



Antidiabetic and Hematological effect of Polyherbal formulation on Streptozocin Induced diabetic Wistar Rats

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Abstract

The study focuses on polyherbal anti-diabetic extracts from different plants that are used at different doses to manage type 2 diabetes mellitus. The effectiveness, safety, affordability, ubiquity, and acceptance of ayurvedic treatments have directed to their widespread acceptance. Throughout world, long utilization of polyherbal remedies to treat diabetes because they contain glycosides, alkaloids, flavonoids, and other chemicals with distinct modes of action. This study was conducted on diabetic rats induced with streptozotocin (STZ) to examine the antidiabetic and haematological effect of polyherbal formulation (PHF).

Objective

To examine the antidiabetic effects of Polyherbal formulation on hematological 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.

Keywords: Diabetis, Polyherbal formulation, Streptozotocin, Wistar Albino Rats, Haematological parameters

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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] Diabetes that is not under control can cause difficulties in numerous organs. Amputations of the lower limbs are caused by heart attacks, strokes, loss of function in the kidneys, and damage to tiny and major blood vessels as well as nerves. Diabetes shortens life and creates disabilities. Although diabetes has been acknowledged as a dangerous illness and has been documented in ancient texts, it does not seem that doctors or healers have regularly faced it. ^[2] Severe microvascular challenges including diabetic neuropathy, diabetic retinopathy, and diabetic kidney disease, as well as devastating macrovascular complications like heart disease, cause kidney failure, blindness, and a general reduction in quality of life in those with diabetes. ^[3] At the moment, insulin and several oral hypoglycemic medications such biguanides and sulfonylureas are available as treatments for DM. These medications are used to treat DM, although they have some drawbacks, like side effects and high secondary failure rates. The wide range traditional plant kingdom has a lot of promising therapeutic values to satisfy this requirement. Numerous natural remedies have been advocated for the treatment of diabetes. ^[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 chemopharmaceutical semi-synthesis," according to the World Health Organisation (WHO). The ancient literature contains extensive documentation of the polyherbal 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. In contrast to allopathic hypoglycaemic medicines, which have a narrow therapeutic range, PH has a wide therapeutic index, making it safe at high doses while still being effective at low doses (better risk to benefit ratio). Because of their effectiveness, safety, low cost, accessibility, and acceptance, pH is perfect for medical treatments. By using PH properly and sensibly, it can have the most beneficial therapeutic effects on human health. The prevalence of DM is rising in the population, placing a financial strain on both those with the condition and the healthcare system as a whole. ^[6] Previous research has shown that *S. grandiflora* has antioxidant and antiurolithiatic activity, as well as anticancer and chemopreventive activity, anxiolytic activity and anticonvulsive effect, hepatoprotective activity, cardioprotective effect, antiulcer activity, antimicrobial activity, analgesic and antipyretic activity, diuretic, CNS depressant and laxative hypolipidemic activity, and anthelmintic activity. Following a thorough evaluation of the literature, there is a lack of study effort documented on the leaves component of for anti-diabetic effects ^[7,8]. While genus *Beta vulgaris* L. has been shown to have a wide range of

pharmacological effects including anti-inflammatory, antioxidant, neuroprotective, hyperglycemic, and anticancer properties. Also prior research has shown that *Beta vulgaris* L. has anticancer activity against tumor cells, specifically breast cancer. *B. vulgaris* subsp. *maritima* is an ancient medicinal herb as well as a traditional meal. It is used in folk medicine to treat a variety of ailments, including leukemia, esophageal cancer, glandular cancer, prostate cancer, and breast cancer.^[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, Agharkar 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. 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 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 number 1409/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 no: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 behavioral (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.

Table no: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 Glibenclamidee (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 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.

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)

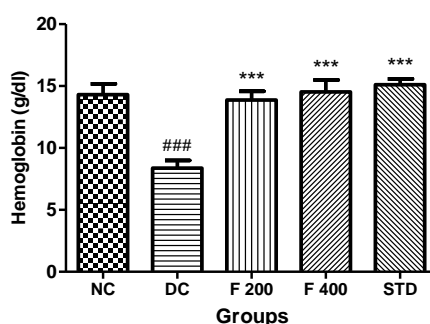


Figure 1: Effect of PHF 200 and 400 on Hemoglobin (g/dl) in STZ-induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### $p < 0.001$ versus NC rats and *** $p < 0.001$ versus DC rats.

