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Research Article

# Hematoprotective Properties of Polyherbal Formulation on Streptozocin-Induced Diabetic Wistar Rats

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## Abstract

To treat type 2 diabetes mellitus, the study focuses on polyherbal anti-diabetic extracts from several plants, administered at varying dosages. Ayurvedic medicines are widely accepted due to their efficacy, safety, affordability, ubiquity, and acceptance. Because polyherbal medicines contain glycosides, alkaloids, flavonoids, and other compounds with diverse modes of action, they have long been used to treat diabetes worldwide. This study examined the Antidiabetic and haematological effects of a polyherbal formulation (PHF) in streptozotocin (STZ)- induced diabetic rats.

**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 reduced by the extract, with the greatest reduction observed at 400 mg/kg body weight. After extract administration at both doses, the quantities of red blood cells, white blood cells, and their functional indices all increased considerably. Also, in diabetic rats, water and feed consumption were intensely decreased, and weight loss was minimised at both dosages.

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

## 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 the most concerning trends in this rapid increase.[1] 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 concept of polyhedral formulation. The medicinal potential of the polyherbal formulation is greater and more extensive than that of the single plant. To create and standardise a polyherbal formulation employing a plant known to have antidiabetic activity, 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 the root of Beta Vulgaris were collected from the local area of Ale, Junnar, Pune, Maharashtra. Taxonomically identified leaves of Sesbania Grandiflora and the root of Beta Vulgaris were identified and authenticated by Dr R.K. Chaudhary, Scientist, Agharkar Research Institute, Autonomous Body under DST, GOI, Pune. Herbarium specimens have been preserved in a laboratory.

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

### Preparation of Methanolic Extract of *Sesbania Grandiflora* and *Beta Vulgaris.*

Methanolic extracts of *Sesbania Grandiflora* and the root of Beta Vulgaris were obtained by the 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 three batches of the polyherbal formulation contained the methanolic extract of the leaves of Sesbania grandiflora and the methanolic extract of Beta vulgaris root at different ratios, as mentioned below in Table 1. Batches were tested for quality in accordance with WHO guidelines for the quality control of herbal medicine. Optimized batch was selected for further *Invivo* 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 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, using 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 at increasing doses of 5mg, 50mg, 300 mg and 2000 mg/kg body weight. The animals were observed continuously for 24 h for their behavioural (alertness, restlessness, irritability, and fearfulness), neurological (spontaneous activity, Reactivity, touch response, pain response, and gait), and autonomic (defecation and urination) profiles. After a 24-h period, the animals were observed for 14 days for mortality.[8]

### *In-vivo* 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 a 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 5% w/v of glucose solution to prevent hypoglycaemic mortality and allowed access to a 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 4 hours of administration of the polyherbal formulation, the rats were allowed free access to food (standard rodent pellets).

#### 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 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) |

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

**Table 2: Observation of changes in Clinical Signs in PHF (2000mg/kg) administered in Acute Toxicity Group**

| Observation | 30 mins | 4 hrs | 14 hrs | 24 hrs |
|---|---|---|---|---|
| Body weight | No change | No change | No change | No change |
| Preterminal deaths | No | No | No | No |
| Motor activity | No change | No change | No change | No change |
| Convulsions | No change | No change | No change | No change |
| Salivation | No change | No change | No change | No change |
| Skin colour | No change | No change | No change | No change |
| Diarrhoea | No change | No change | No change | No change |
| Aggression | No change | No change | No change | No change |
| Sedation | No change | No change | No change | No change |
| Excitation | No change | No change | No change | No change |

***In-vivo* Antidiabetic Effect of Polyherbal Formulation on Hematological parameters in Streptozotocin Induced Diabetic Rats**

**Hematological Parameters**

**Hemoglobin (g/dl)**

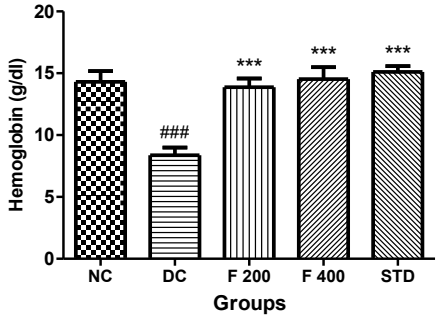


Figure 1: Effect of PHF 200 and 400 on Hemoglobin (g/dl) in STZ-induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and ***$p$<0.001 versus DC rats.

