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PRE-CLINICAL EVALUATION OF ANTIDIABETIC ACTIVITY OF PHYLLANTHUS RETICULATUS FRUIT EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT
Diabetes mellitus is a metabolic illness with numerous etiologists that is defined by chronic hyperglycemia and abnormalities in carbohydrate, lipid, and protein metabolism caused by insulin production, insulin action, or both. Hyperglycemia is a symptom of diabetes mellitus, which is a collection of disorders. Diabetes mellitus is classified as an epidemic by the World Health Organization (WHO), despite the fact that it is a non-infectious disease. This disease is predicted to affect over 203% of the world's population, with an annual growth rate of 4-5%. The powdered material of the fruit of *Phyllanthus reticulates* was extracted separately using 95% methanol by Soxhlet apparatus Phyto-constituentsuents like alkaloids, tannins, cardio glycosides, and traces of flavonoids, etc. The dose selection study was carried out in accordance with the OECD 423 guidelines. Female Wistar rats were used in this experiment. There were two groups, each with six fasting animals. A single dosage of the test drug was given to one group of 300 mg/kg and another group of 2000 mg/kg body weight. Any indicators of toxicity should be detected in the animals prior to dosing and for a total of 14 days. Following that, the test dose was selected as 300 mg/kg and 600 mg/kg. Group-I: Normal Control, Group-II: Disease Control (Only Streptozotocin), Group-III: Low Dose of *Phyllanthus reticulates* Fruit Extract (300mg/Kg Body Weight) + Streptozotocin, Group-IV: High Dose of *Phyllanthus reticulatus* Fruit Extract (600mg/Kg Body Weight) + Streptozotocin, Group-V: Standard Drug [Metformin] (0.5gm/Kg Body Weight) + Streptozotocin study biochemical parameters such as lipid profile (Total cholesterol, Triglycerides, HDL, LDL).

KEYWORD: *Phyllanthus reticulatus*, Streptozotocin (STZ), Flavonoids, Anti-Diabetic activity, Herbal Plants.

1. INTRODUCTION

Diabetes mellitus is a metabolic illness with numerous etiologies that is defined by chronic hyperglycemia and abnormalities in carbohydrate, lipid, and protein metabolism caused by insulin production, insulin action, or both. Diabetes is a condition in which your blood sugar levels are abnormally high. Sugar is always present in your blood since your body needs sugar for energy. However, having too much sugar in your blood is harmful to your health. Diabetes is a metabolic illness that affects how our bodies utilize digested food for development and energy. The digestive fluids break down the majority of the food we eat into glucose, a simple sugar that is the body's primary source of energy (Vigneri R et al., 1987).[1,2,3]

Hyperglycemia is a symptom of diabetes mellitus, which is a collection of disorders. Diabetes mellitus is classified as an epidemic by the World Health Organization (WHO), despite the fact that it is a non-infectious disease. This disease is predicted to affect over 203 percent of the world's population, with an annual growth rate of 4-5 percent (Allison DB et al., 1999).[4,5]

Following digestion, glucose enters our bloodstream, where it is available for utilization by body cells for development and energy. Insulin must be present in order for glucose to enter the cells. The pancreas, a big gland behind the stomach, produces insulin, which is a hormone. The pancreas is meant to create the proper quantity of insulin to transfer glucose from our blood into our cells when we eat. Sugar cannot enter cells if your body does not produce enough insulin or if the insulin does not operate properly. It persists in the blood, producing a rise in blood sugar and the development of diabetes. As a result, glucose builds up in the blood, overflows into the urine, and exits the body. As a result, even if the blood contains enormous levels of glucose, the body loses its main source of fuel (Vigneri R et al., 1987).[6]

Diabetes is a metabolic condition, which refers to the body's utilization of digested food for growth and energy. After all of the processes of ingestion, digestion,

assimilation, and absorption, glucose is the end product of our food, which implies that anything we eat eventually breaks down into glucose. Once digestion is complete, the process of assimilation begins. The glucose that gets into the bloodstream after digestion must be utilized by cells for development and energy. To make it easier for glucose to enter the body, we need a hormone called insulin, which is produced by the pancreas of the islets of Langerhans. (Narayan KM et al., 2003).[7,8]

Type 1 or Insulin Dependent Diabetic Mellitus (IDDM) or juvenile-onset diabetes

Type I diabetes mellitus, also known as insulin-dependent diabetes mellitus or juvenile-onset diabetes mellitus, is caused by the destruction of pancreatic beta cells. Its causes are unknown, but it has been linked to viral infection in some cases, and current research suggests it could also be caused by autoimmunity. Islets cell autoantibodies are found in 90% of newly diagnosed sufferers' serum. Antibodies are made against a variety of cell components, including cytoplasm and membrane antigens, as well as insulin (Daneman D et al., 2006).[9]

Type II or Non-Insulin Dependent Diabetes Mellitus (NIDDM) or maturity-onset diabetes