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)

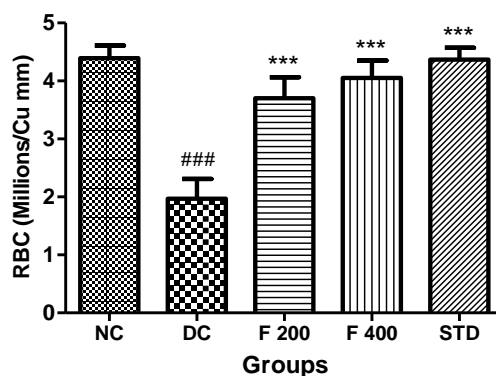


Figure 2: Effect of PHF 200 and 400 on RBC (Million/Cu mm) in STZ-induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### p <0.001 versus NC rats and *** p <0.001 versus DC rats. The effects of PHF 200 and 400 on Total RBCs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

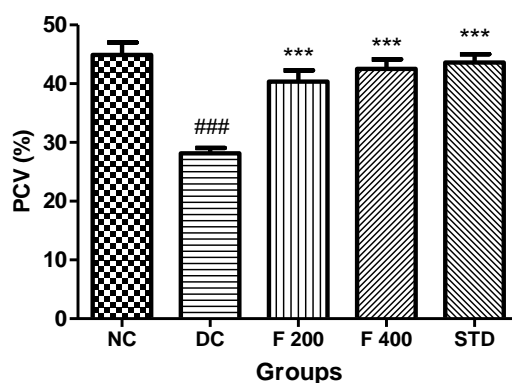


Figure 3: Effect of PHF 200 and 400 on PCV (%) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### p <0.001 versus NC rats and *** p <0.001 versus DC rats.

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)

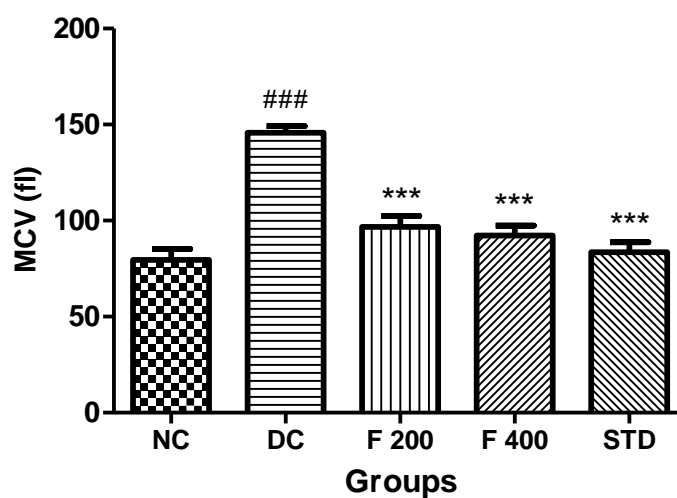


Figure 4: Effect of PHF 200 and 400 on MCV (fl) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### p <0.001 versus NC rats and *** p <0.001 versus DC rats. The effects of PHF 200 and 400 on MCV (fl) in STZ induced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

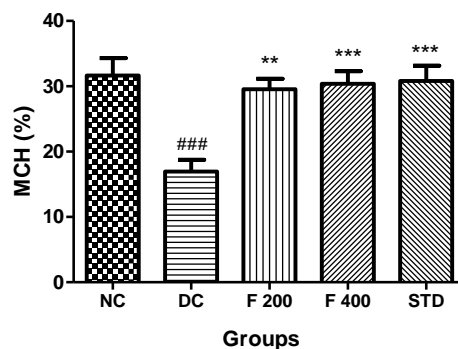


Figure 5: Effect of PHF 200 and 400 on MCH (%) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### p <0.001 versus NC rats and ** p <0.01; *** p <0.001 versus DC rats. The effects of PHF 200 and 400 on MCH (%)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

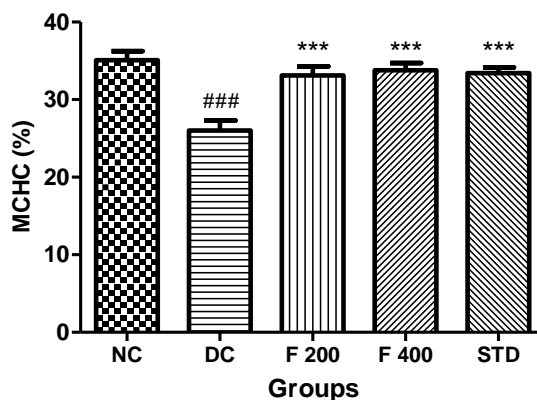


Figure 6: Effect of PHF 200 and 400 on MCHC (%) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### p <0.001 versus NC rats and *** p <0.001 versus DC rats. The effects of PHF 200 and 400 on MCHC (%) in STZ induced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

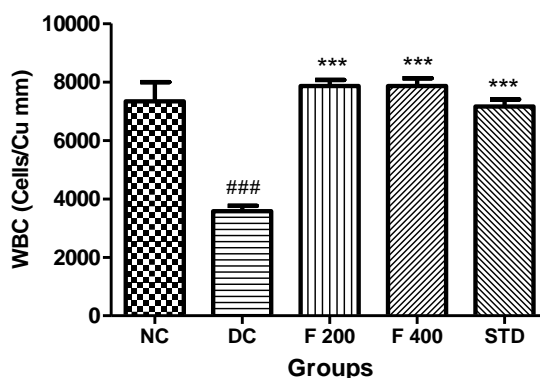


Figure 7: Effect of PHF 200 and 400 on WBC (Cells/Cumm) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### p <0.001 versus NC rats and *** p <0.001 versus DC rats. The effects of PHF 200 and 400 on WBC count in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