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)**

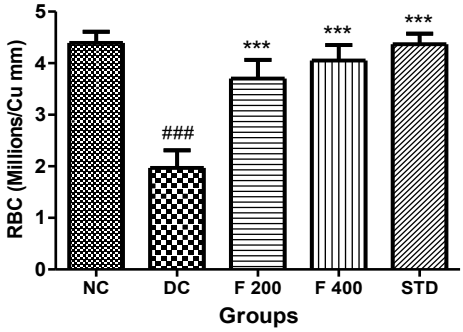


Figure 2: Effect of PHF 200 and 400 on RBC (Million/Cu mm) in STZ-induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and ***$p$<0.001 versus DC rats.

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 (%)**

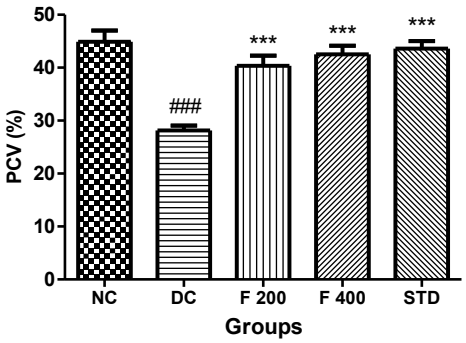


Figure 3: Effect of PHF 200 and 400 on PCV (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and ***$p$<0.001 versus DC rats.

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)**

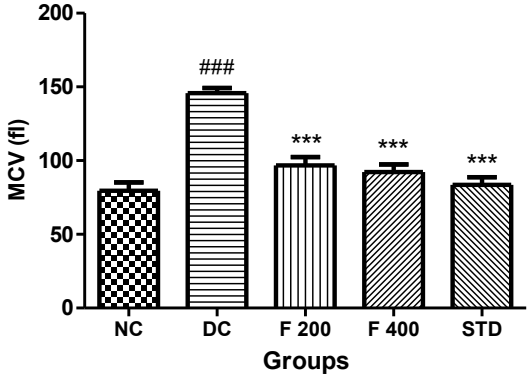


Figure 4: Effect of PHF 200 and 400 on MCV (fl) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and ***$p$<0.001 versus DC rats.

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 (%)**

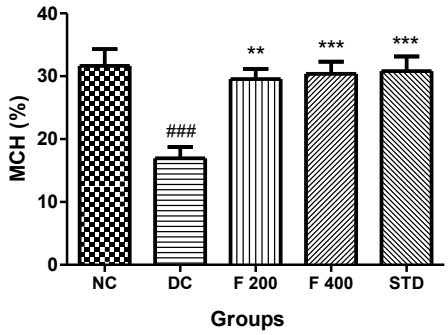


Figure 5: Effect of PHF 200 and 400 on MCH (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and **$p$<0.01; ***$p$<0.001 versus DC rats.

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**

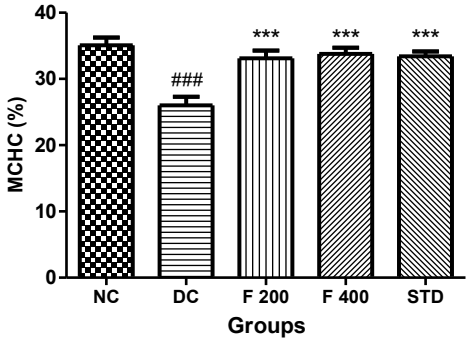


Figure 6: Effect of PHF 200 and 400 on MCHC (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and ***$p$<0.001 versus DC rats.

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)**

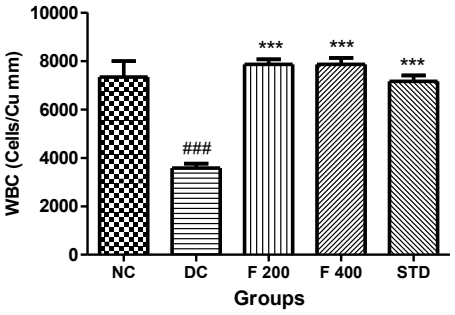


Figure 7: Effect of PHF 200 and 400 on WBC (Cells/Cumm) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and ***$p$<0.001 versus DC rats.

