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# Evaluating of Anti-Diabetic Potential and Beneficial Effect of Nutraceutical Powder in Management of Various Diabetic Complication

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## Abstract

Diabetes mellitus is a chronic disorder that generates raised more blood glucose levels due to insufficient insulin production or activity. This illness's metabolic imbalance raises the likelihood of long-term problems in blood vessels, resulting in frequent hospitalization and increased risk of cardiovascular disease. Providing appropriate treatment to diabetic patients is vital to avert complications. Female Wistar rats were divided into different groups (n = 6). Eight groups each one induced alloxan to type1 diabetes and treated with different doses of nutraceutical powder. 24 h after the last dose, animals were subjected to behavioural, biochemical, and histopathological evaluations. The data were analysed by one-way ANOVA. In in-vitro model of alpha amylase inhibition assay the absorbance of sample increased as the concentration of nutraceutical powder will increase. In alloxan induced in vivo diabetic model in Wistar rats the body weight, the water intake observed the increasing in disease control as comparing to normal, standard, Test drug group1 lower dose(200mg/kg) and Test drug group 2(500mg/kg), the food intake observed the decreasing in disease control as comparing to normal, standard, Test drug group1 lower dose(200mg/kg) and Test drug group 2(500mg/kg).After 45 days of study, all biochemical parameters the blood, LDL, insulin and creatinine level observed the increasing in disease control group with comparing to normal control, standard control and tests groups. The study will conclude our formulation has potential for managing diabetes by stimulating the regeneration of pancreatic β-cells and further research is needed to understand the active constituents and mechanism of action.

**Keywords:** DPPH, alpha amylase inhibition, Alloxan induced, nutraceutical powder, Diabetes, Wistar rats

## Introduction

Diabetes mellitus is a chronic condition that causes elevated blood glucose levels due to insufficient insulin production or activity. This illness's metabolic imbalance raises the likelihood of long-term problems in blood vessels, resulting in frequent hospitalization and increased risk of cardiovascular disease. Providing high-quality treatment to diabetic patients is vital to avoid complications (National Institute for Health and Care Excellence, 2015; World Health Organization, 2024). Diabetes mellitus is a collection of illnesses that cause excessive blood sugar levels due to inadequate insulin secretion or activity.

Type 1 diabetes is characterized by low insulin production. Type 2 and gestational diabetes are caused by insulin resistance, which results in hyperglycemia (Castell et al., 1997). Diabetes prevalence is anticipated to rise across all age categories, from 2.8% in 2000 to 4.4% in 2030. It is anticipated that by 2030, there will be 366 million diabetics globally, up from 171 million in 2000. Men are more likely than women to have diabetes.

Between 2000 and 2030, the urban population in emerging nations is predicted to quadruple. Additionally, the number of persons aged 65 and older has grown, making it the most major demographic factor influencing diabetes prevalence globally (Roglic, 2016; Lin et al., 2018). Untreated diabetes symptoms often include weight loss, frequent urination, thirst, and hunger. These symptoms emerge rapidly within weeks or months in type 1 diabetes, however in type 2 diabetes, they may appear gradually. When the renal threshold for glucose resorption is surpassed, glucose enters the urine, resulting in osmotic diuresis, dehydration, increased thirst, and increased drinking.

Although all varieties of diabetes have been treated since insulin became medically available in 1921, there is presently no cure. Type 1 diabetes is treated primarily with injections administered by a syringe, insulin pump, or insulin pen. Type 2 diabetes is managed with a mixture of diet, exercise, medication, and insulin (Lakhtakia, 2013).

Medicinal plants are commonly used as alternative therapeutic methods to prevent or treat a range of diseases globally. Over 800 plant species in India have been found as having antidiabetic properties. While complementary and alternative medicine (CAM) methods are popular, there is no scientific evidence to support their efficacy in treating diabetes. Natural therapies have been gaining popular worldwide. because of their natural origins and few side effects (Jarald et al., 2008). Euterpe oleracea is a large palm tree found in South America, namely along the Amazon River and its tributaries and estuaries. The acai fruit is extremely valuable to indigenous communities. Anthocyanins (ACNs), proanthocyanidins (PACs), and other flavonoids were found to be the most abundant photochemical in this study, which conducted all analyses and testing on a standardized freeze-dried acai fruit pulp/skin powder. It decreased blood glucose, insulin resistance, leptin and IL-6 levels, lipid profile, and vascular dysfunction. ASE boosted the expression of insulin signaling proteins in skeletal muscle and adipose tissue, as well as plasma GLP-1 levels. Exercise training improved glycemic control by lowering TNF-α levels, boosting pAKT and adiponectin expressions in adipose tissue, and IR and pAMP expressions in skeletal muscle in diabetic rats (de Bem et al., 2018). The noni tree (Morinda citrifolia) is found only in Southeast Asia and Australasia. It produces fruit. Previously, it was used to treat cancer, tumors, diabetes, anxiety, high blood pressure, and other conditions. One of the most notable advantages is that there are no recognized contraindications.

