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Original Article

Hematoprotective Properties of Polyherbal Formulation on Streptozocin Induced Diabetic Wistar Rats

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ABSTRACT

Background: In order to treat type 2 diabetes mellitus, the study focuses on polyherbal anti-diabetic extracts from several plants that are administered at varying dosages. Ayurvedic medicines are widely accepted due to its efficacy, safety, affordability, ubiquity, and acceptance. Because polyherbal medicines comprise glycosides, alkaloids, flavonoids, and other compounds with different modes of action, they have been used for a long time to treat diabetes worldwide. This study examined the antidiabetic and haematological effects of polyherbal formulation (PHF) in diabetic rats induced with streptozotocin (STZ).
Objective: To examine the antidiabetic effects of Polyherbal formulation on haematological parameters in streptozotocin-induced diabetic rats.
Method: In this study,Wistar albino rats (n=6) were split up into five groups Streptozotocin was injected intraperitoneally to male Wistar rats to cause diabetes. After being confirmed diabetic, animals were treated orally with distilled water or extracts at 200 or 400 mg/kg body weight daily for 30 days.
Results: Blood glucose levels were significantly decreased by the extract, with the highest results being at 400 mg/kg body weight. After extract administration at both doses, the quantities of red blood cells, white blood cells, and their functional keys all considerably increased. Also in diabetic rats, water and feed consumption were intensely decreased, and weight loss was minimized at both dosages.

Introduction

Over the past 20 years, the number of persons with diabetes has more than doubled globally. The rise of type 2 diabetes in kids, teens, and young people is one of this rapid increase's most concerning trends [1].

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Uncontrolled diabetes can lead to complications in several organs. Lower limb amputations can result from heart attacks, strokes, kidney failure, and injury to both large and small blood arteries as well as nerves. Diabetes causes impairments and shortens life. Despite the fact that diabetes has been recognized as a serious condition and mentioned in ancient writings, it does not appear that medical professionals or healers have frequently dealt with it [2]. Kidney failure, blindness, and a general decline in quality of life are caused by severe microvascular problems such as diabetic neuropathy, diabetic retinopathy, and diabetic kidney disease, as well as debilitating macrovascular issues like

heart disease[3] Insulin and a number of oral hypoglycemic drugs, including biguanides and sulfonylureas, are currently available as therapies for diabetes mellitus. These drugs are used to treat diabetes mellitus, but they have a number of disadvantages, including high secondary failure rates and adverse effects.To meet this need, the diverse traditional plant kingdom offers several intriguing therapeutic benefits. Diabetes has been treated with a variety of natural therapies [4] "A medicinal plant is a plant that, 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," according to the World Health Organisation (WHO). The ancient literature contains extensive documentation of the polyhedral formulation concept. The medicinal potential of the polyherbal formulation is greater and more extensive than that of the single plant. In order to create and standardise a polyherbal formulation employing a plant known to have antidiabetic action, the current study was designed to assess its therapeutic benefits in rodents.[5] The medicine formulation in Ayurveda is based on two principles:

1. Polyherbal (PH) formulations use multiple herbs to create a single product. To achieve therapeutic effectiveness, it combines many herbs.

2. With a broad therapeutic index, PH is safe at high dosages and remains effective at low doses (better risk to benefit ratio) than allopathic hypoglycemic medications, which have a limited therapeutic range. pH is ideal for medical therapies due to its efficacy, safety, affordability, accessibility, and acceptance. PH can have the most positive therapeutic effects on human health when used appropriately and prudently. Diabetes mellitus is becoming more common in the community, which puts a financial burden on both those who have the illness and the healthcare system overall[6] Antioxidant and antiurolithiatic properties, anticancer and chemopreventive properties, anxiolytic and anticonvulsive properties, hepatoprotective and cardioprotective properties, antiulcer properties, antimicrobial properties, analgesic and antipyretic properties, diuretics, CNS depressant and laxative hypolipidemic properties, and anthelmintic properties have all been demonstrated in prior studies on S. grandiflora. After a careful review of the literature, it was found that little research had been done on the leaves' potential to prevent diabetes [7,8]. However, a variety of pharmacological activities, such as anti-inflammatory, antioxidant, neuroprotective, hyperglycemic, and anticancer capabilities, have been demonstrated for the genus Beta vulgaris L. Additionally, earlier studies have demonstrated the anticancer properties of Beta vulgaris L. against tumor cells, particularly breast cancer. B. vulgaris subsp. maritima is both a traditional food and an old medicinal herb. Folk medicine uses it to treat a number of illnesses, such as breast cancer, prostate cancer, glandular cancer, esophageal cancer, and leukemia [9,10,11,12,]