Type-II Diabetes Mellitus, also known as non-insulin-dependent diabetes mellitus or adult-onset diabetes mellitus, is characterized by impaired insulin release and basal insulin output. Insulin resistance occurs in this situation as a result of a malfunction in the tissue response to insulin-mediated by faulty insulin receptors on target cells. Diabetes type II is the most frequent type, accounting for 90-95 percent of cases. Insulin resistance is a disorder that occurs in Type II diabetes when the body does not respond appropriately to insulin (Goldfine et al., 2001).[10]

Gestational diabetes

Gestational diabetes is a kind of diabetes that appears in certain pregnant women in the latter stages of the pregnancy. Women who have had gestational diabetes have a 40% to 60% chance of developing Type-II diabetes in the next 5 to 10 years, even though it often resolves after giving a child. Maintaining a healthy body weight and remaining physically active can help you avoid type 2 diabetes. Approximately 5% of pregnant women develop gestational diabetes in their third trimester, a kind of Type 2 diabetes that is usually only temporary (American Diabetes Association, 2005).[11]

2. MATERIALS AND METHODS

2.1 Plant materials

The plant *Phyllanthus reticulatus* fruit was collected in Merch, 2022 from Kerala and utter Pradesh and was identified and authenticated by Mr. Subir Samanta, Head, Department of Botany, A.K.P.C College, Bengai, Hooghly, pin-712611, West Bengal, India. Voucher specimens were deposited at our College Museum for future reference.

2.2 Extraction of plants

The powdered material of the fruit of *Phyllanthus reticulates* was extracted separately using 95% methanol by Soxhlet apparatus (HarVarleyrley *et al.,* 1983). The extracts were kept in a hot air oven maintaining the temperature and pressure to remove the solvent part. The extract was stored in a desiccator and was subjected to various chemical tests to detect the presence of different Phyto-constituentsuents like alkaloids, tannins, cardio glycosides, traces of flavonoids, etc.

2.3 Drugs and Chemicals

Methanol and normal saline (0.9% NaCl) were obtained from the Central Medical Store of the Institution. STZ, thiobarbituric acid, citric acid, sodium citrate, sodium hydroxide, and glucose estimation kits were obtained from Hi Media Laboratories Pvt., Limited, Mumbai, Maharashtra, India. Kits for estimation of lipid profile were obtained from Crest Udani chemical store, Goa, India. Ether, thiopentone sodium, potassium phosphate buffer, hydrogen peroxide solution, and tricarboxylic acid were obtained from Sigma-Aldrich India, Bengaluru, Karnataka, India. Crude powder of metformin was obtained from Aventis Pharma Limited, Goa, India. RIA kits for insulin assay were supplied by the Board of Radiation and Isotope Technology, Bhabha Atomic Research Centre (Navi Mumbai, Maharashtra, India)

2.4 Preliminary phytochemical screening

The methanol extract of the fruit of *Phyllanthus reticulatus* was subjected to preliminary screening for various active phytochemical constituents by the following tests.[12]

3. Pharmacological screening

Male Wistar albino rats, 9-12 weeks old with an average weight of 150-180 g were purchased from the animal house, NSHM Knowledge Campus, Kolkata-53, West Bengal, India, and used for the study. They were housed in polypropylene cages and fed with a standard chow diet and water *ad libitum.* The animals were exposed to an alternate cycle of 12 h of darkness and light each. Before each experiment, the animals were fasted for at lealeast 18 The experimental protocols were approved by Institutional Animal Ethics Committee (Regd. No. CPCSEA/1458/PO/Re/S/11/) and were in accordance with IAEC.

3.1 Acute toxicity studies

The dose selection study was carried out in accordance with the OECD 423 guidelines. [13] Female Wistar rats were used in this experiment. There were two groups, each with six fasting animals. A single dosage of the test drug was given to one group of 300 mg/kg and another group of 2000 mg/kg body weight. Any indicators of toxicity should be detected in the animals prior to dosing and for a total of 14 days. Following that, the test dose was selected as 300 mg/kg and 600 mg/kg.

3.2 Estimation of blood glucose levels

Blood was collected from the tip of the tail vein and fasting blood glucose levels were measured using a commercial glucometer and glucose-oxidase strips.

3.3 Streptozotocin-Induced diabetes mellitus

Wistar male rats were divided into 5 groups.

Group-1 will serve as normal control and receive regular rat food and drinking water at the libitum.

Groups- 2 to 5 overnight fasted animals were administered a single intraperitoneal injection of 60 mg/kg STZ dissolve 0.1 (M) cold sodium citrate buffer at PH 4.5. Fasting blood glucose level was estimated after 72 hours. Rats showing blood glucose levels of more than 250 mg/were included in the study. Per group 6 animals were there.

Group-2 was the diseased control group (only streptozotocin)

Groups 3 and 4 received low and high doses of the test drug [Phyllanthus reticulatus fruit extract]

Group-5 received the standard anti-diabetic drug, metformin.

