transaminase (IU/L)

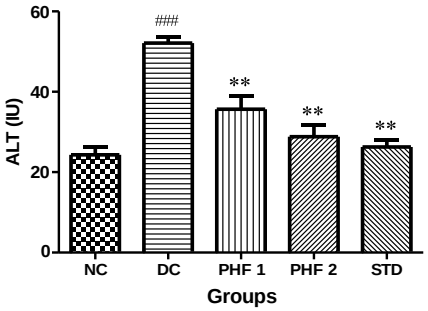


Figure 4: Effect of PHF 200 and 400 on Alanine transaminase level (IU/L) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in alanine transaminase levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.001$) decrement in alanine transaminase levels when compared with DC rats.

Aspartate aminotransferase (IU/L)

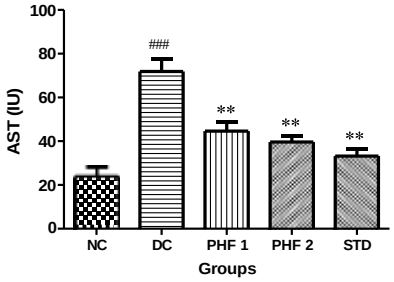


Figure 5: Effect of PHF 200 and 400 on Aspartate transaminase level (IU/L) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in aspartate transaminase levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.001$) decrement in aspartate transaminase levels when compared with DC rats.

Blood Urea Nitrogen (mg/dl)

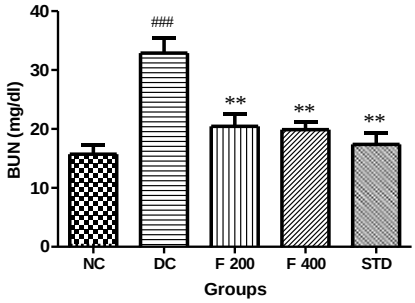


Figure 6: Effect of PHF 200 and 400 on BUN (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in BUN levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.001$) decrement in BUN levels when compared with DC rats.

Total Cholesterol (mg/dl)

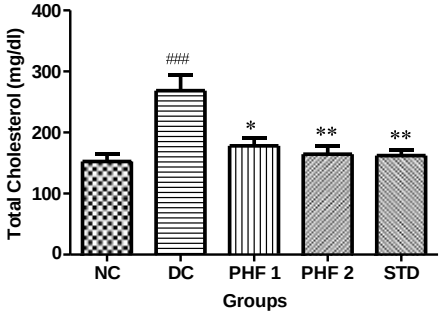


Figure 7

Figure 7: Effect of PHF 200 and 400 on Total Cholesterol (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 200 and 400 on total cholesterol (mg/dl) in STZ induced diabetes in rats are shown in Figure 7. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in total cholesterol levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) decrease in total cholesterol levels when compared with DC rats.

Triglycerides (mg/dl)

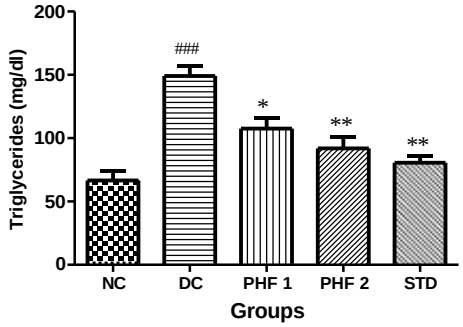


Figure 8

Figure 8: Effect of PHF 200 and 400 on Triglycerides (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in triglycerides levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) decrement in triglycerides levels when compared with DC rats.

HDL Cholesterol (mg/dl)

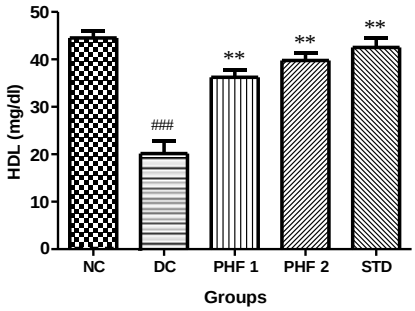


Figure 9: Effect of PHF 200 and 400 on High Density Lipoprotein (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant (p<0.001) decrement in HDL levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant (p<0.001) increment in HDL levels when compared with DC rats.

LDL Cholesterol (mg/dl)

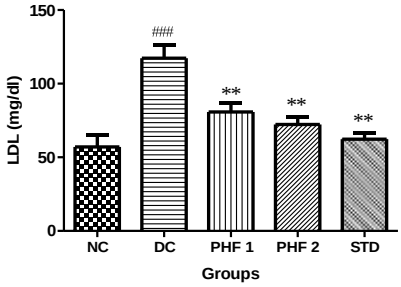


Figure 10: Effect of PHF 200 and 400 on Low Density Lipoprotein (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant (p<0.001) increment in LDL levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant (p<0.001) decrement in LDL levels when compared with DC rats.

VLDL Cholesterol (mg/dl)

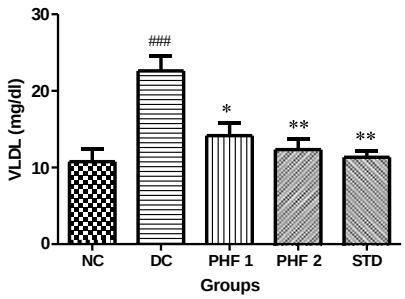


Figure 11

Figure 11: Effect of PHF 200 and 400 on Very Low Density Lipoprotein (mg/dl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in VLDL levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) decrement in VLDL levels when compared with DC rats.

Conclusion

When compared to normal control, the results showed a progressive loss of body weight in diabetic control. This could be the result of an excessive breakdown of fatty acids and tissue proteins brought on by a drop in plasma insulin levels. A lack of insulin can slow down the synthesis of proteins and quicken the breakdown of metabolites, raising blood levels of amino acids that are used in the process of gluconeogenesis. Body weight increased following administration of PHF 400 mg/kg of the extract compared to Group 2. .. , The treatment of rats with PHF2 (200 and 400 mg/kg, p.o.) and Glibenclamide exhibited significant ($p<0.001$) changes in Biochemical parameters such as hemoglobin, glycosylated hemoglobin, high-density lipoprotein, low-density lipoprotein, glucose, urea, creatinine, serum cholesterol, serum triglyceride, and it was discovered that PHF's methanol extracts had strong antihyperglycemic effects. We appeal to the conclusion that the plant fraction and extract that were tested for their antidiabetic properties significantly reduced serum glucose levels and other diabetes-related

complications. The results of this study lend support to the use of this plant in conventional antidiabetic preparations; formulations based on the plant's fraction and identified effective extract may be more effective than those currently on the market that use crude aqueous extract.

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Conflict of interest

No conflict of interest in the present study

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