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 identified leaves of Sesbania Grandiflora and root of Beta Vulgaris was Identified and authenticated by Dr. R. K Chaudhary, Scientist, Agarak Research Institute, Autonomous Body under DST, GOI, Pune. Herbarium specimen has been preserved in laboratory.

Chemicals

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

Animals

Adult male Wistar rats (180-250 g) of were procured from Lachmi Biofarms 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. The work was approved from the Institutional Animal Ethical Committee (IAEC). Institution registration number1409/PO/RE/S/11/CPCSEA. Proposal number: PIPH 07/01, of Acclimatization of Animals Vishal Institute of Pharmaceutical Education and Research Ale

Preparation of Methanolic Extract of Sesbania Grandiflora and Beta Vulgaris

Methanolic extracts of Sesbania Grandiflora and root of Beta Vulgaris was obtained by Soxhlet extraction method in methanol solvent for 48 hours. The extracts

were evaporated to dryness (resinous material) under reduced pressure at 60°C and stored at 4°C until use.

Preparation of Polyherbal formulation

The different three batches of polyherbal formulation containing methanolic extract of leaves of Sesbania Grandiflora and methanolic extract of Beta Vulgaris root with different ratio as mentioned below in table no 1. Batches were tested for quality as per WHO guidelines for quality control of herbal medicine. Optimized batch was selected for further In vivo studies for antidiabetic studies.

Table 1: Polyherbal Formulation design.

Name of formulation	Drug combination	Ratio
PHF 1	MESG+MEBV	1:2
PHF2	MESG+MEBV	1:1
PHF3	MESG+MEBV	2:1

PHF: Polyherbal Formulation, MESG: methanolic extract of Sesbania Grandiflora leaves, MEBV- methanolic extract of Beta Vulgaris Root [13].

Acute toxicity studies

Acute Toxicity Studies: 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 male Wistar rats (3 animals/dose) were used for the experiment. Overnight fasted rats were orally fed with the

Polyherbal formulation in increasing dose was 5mg, 50mg, 300 mg and 2000 mg/kg body weight. 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]

In vivo Antidiabetic Activity Antidiabetic Effect of Polyherbal Formulation in Streptozotocin Induced Diabetic Rats

Streptozotocin (STZ) & Glibenclamide (GLB) administration Diabetes was induced in overnight-fasted Wistar Albino rats by administering single intraperitoneal (i.p.) dose of freshly prepared streptozotocin (STZ) 45 mg/kg in 0.1 M citrate buffer (pH 4.5) .After 24 h of STZ administration, the rats were given 20% w/v of glucose solution to prevent hypoglycaemic mortality and allowed access to standard diet. Diabetes was confirmed in STZ treated animals by measuring fasting blood glucose levels after 48 h of induction. The standard glibenclamide were suspended in 0.5% w/w distilled water and administered once daily through oral gavage for 30 consecutive days [13,14,15].

Administration of Polyherbal Formulation

PHF2 extract was suspended in 1ml of sterile water and administered orally for 30 days; while the control group received water as a vehicle. After 4hours of Polyherbal formulation administration, the rats were allowed free access to food (standard rodent pellet).