Treatment will go on for 15 days. On the 15th day, animals will be sacrificed. reticulatus

Group-I: Normal Control

Group II: Disease Control (Only Streptozotocin)

Group III: Low Dose of *Phyllanthus reticulatus* Fruit Extract (300mg/Kg Body Weight) + Streptozotocin

Group IV: High Dose of *Phyllanthus reticulatus* Fruit Extract (600mg/Kg Body Weight) + Streptozotocin

Group-V: Standard Drug [Metformin] (0.5gm/Kg Body Weight) + Streptozotocin.[55]

4. Biochemical studies

At the end of the study, the blood samples were collected by bleeding of the retro-orbital plexus using the microcapillary technique from all the groups of rats, and serum was separated to study biochemical parameters such as lipid profile (Total cholesterol, Triglycerides, HDL, LDL).

Body weight changes: The body weight of the animals in each group was recorded using a standard electronic weighing machine on day 1 and day 15 of the experiment.

Method of blood glucose estimation: Fasting blood glucose was estimated by the glucose oxidase method using glucose estimation kits.

Histopathological studies: Pancreatic tissues from all groups were subjected to histopathological studies. The whole pancreas from each animal was removed after sacrificing the animal under anesthesia, collected in 10% formalin solution, and immediately processed by the paraffin technique. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H and E) for histological examinations. Stained sections were qualitatively evaluated using a photo microscope equipped with Canon Zoom Browser EX digital camera. (Japan)

5. RESULTS

5.1 Statistical analysis

Statistical analysis was done using Graph Pad Prism Software version 4 [Graph Pad I NC., USA]. One-Way ANOVA followed by Bonferroni's Test was applied. The confidence interval was taken at 95%.

5.2 Pharmacognostic-Study

5.2.1 Qualitative Determination: The qualitative determinations of the methanolic extract of the fruit of *Phyllanthus reticulatus* were shown in Table No.1. In qualitative determination, the Phytoconstituents analysis was performed and which confirms the presence of alkaloids, glycosides, flavonoids, saponin, and tannins.

Table No. 1: Phytoconstituents analysis of methanolic fruit extract of *Phyllanthus reticulates.*

Sl. No	Plant constituents	*Phyllanthus reticulatus*
1	Test for alkaloids	+
2	Test for glycosides	+
3	Test for carbohydrates	-
4	Test for phytosterols	-
5	Test for sterols	-
6	Test for flavonoids	+
7	Test for saponins	+
8	Test for tannins	+
9	Test for terpenoids	+
10	Test for fixed oils and fats	-

- Absent, + Present

5.2.2 Acute toxicity studies

This research aids in determining the therapeutic index, and the extract's safety has been established. The OECD criteria were followed for conducting the acute toxicity investigation. Depending upon OECD 423 Guidelines, low dose, and high dose were found 300 mg/kg and 600 mg/kg body weigh.

Table No. 2: The effects of methanolic root extract of *Phyllanthus reticulum* general behavioral observation in acute toxicity studies.

Sl. No.	General behavior	Observation after the drug administration
1	Sedation	-
2	Hypnosis	-
3	Convulsion	-
4	Ptosis	-
5	Analgesia	-
6	Stupor reaction	-
7	Motor activity	-
8	Muscle relaxant	-
9	CNS stimulant	-
10	CNS depressant	-
11	Pilo erection	-
12	Skin color	-
13	Lacrimation	-
14	Stool consistency	-

- Absent, + Present

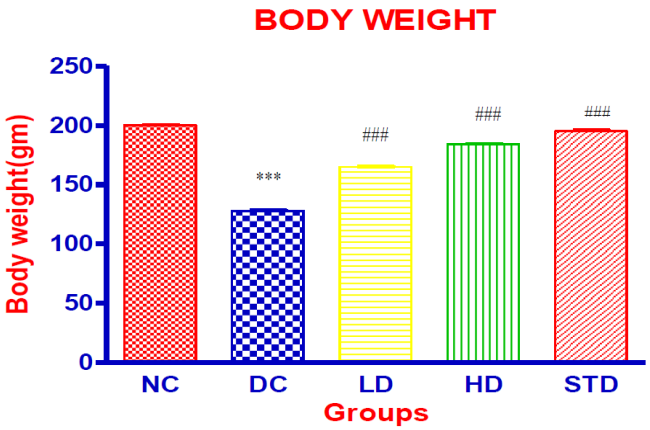
5.2.3 Body weight

When compared to the control group, the body weight in the STZ-treated group decreased considerably. Then STZ-induced rats were given Phyllanthus reticulatus methanolic fruit extracts at doses of 300 mg/kg/oral and 600 mg/kg/oral. Treatment with Phyllanthus reticulatus methanolic fruit extract at a dose of 300 mg/kg/oral results in a marginal reduction in body weight and it gives a highly significant value(165±0.27, P<0.001). Treatment with Phyllanthus reticulatus methanolic fruit extract at a dose of 600 mg/kg/oral results that it is also increased body weight and also gives a highly significant value (184±0.38, P <0.001) when compared to diseases control. Between the first and second weeks, treatment with metformin (0.5gm/kg b.w/oral) resulted in a substantial (p<0.001) increase the body weight.