The plant contains significant amounts of anthraquinones, flavanol glycosides, iridoid glycosides, lipid glycosides, and triterpenoids. Research indicates that Diabetes Mellitus is a chronic metabolic condition characterized by insulin resistance. Noni shows potential as a natural medication for lowering blood glucose levels. his study intends to analyses the effectiveness of noni juice on diabetes individuals' blood sugar levels (Dafriani et al., 2020). Soursop is sometimes known as graviola or Guaynabo. It is the fruit of Annona muricata. It is endemic to the Americas' tropical areas and the Carrabin, where it is abundantly distributed. It includes a high concentration of vitamin C and antioxidants, which enhance immune health and may have anti-carcinogenic qualities. Soursop (fruit and leaves) also contains phytosterol, tannins, and flavonoids, which are all antioxidants. Annona muricata (AM) is an evergreen plant from the Annonaceae family with anticancer and antidiabetic properties (Son et al., 2021).

## Methods

### Plant Collection and Identification

Noni plant, Soursop plant and Acai berry was purchased from Full moon company Ahmadabad and It was authenticated by the Faculty of Science, RK University.

**Preliminary Phytochemical Screening**

The extract was analyzed for secondary metabolites including phenols, tannins, flavonoids, glycosides and alkaloids using the standard method as referenced in sources (Sheikh & Patil, 2020).

**In Vitro DPPH Assay**:

Pancreatic-amylase, an important digestive enzyme, hydrolyzes starch into smaller oligosaccharides such as maltose, malt triose, and oligoglucan. Glucosidase subsequently converts these oligosaccharides into glucose, which is taken into the circulation. As a result, postprandial hyperglycemia (PPHG) increases. Therefore, it is critical to address these two parameters while treating diabetes.

In this investigation, different quantities of plant extracts (200 ,400, 600) will produce in a phosphate buffer solution with a stock solution of 1 mg/ml. Mix 500 µl of alpha-amylase (0.5 mg/ml) with Add 500 milliliters of the experiment sample or standard and incubate for 10 minutes at room temp.

Add 500 µg of 1% starch solution and incubate for an additional 10 minutes.

After that, 1 ml of coloring agent will add, and the reaction mixture was made by combining96 mM of 3,5-Dinitrosalicylic acid with a sodium potassium tartrate solution (12 g dissolved in 8 mL of 2M NaOH) and heating in a boiling water bath for 15 minutes. The mix will allow to cool, and 10 mL of distilled water will add to evaluate the absorbance of the color extracts for each set of test sample concentrations, a blank test sample will create by substituting the enzyme with buffer, while control incubations representing 100% enzyme activity will create by substituting the test drug with buffer.

The absorbance of 540 nm will measure (Ishnava & Motisariya, 2018).

**Experimental Animals**

In this study, we followed the CCSEA (Committee of Control and Supervision of Experiments on Animals) guidelines for animal handling. Approval for all animal procedures was granted by the institutional Ethics Committee at the School of Pharmacy, RK University with reference no. RKCP/COL/RE/23/135, on December 30, 2023(figure 4). The experiments followed authorized protocols and the Guide for Care and Use of Laboratory Animals as per CCSEA guidelines. Forty-eight female albino Wistar rats, aged three months old, were used in total for the experiments. The rats were housed in standardized cages, with 2–3 rats per cage, in a room maintained at a controlled temperature (25 ± 3 °C), humidity (30-70%), and light (12:12 light/dark cycle, lights on at 6:30 a.m.), with ad libitum access to food and water. The experimental rats were randomly assigned to eight groups, each containing six animals.

**Induction of diabetes**

Following an overnight fast, Alloxan (150 mg/kg) in 0.1 M cold citrate of sodium buffer (pH 4.5) will be administered intraperitoneally. The control group receives only the automobile. Within a week, rat having moderate diabetes, as evidenced by glycosuria and hyperglycemia (blood glucose levels more than 250 mg/dl), will be selected for the experiment (Anoop & Firdous, 2016; Sayeli & Shenoy, 2021).

**Treatment Design**

The rats were divided into 8 groups with 6 animals (n = 6) each as below

Group I- Normal control (received ml normal distilled water)

Group II- Disease control untreated Gave 5%glucose before two days and after two days Induced 120 mg/kg of alloxan

Group III- Standarad group Gave 5%glucose before two days and after two days Induced 120 mg/kg of alloxan and treated with standard drug metformin (500mg/kg) for 28 days.