Experimental Design

Diabetes was confirmed in STZ-treated animals by measuring fasting blood glucose levels after 48 h of induction. Wistar albino rats measuring above 200 mg/dl of blood glucose levels were considered as diabetics and randomly divided into Group II- Group V.

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 herbal 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 haematological studies.

Table 2: Experimental Design of Antidiabetic Polyherbal Formulation.

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

Result

Acute Toxicity Study

Acute toxicity trials up to 2000 mg/kg administered as a single oral dosage revealed no deaths. As a result, the study was conducted at dose levels of 200 and 400 mg/kg

***In vivo* Antidiabetic Effect of Polyherbal Formulation on Haematological Parameters in Streptozotocin Induced Diabetic Rats**

Hematological Parameters

Hemoglobin (g/dl)

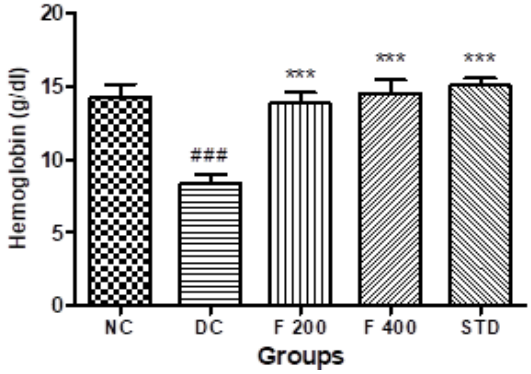

Figure 1: Effect of PHF 200 and 400 on Hemoglobin (g/dl) in STZ-induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###p<0.001 versus NC rats and ***p<0.001 versus DC rats.

The effects of PHF 200 and 400 on Hemoglobin (g/dl) in STZ induced diabetes in rats are shown in Figure 1. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) decrease in Hemoglobin count 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 Hemoglobin count when compared with DC rats (Figure 1).

Total RBC Count (millions /Cu mm)

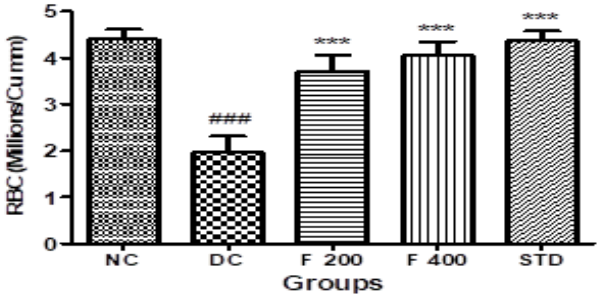

Figure 2: Effect of PHF 200 and 400 on RBC (Million/Cu mm) in STZ-induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###p<0.001 versus NC rats and ***p<0.001 versus DC rats.

The effects of PHF 200 and 400 on Total RBCs in STZ induced diabetes in rats are shown in Figure 2. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) elevation in Total RBC count 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 ($p<0.001$) significant ($p<0.001$) increment in Total RBC count when compared with DC rats.

Packed Cell Volume (%)

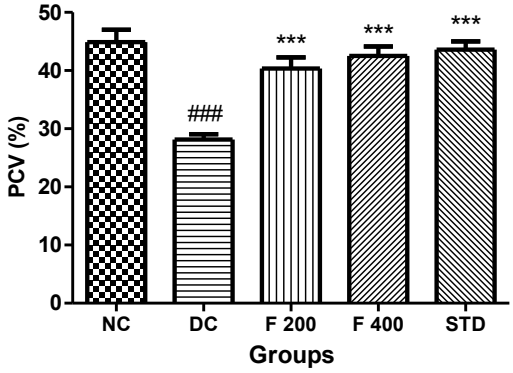

Figure 3: Effect of PHF 200 and 400 on PCV (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 200 and 400 on PCV (%) in STZ induced diabetes in rats are shown in Figure 3. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) decrease in PCV count 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 PCV count when compared with DC rats.