Table No. 3: The effect of methanolic fruit extract of *Phyllanthus reticulatus* body weight STZ-induced diabetic rats.

Groups	Values (gm)
Normal Control	200±0.46
Diabetic Control	128±0.53***
Prld(300mg/kg b.w.)	165±0.27$^{###}$
Prhd(600mg/kg b.w.)	184±0.38$^{###}$
Standard|metformin (0.5gm/kgB.w.)	195±0.94$^{###}$

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. $^{#}$p<0.05, $^{##}$p<0.01, $^{###}$p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. $^{#}$p<0.05, $^{##}$p<0.01, $^{###}$p<0.001 when compared with diseased control group.

Figure No. 1: The effect of methanolic fruit extract of *Phyllanthus reticulates* on body weight in STZ-induced diabetic rats.

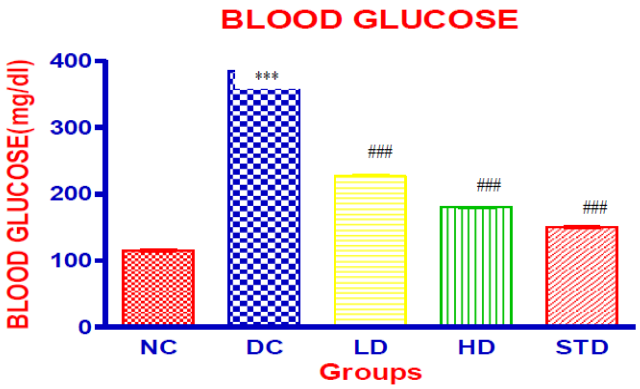
5.2.4 Antidiabetic activity

When compared to the control group, the blood glucose level in the STZ-treated group increased considerably. Then STZ-induced rats were given Phyllanthus reticulatus methanolic fruit extracts at doses of 300 mg/kg/oral and 600 mg/kg/oral. After Treatment, with Phyllanthus, reticulatus methanolic fruit extract at a dose of 300 mg/kg/oral results decreased blood glucose levels it giving a highly significant value (227±0.73, P<0.001) compared with the diseases group. Treatment with Phyllanthus reticulatus methanolic fruit extract at a dose of 600mg/kg/oral resulted in it is significantly decreased blood glucose level it also gives a highly significant value (180±0.41 P <0.001). Between the first and second weeks, treatment with metformin (0.5gm/kg b.w/oral) resulted in a substantial (150±0.22, P<0.001) reduction in blood glucose levels.

Table No. 4: The effect of methanolic fruit extract of *Phyllanthus reticulatus* blood glucose level in STZ-induced diabetic rats.

Groups	Values (mg/dl)
Normal Control	115±0.62
Diabetic Control	385±0.98***
Prld (300mg/kg b.w.)	227±0.73##
Prhd (600mg/kg b.w.)	180±0.41###
Standard |metformin (0.5gm/kg)	150±0.22###

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.

Figure No. 2: The effect of methanolic fruit extract of *Phyllanthus reticulates* on blood glucose level in STZ-induced diabetic rats.

5.3 Levels of the antioxidant parameters

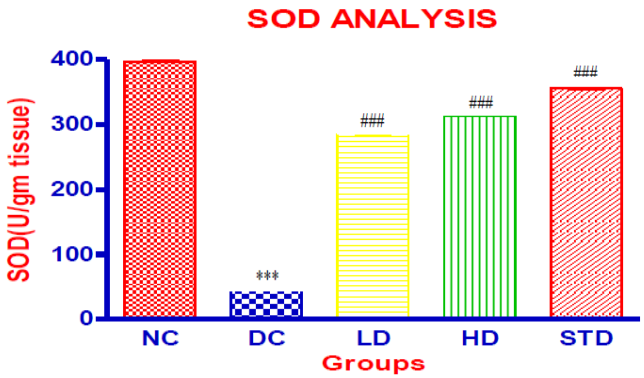
On the opposite side, the antioxidant GSH, SOD, and CAT levels of diabetic rats were found to be highly significant values (55±0.63, 4±0.25, 1±0.29 and P<0.001, P <0.001, P<0.001) in comparison with control values. Treatment of diabetic rats by using the low(300mg/kg b.w) and high dose(600mg/kg b.w) the drug significantly improved and its value is highly significant(P<0.001), the concentration of the antioxidant GSH, SOD and CAT levels in comparison to the diabetic control values of these parameters. On the other hand, the MDA level significantly increased (79±0.24, P<0.001) compare to the control value. Treatment of diabetic rats by using the low(300mg/kg b.w) and high dose(600mg/kg b.w) of the drug significantly decreased and it is also given highly significant value(P<0.001), the concentration of the antioxidant MDA levels in comparison to the diabetic control values of these parameters.