Group IV-Low dose group Gave 5%glucose before two days and after two days Induced 120 mg/kg of alloxan and treated with nutraceutical powder extract low dose(200mg/kg) for 28 days.

Group V- High dose group Gave 5%glucose before two days and after two days Induced 120 mg/kg of alloxan and treated with nutraceutical powder extract low dose(200mg/kg) for 28 days.

**Evaluation of Biochemical parameters** (Katiyar, 2019)

The blood glucose levels and body weight were measured every eight days. The biochemical parameters total cholesterol, liver function tests (serum), and kidney function tests (blood creatinine) were measured at study's end. Miracle Laboratory, located Shop no.1, Kalpvan business plaza B/H Toyota Show-room, Gondal Rd, Kangasiyali, Rajkot, Gujarat 360022, was the laboratory source used to determine the MB level. Every day, physical measurements were made of a number of indicators, including water intake, and food intake.

**Body weight measurement**

Animals managed and weigh using a balance. The animals' weight is displayed at the tip of the weight balance; daily body weight measurements are taken, and rat tips will yield blood samples. weekly blood glucose analysis using the tail (0, 7, 14, 21, 28 days).

**Measuring Food Consumption**

Throughout the study period, we monitored the food consumption of each group. We serve 100gm-gram lunches every day. The residual food weight will be measured the following day, and we will conclude that, in contrast to the standard and test groups, the rats in the illness group consumed more food over the course of the day. We found that the rise in the consumption of animal foods is due to diabetes. Therefore, we plan to gather data for 45 days, after which we utilized statistical software to produce graphs that each group can compare to the standard control.

**Measurement of Water consumption**

We measured water usage for all groups from Day 1 to the completion of the research period. Every day, there is 250 milliliters of water. The next day, we used a measuring cylinder to measure and weigh the bottle's remaining water. We deduced that as the day progresses, rats' water consumption gradually grew.

**Histopathology study**

When the treatment plan was finished, the rats were slaughtered, and their liver, kidney, and pancreas were taken out. After that, the tissues were promptly preserved in a 10% formalin solution to prevent deterioration. The process of embedding in paraffin wax involved removing water using alcohol dehydration and xylene penetration. After that, sections were photographed using photomicrography, examined under a nitro scope, and stained with hematoxylin-eosin(H&E).

**Statistical Analysis**

Collected data were analyzed by using one-way ANOVA (analysis of variance) followed by Dunnett's test for multiple comparisons. Results are expressed as Mean±SEM and a p-value of less than 0.05 was considered statistically significant.

**Results and Discussion**

Phytoconstituents Test:

Table 1. Phytochemical Tests

| Sr.no. | Chemical tests | Results |
|---|---|---|
| 1. | Alkaline reagent test | ++ |
| 2. | Modified Bontrager's Test | ++ |
| 3. | Phenolic compound Test | ++ |
| 4. | Gelatin Test | ++ |
| 5. | Shinoda Test | ++ |

+ denotes the presence of a phytoconstituent, whereas - denotes its absence.

The powder of nutraceuticals powder underwent phytochemical screening, which identified Flavonoids, Alkaloids, Tannins and Glycosides.

**In vitro alpha amylase assay**

As contrast to the test groups, the Standard control group's percentage of inhibition increased significantly and gradually, as seen in [Fig]. According to this model's findings, the percentage inhibition in the standard control was significantly higher (90.59+ 0.066) than in Tests 1 (89.84 + 0.0476), 2 (89.95 + 0.0355), and 3 (90.11 + 0.05366).

Table 2. Results of Alpha amylase assay

| Conc.(mg/ml) | %inhabitation of alpha amylase (Mean±SEM) |
|---|---|
| 200 | 89.84 ±0.066 |
| 400 | 89.95 ±0.0476 |
| 600 | 90.11 ± 0.0355 |