Mean Corpuscular Volume (fl)

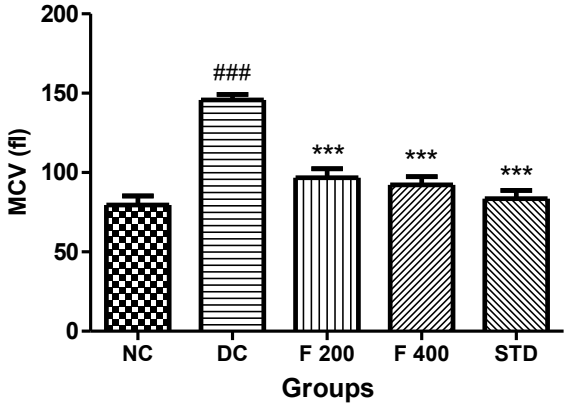

Figure 4: Effect of PHF 200 and 400 on MCV (fl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 200 and 400 on MCV (fl) in STZ induced diabetes in rats are shown in Figure 4. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in MCV count 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 MCV count when compared with DC rats.

Mean Corpuscular Hemoglobin (%)

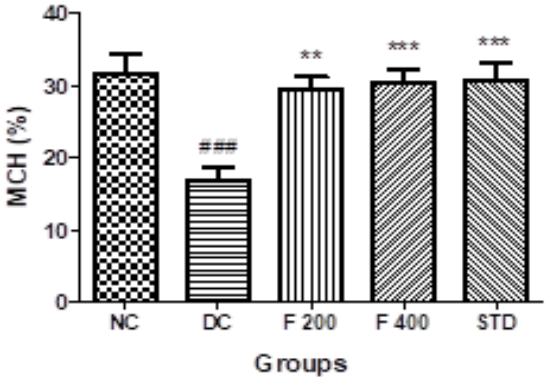

Figure 5: Effect of PHF 200 and 400 on MCH (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###p<0.001 versus NC rats and **p<0.01; ***p<0.001 versus DC rats.

The effects of PHF 200 and 400 on MCH (%) in STZ induced diabetes in rats are shown in Figure 5. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant (p<0.001) decrease in MCH count 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) increase in MCH count when compared with DC rats.

Mean Corpuscular Hemoglobin Concentration

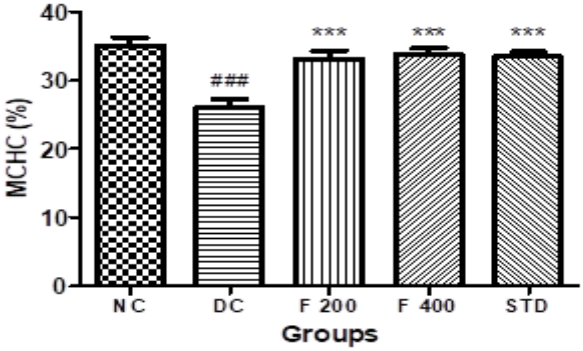

Figure 6: Effect of PHF 200 and 400 on MCHC (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###p<0.001 versus NC rats and ***p<0.001 versus DC rats.

The effects of PHF 200 and 400 on MCHC (%) in STZ induced diabetes in rats are shown in Figure 6. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant (p<0.001) decrease in MCHC count 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) increase in MCHC count when compared with DC rats.

Total WBC Count (millions /Cu mm)

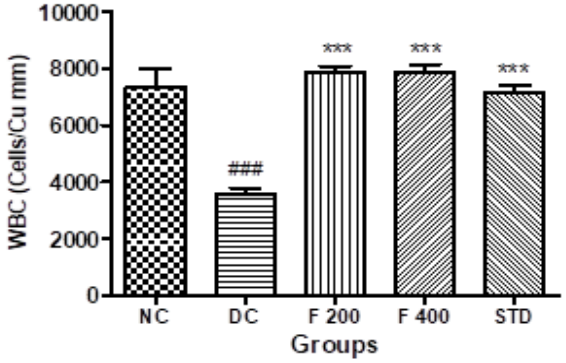

Figure 7: Effect of PHF 200 and 400 on WBC (Cells/Cumm) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###p<0.001 versus NC rats and ***p<0.001 versus DC rats.