5.3.1 Sod analysis

Table No. 5: The effect of methanolic fruit extract of *Phyllanthus reticulates* on an antioxidant parameter in (SOD analysis STZ-induced diabetic rats.

Groups Treatment/ Dose	Sod (U/gm tissue)
Normal control	397±0.17
Diabetic control	55±0.63***
PRLD (300mg/kg b.w.)	286±0.42###
PRHD (600mg/kg b.w.)	314±0.28 ###
Standard |Metformin (0.5gm/kg b.w.)	355±0.94 ###

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001 when compared with normal control group. #p<0.05,##p<0.01,###p<0.001 when compared with diseased control group.

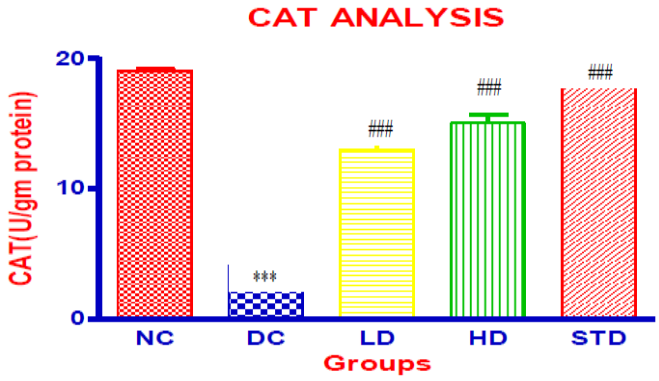
Figure No. 3: The effect of methanolic fruit extract of *Phyllanthus reticulates* on the antioxidant parameter (SOD analysis) STZ-induced diabetic rats.

5.3.2 Cat analysis

Table No. 6: The effect of methanolic fruit extract of *Phyllanthus reticulates* on an antioxidant parameter in (CAT analysis-induced diabetic rats.

Groups Treatment/ Dose	CAT (U/gm protein)
Normal control	19±0.17
Diabetic control	4±0.25***
PRLD (300mg/kg b.w.)	13±0.98###
PRHD (600mg/kg b.w.)	15±0.66###
Standard |Metformin (0.5gm/kg b.w.)	18±0.35###

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group compared with the diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.

Figure No. 4: The effect of methanolic fruit extract of *Phyllanthus reticulates* on the antioxidant parameter (CAT analysis) in STZ-induced diabetic rats.

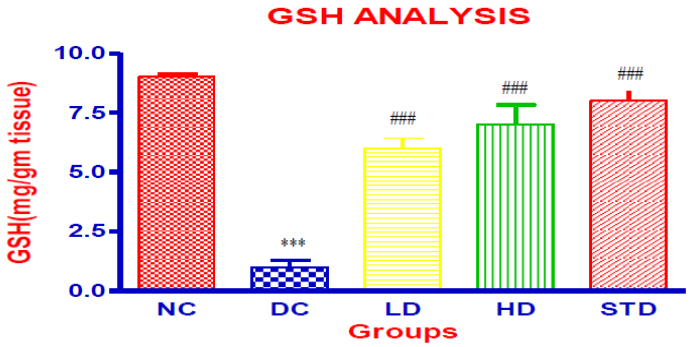
5.3.3 Gsh analysis

Table No. 7: The effect of methanolic fruit extract of *Phyllanthus reticulates* on an antioxidant parameter in (GSH analysis) STZ-induced diabetic rats.

Groups Treatment/ Dose	GSH (mg/gm tissue)
Normal control	9±0.14
Diabetic control	1±0.29***
PRLD (300mg/kg b.w.)	6±0.47###

PRHD (600mg/kg b.w.)	7±0.83$^{###}$
Standard |Metformin (0.5gm/kg b.w.)	8±0.92$^{###}$

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.

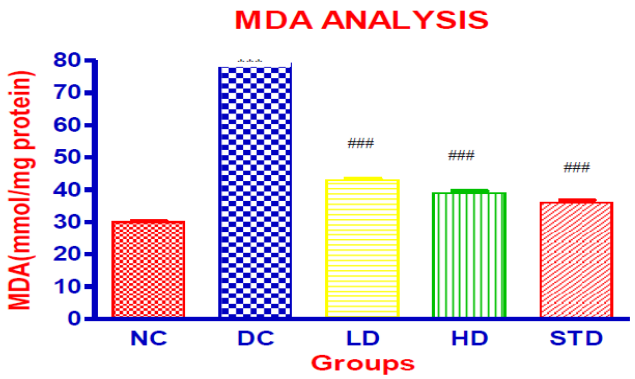
Figure No. 5: The effect of methanolic fruit extract of *Phyllanthus reticulates* on the antioxidant parameter (GSH analysis) STZ-induced diabetic rats.