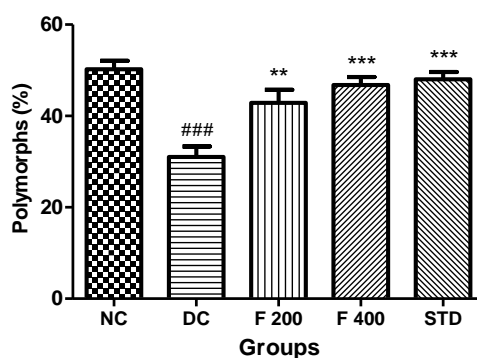


Figure 8: Effect of PHF 200 and 400 on Polymorphs (%) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### p <0.001 versus NC rats and ** p <0.01; *** p <0.001 versus DC rats. The effects of PHF 200 and 400 on polymorphs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

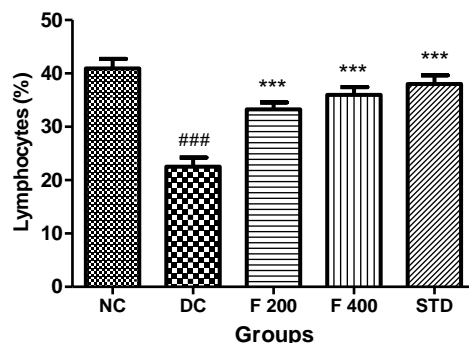


Figure 9: Effect of PHF 200 and 400 on Lymphocytes (%) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM ($n = 6$) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### $p < 0.001$ versus NC rats and *** $p < 0.001$ versus DC rats. The effects of PHF 200 and 400 on Lymphocytes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

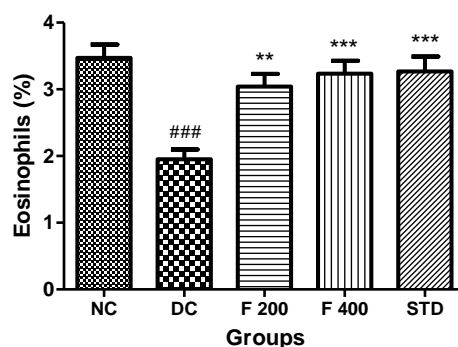


Figure 10: Effect of PHF 200 and 400 on Eosinophils (%) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM ($n = 6$) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### $p < 0.001$ versus NC rats and ** $p < 0.01$; *** $p < 0.001$ versus DC rats. The effects of PHF 200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

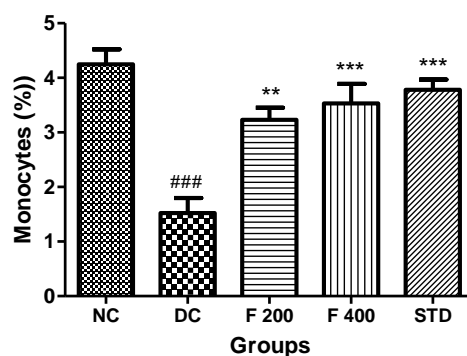


Figure 11: Effect of PHF 200 and 400 on Monocytes (%) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM ($n = 6$) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ### $p < 0.001$ versus NC rats and * $p < 0.01$; *** $p < 0.001$ versus DC rats.

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 ($p < 0.01$; $p < 0.001$) increase in monocytes count when compared with DC rats.

Basophils (%)

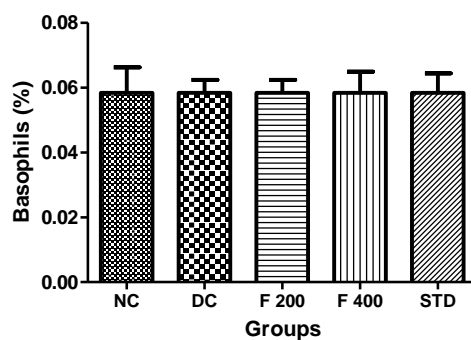


Figure 12: Effect of PHF 200 and 400 on Basophils (%) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $###p < 0.001$ versus NC rats and $**p < 0.01$; $***p < 0.001$ versus DC rats.

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)

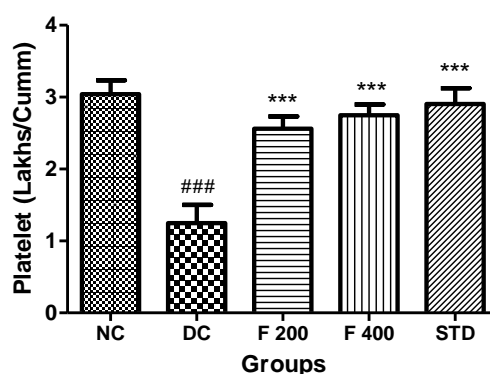


Figure 13: Effect of PHF 200 and 400 on Platelet (Lakhs/Cumm) in STZ induced Diabetes in rats. Values are expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $###p < 0.001$ versus NC rats and $***p < 0.001$ versus DC rats. The effects of PHF 200 and 400 on platelet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

Results

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

No conflict of interest in the present study

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