The effects of PHF 200 and 400 on WBC count in STZ induced diabetes in rats are shown in Figure 7. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) decrease in WBC count 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 ($p<0.001$) increase in WBC count when compared with DC rats.

Polymorphs (%)

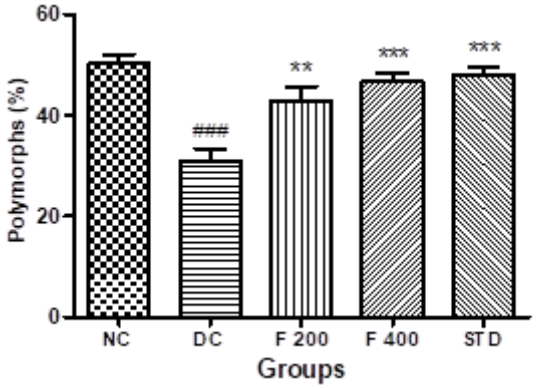

Figure 8: Effect of PHF 200 and 400 on Polymorphs (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{**}p<0.01$; $^{***}p<0.001$ versus DC rats.

The effects of PHF 200 and 400 on polymorphs (%) in STZ induced diabetes in rats are shown in Figure 8. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) decrease in polymorphs count 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 ($p<0.01$; $p<0.001$) increase in polymorphs count when compared with DC rats.

Lymphocytes (%)

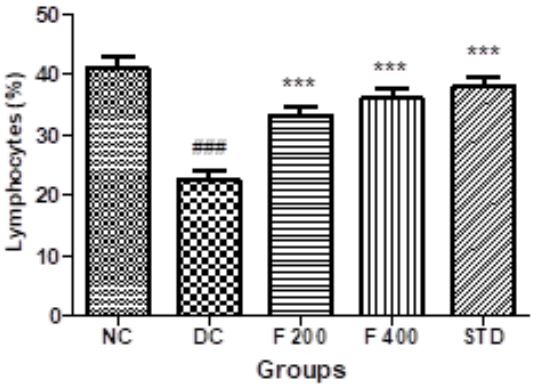

Figure 9: Effect of PHF 200 and 400 on Lymphocytes (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 200 and 400 on Lymphocytes (%) in STZ induced diabetes in rats are shown in Figure 9. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) decrease in lymphocytes count 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 ($p<0.001$) increase in lymphocytes count when compared with DC rats.

Eosinophils (%)

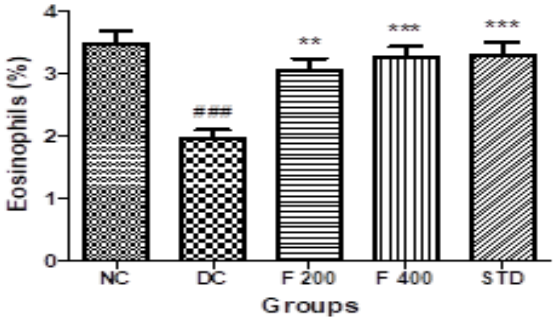

Figure 10: Effect of PHF 200 and 400 on Eosinophils (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###p<0.001 versus NC rats and **p<0.01; ***p<0.001 versus DC rats.

The effects of PHF 200 and 400 on Polymorphs (%) in STZ induced diabetes in rats are shown in Figure 10. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant (p<0.001) decrease in eosinophils count 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) increase in eosinophils count when compared with DC rats.