5.3.4 Mda analysis

Table No. 8: The effect of methanolic fruit extract of *Phyllanthus reticulates* on an antioxidant parameter in (MDA analysis-induced-diabetic rats.

Groups Treatment/ Dose	MDA (mmol/mg protein)
Normal control	30±0.31
Diabetic control	79±0.24***
PRLD (300mg/kg b.w.)	43±0.47$^{###}$
PRHD (600mg/kg b.w.)	39±0.68$^{###}$
Standard Metformin (0.5gm/kg b.w.)	36±0.75 $^{###}$

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.

Figure No. 6: The effect of methanolic fruit extract of *Phyllanthus reticulates* on the antioxidant parameter (MDA analysis) in STZ-induced diabetic rats.

5.4 Estimation of lipid profile

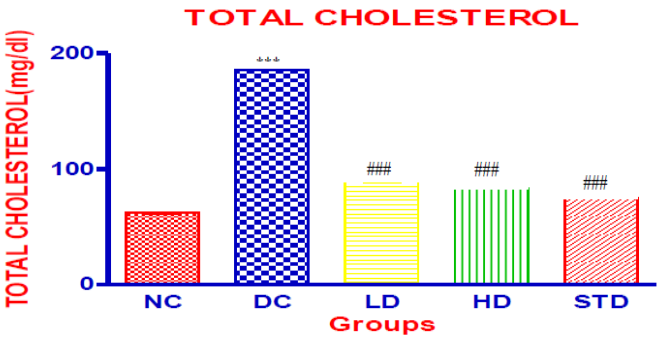
When compared to control rats, blood total cholesterol, triglyceride, LDL, and VLDL levels were significantly higher in STZ-induced diabetic rats, while HDL levels were significantly lower. When compared to STZ-induced diabetic animals, serum total cholesterol, triglyceride, and LDL, levels of diabetic animals treated with PRLD 300mg/kg/oral and 600mg/kg/oral showed significant decreases (p<0.001) and HDL levels of diabetic animals treated with Phyllanthus reticulatus showed significant increases (p<0.01). When compared to STZ-induced diabetic rats, metformin (0.5gm/kg/oral) exhibited a substantial drop (p<0.001) in serum total cholesterol, triglyceride, and LDL, while HDL was considerably enhanced.

5.4.1 Total cholesterol

Table No. 9: The effect of methanolic fruit extract of *Phyllanthus reticulates* on lipid profile (TOTAL CHOLESTEROL) in STZ-induced diabetic rats.

Groups Treatment/ Dose	Total cholesterol (mg/dl)
Normal control	62±0.22
Diabetic control	186±0.1 ***
PRLD (300mg/kg b.w.)	89±0.17 ###
PRHD (600mg/kg b.w.)	84±0.39 ###
Standard |Metformin (0.5gm/kg b.w.)	75±0.42 ###

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.

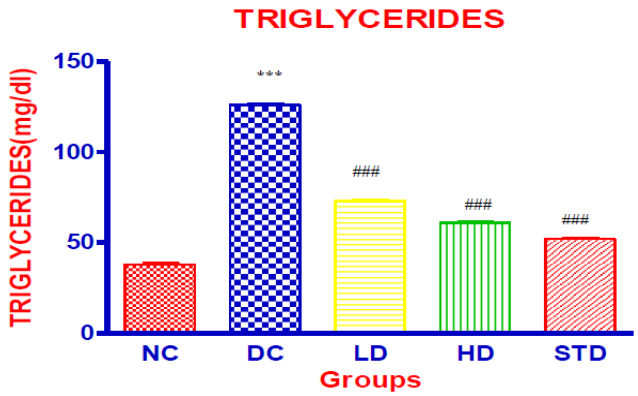
Figure No. 7: The effect of methanolic fruit extract of *Phyllanthus reticulates* on lipid profile (TOTAL CHOLESTEROL analysis) in STZ-induced diabetic rats.

5.4.2 Triglycerides

Table No. 10: The effect of methanolic fruit extract of *Phyllanthus reticulates* on lipid profile (TRIGLYCERIDES) in STZ-induced diabetic rats.

Groups Treatment/ Dose	Triglycerides (mg/dl)
Normal control	36±0.42
Diabetic control	126±0.39 ***
PRLD (300mg/kg b.w.)	73±0.34 ###
PRHD (600mg/kg b.w.)	61±0.26 ###
Standard |Metformin (0.5gm/kg b.w.)	52±0.54 ###

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.