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 (%)**

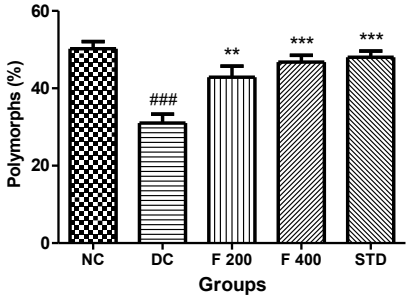


Figure 8: Effect of PHF 200 and 400 on Polymorphs (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and **$p$<0.01; ***$p$<0.001 versus DC rats.

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 (%)**

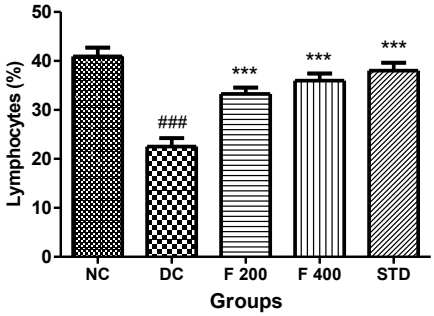


Figure 9: Effect of PHF 200 and 400 on Lymphocytes (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and ***$p$<0.001 versus DC rats.

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 (%)**

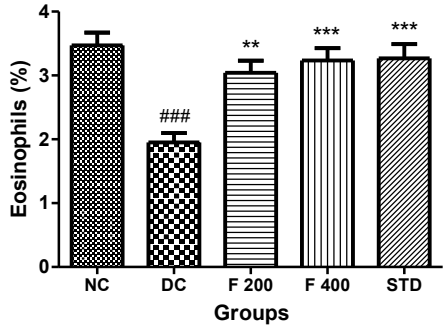


Figure 10: Effect of PHF 200 and 400 on Eosinophils (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and **$p$<0.01; ***$p$<0.001 versus DC rats.

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 (%)**

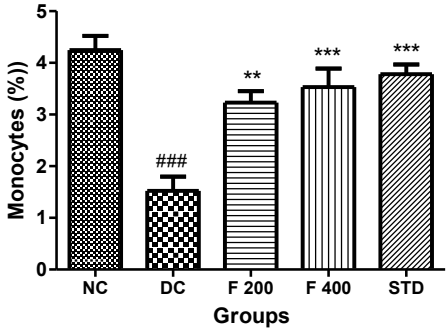


Figure 11: Effect of PHF 200 and 400 on Monocytes (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and **$p$<0.01; ***$p$<0.001 versus DC rats.

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) increase in monocytes count when compared with DC rats.

**Basophils (%)**

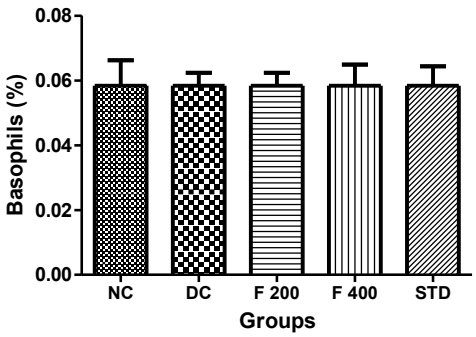


Figure 12: Effect of PHF 200 and 400 on Basophils (%) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and **$p$<0.01; ***$p$<0.001 versus DC rats.

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)**

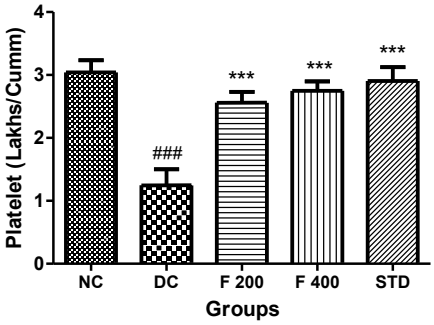


Figure 13: Effect of PHF 200 and 400 on Platelet (Lakhs/Cumm) in STZ induced Diabetes in rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. ###$p$<0.001 versus NC rats and ***$p$<0.001 versus DC rats.

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.

## DISCUSSION

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 showed no significant change in basophil count compared with NC rats.

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