Monocytes (%)

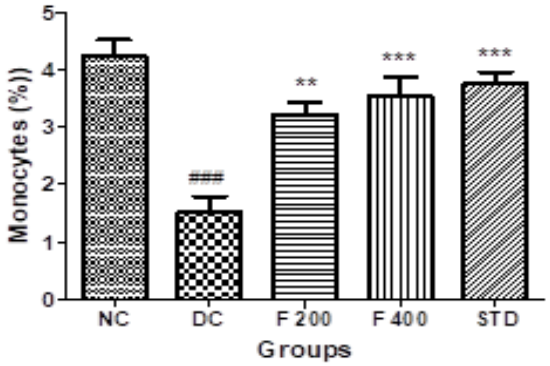

Figure 11: Effect of PHF 200 and 400 on Monocytes (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###p<0.001 versus NC rats and **p<0.01; ***p<0.001 versus DC rats.

The effects of PHF 200 and 400 on monocytes (%) in STZ induced diabetes in rats are shown in Figure 11. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant (p<0.001) decrease in monocytes count 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) in monocytes count when compared with DC rats.

Basophils (%)

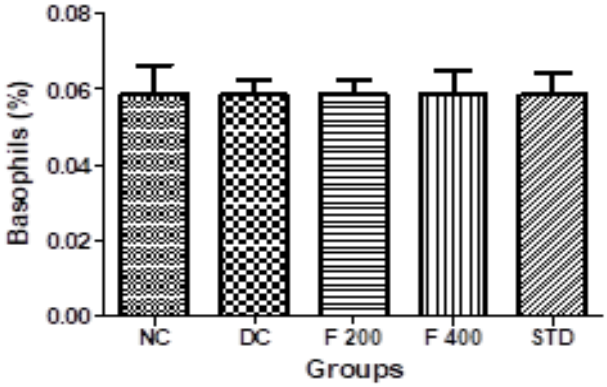

Figure 12: Effect of PHF 200 and 400 on Basophils (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###p<0.001 versus NC rats and **p<0.01; ***p<0.001 versus DC rats.

The effects of PHF 200 and 400 on basophils (%) in STZ-induced diabetes in rats are shown in Figure 12. The treatment of rats with STZ (45 mg/kg, i.p.), PHF2 (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited non-significant change in basophils count when compared with NC rats.

Platelet Count (Lakhs/Cu mm)

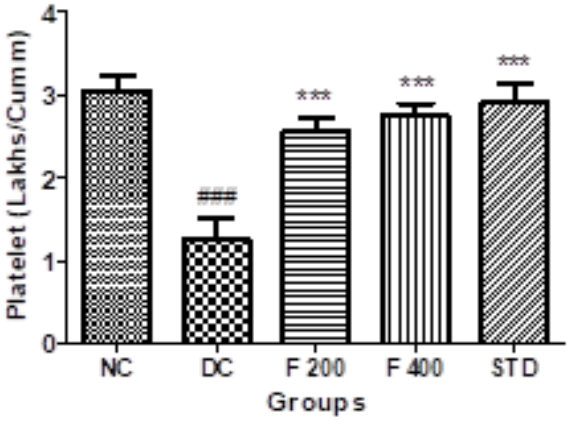

Figure 13: Effect of PHF 200 and 400 on Platelet (Lakhs/Cumm) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 200 and 400 on platelet (Lakhs/Cumm) in STZ induced diabetes in rats are shown in Figure 11. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) decrease in platelet count 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 ($p<0.01$; $p<0.001$) increase in platelet count when compared with DC rats.

Conclusion

The result revealed a progressive body weight loss in diabetic control as compared to Normal control. This may be due excessive breakdown of tissue protein and fatty acids due to decrease in plasma insulin level. Insulin deficiency may impede protein synthesis and accelerate metabolite breakdown, resulting in higher amino acid levels in the blood, which are then used for gluconeogenesis.[11] 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 ($p<0.001$) increase in Hemoglobin count, Total RBC count, MCH count,,PCV count, MCHC count, WBC count, polymorphs count ,lymphocytes, eosinophils, monocytes count ,platelet count, while PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.001$) decrement in MCV count when compared with DC rats. But the treatment of rats with STZ, PHF2, and Glibenclamide exhibited a non-significant change in basophil count when compared with NC rats.

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Conflict of Interest

None declared.

Author Contributions

All the authors contributed to the study.

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