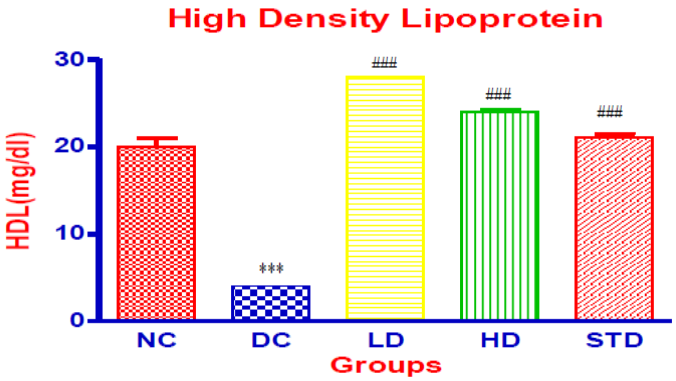
Figure No. 8: The effect of methanolic fruit extract of *Phyllanthus reticulates* on lipid profile (TRIGLYCERIDE analysis) in STZ-induced diabetic rats.

5.4.3 HDL (HIGH-DENSITY LIPOPROTEIN)

Table No. 11: The effect of methanolic fruit extract of *Phyllanthus reticulates* on lipid profile (HIGH-DENSITY LIPOPROTEIN) in STZ-induced diabetic rats.

Groups Treatment/ Dose	HDL (mg/dl)
Normal control	19±0.96
Diabetic control	04±0.82 ***
PRLD (300mg/kg b.w.)	28±0.11###
PRHD (600mg/kg b.w.)	24±0.23 ###
Standard |Metformin (0.5gm/kg b.w.)	21±0.45###

Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001 when compared with normal control group. #p<0.05,##p<0.01,###p<0.001 when compared with diseased control group.

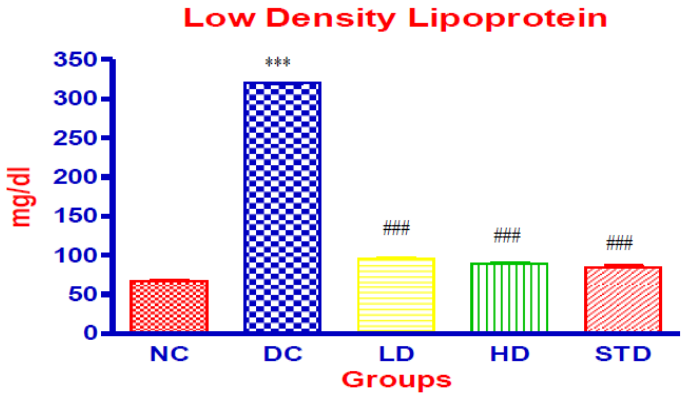
Figure No. 9: The effect of methanolic fruit extract of *Phyllanthus reticulates* on lipid profile (HIGH DENSITY LIPOPROTEIN analysis) in STZ-induced diabetic rats.

5.4.4 LDL (LOW-DENSITY LIPOPROTEIN)

Table No. 12: The effect of methanolic fruit extract of *Phyllanthus reticulates* on lipid profile (LOW-DENSITY LIPOPROTEIN) in STZ-induced diabetic rats.

Groups Treatment/ Dose	LDL (mg/dl)
Normal control	65±0.21
Diabetic control	325±0.22 ***
PRLD (300mg/kg b.w.)	98±0.68###
PRHD (600mg/kg b.w.)	89±0.75###
Standard |Metformin (0.5gm/kg b.w.)	81±0.93 ###

Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001 when compared with normal control group. #p<0.05,##p<0.01,###p<0.001 when compared with diseased control group.



Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group. #p<0.05, ##p<0.01, ###p<0.001 when compared with diseased control group.

Figure No. 10: The effect of methanolic fruit extract of *Phyllanthus reticulates* on lipid profile (LOW-DENSITY LIPOPROTEIN analysis) in STZ-induced diabetic rats.

5.5 Histopathology

Streptozotocin caused severe necrotic changes in pancreatic islets, especially in the center of islets. Nuclear changes, karyolysis, and severe reduction of beta cells were observed in diabetic control rats. However, diabetic rats treated with the test drug and the standard drug showed restoration of the altered architecture and number of islets, ameliorated karyolysis, and necrosis toward the normal morphology of the pancreas.

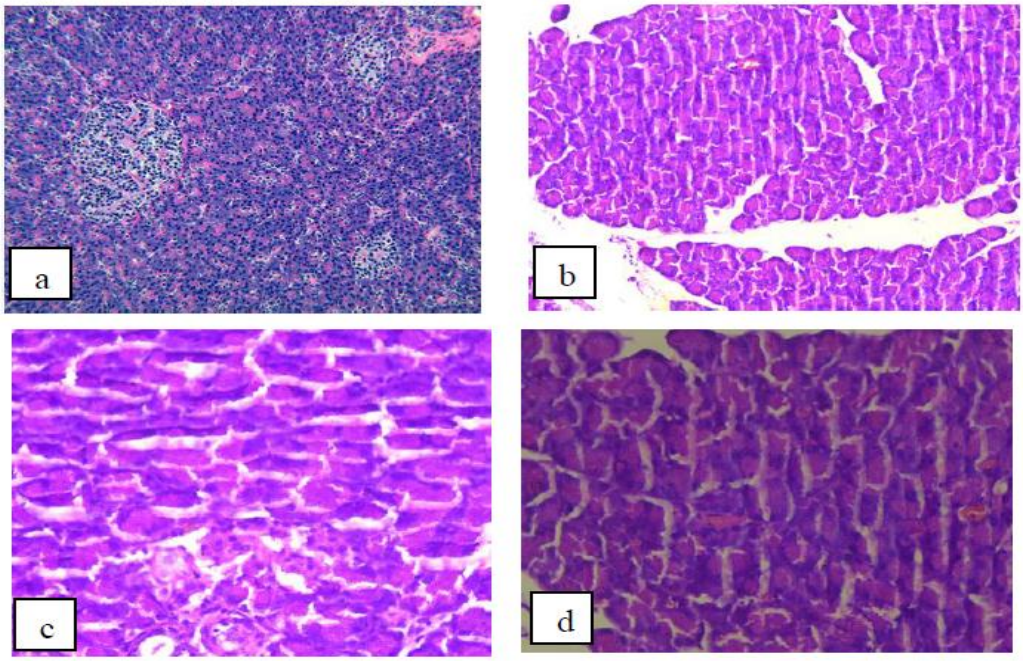


Figure 11: Histopathology of (a) Pancreas in a normal rat, (b) Pancreas in STZ diabetes rat, (c) Pancreas taking the high dose of a drug in rat, (d) Pancreas taking the standard drug (Metformin) in the rat.

6. DISCUSSION

Due to their natural origin, medicinal plants serve an important role in the treatment of diabetes, especially for those with low finances. According to Ethnobotany, over 800 medicinal plants have anti-diabetic activity, and their phytochemical constituents such as alkaloids, glycosides, terpenoids, flavonoids, and others have shown to be quite efficient in both preclinical and clinical investigations. The current study found that the methanolic extract of Phyllanthus reticulatus is nontoxic when given orally to Wister1 albino rats at doses up to 600mg/kg body weight.

Literature review and phytochemical evaluation showed the presence of flavonoids, alkaloids, glycosides, and sterols. It is possible that the anti-diabetic property of the methanolic extract of *Phyllanthus reticulates* could be mediated by the synergistic effect of these phytochemicals. The STZ-induced diabetic model is one of the best models in which Streptozotocin is an alkylating agent which causes DNA damage which results in the activation of poly (ADP-ribose) synthetase that leads to the depletion of NAD and ATP virtually causes beta cell necrosis in the experimental rats. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to a stable hyperglycemic state. STZ is the most commonly used chemical for the induction of experimental diabetes for both IDDM and NIDDM.

The purpose of this study was to assess the anti-diabetic potential of Phyllanthus reticulatus in both normal and STZ-induced diabetic rats. In STZ-induced diabetic rats, the methanolic extract of Phyllanthus reticulatus was found to have blood glucose-reducing properties. Oral administration of Phyllanthus reticulatus methanolic extract protected against STZ-induced diabetic mellitus.

The fact that blood urea and serum creatinine levels were normal implies that the methanolic extract of Phyllanthus reticulatus had no effect on renal function and that renal integrity was retained, as well as the absence of any substantial anomalies. There were no significant changes in liver function tests, according to the study. The normal values of ALP, AST, and ALT were affected by the methanolic extract of Phyllanthus reticulatus, indicating that the extract is hepatotoxic. The lipid profile of triglycerides, total cholesterol, HDL, and LDL was also changed by the methanolic extract of Phyllanthus reticulatus, according to the study.

7. SUMMARY AND CONCLUSION

The increased popularity of herbal remedies for a variety of chronic illnesses can be attributed to a number of causes. People that use alternative remedies aren't always uniform, which is interesting. Many people who use herbal medications discover that they are more in line with their own values, beliefs, and philosophical approach to health and life.

The fruit of Phyllanthus reticulatus was chosen for testing antidiabetic potential in a diabetic rat model induced by STZ. The fruit extract was extracted using methanol as a solvent in a Soxhlet system, and preliminary phytochemical screening revealed that the Phyllanthus reticulatus methanolic fruit extract includes alkaloids, glycosides, flavonoids, and tannins.

For medications derived from plants, having a high safety profile is critical. Toxicological investigations can be used to determine the level of toxicity. In comparison to the standard medicine metformin, the methanolic fruit extract of Phyllanthus reticulatus demonstrated considerable anti-diabetic action in a dose-dependent manner, according to the findings. when compared to the control group, STZ-induced diabetic rats showed a substantial drop in liver Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), blood urea level, cholesterol, triglycerides, VLDL, and LDL. In STZ-induced diabetic rats, the methanolic fruit extract of Phyllanthus reticulatus demonstrated a substantial impact. More research is needed to determine the underlying mechanism of the hypoglycemic action and to identify the active molecule (s) responsible for the anti-diabetic properties.

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