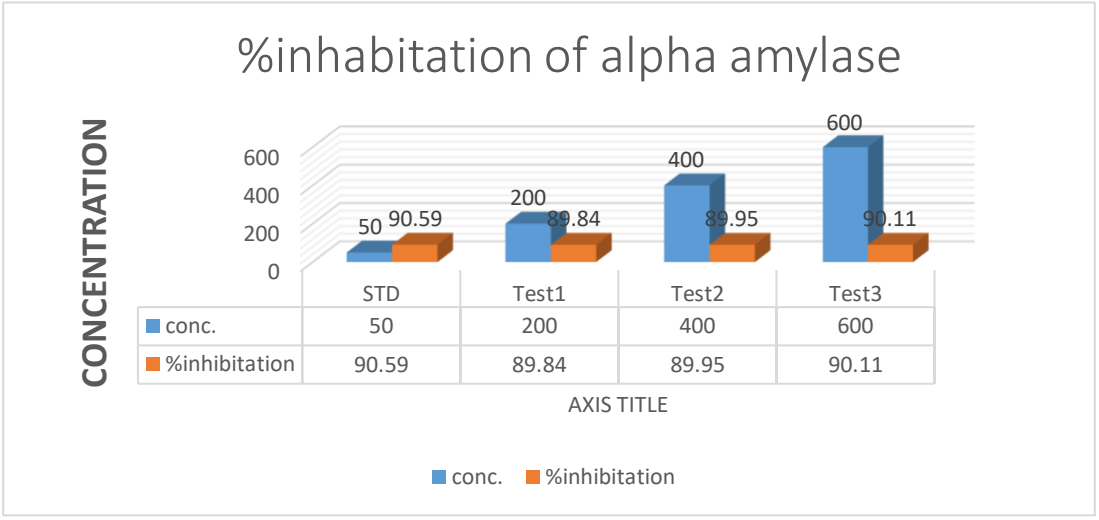



Figure 1. In vitro alpha amylase

**In vivo studies**

The following outcomes of the in vivo investigation are displayed in accordance with the protocol outlined in the Materials and Methodology section:

***Impact of Nutraceutical Powder on Body weight in a Model of Diabetes Induced by Alloxan:***

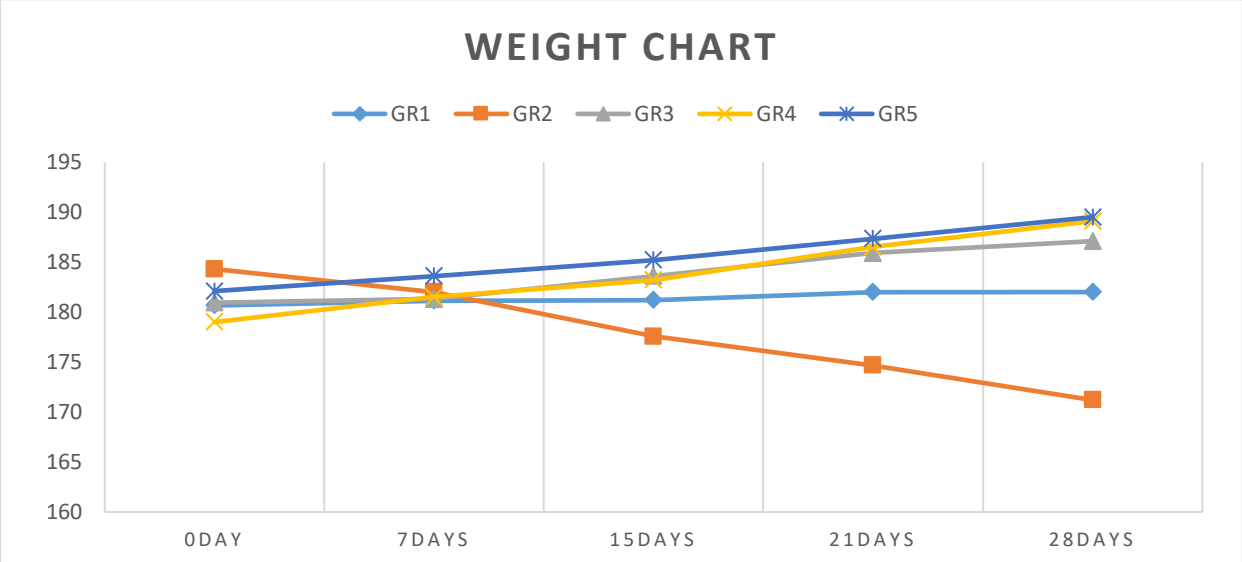



Figure 2. Weight Chart

We monitor the animals' increased body weight in this criterion every eight days to six weeks for all groups expect disease control group. As a standard metformin was employed, Nutraceutical Powder low dose and high dose were present in the test 1 and test 2 groups, respectively. In Test 1, 200 mg/kg, in Test 2, 500 mg/kg extract is used; distilled water is used as a normal control, and the disease control group is left untreated. The aforementioned graph demonstrated that, in contrast to the normal, standard, and test groups, the body weight of the rats in the illness control group decreased gradually, it's one of signs of diabetes type1.

***Impact of Nutraceutical Powder on Food intake in a Model of Diabetes Induced by Alloxan***

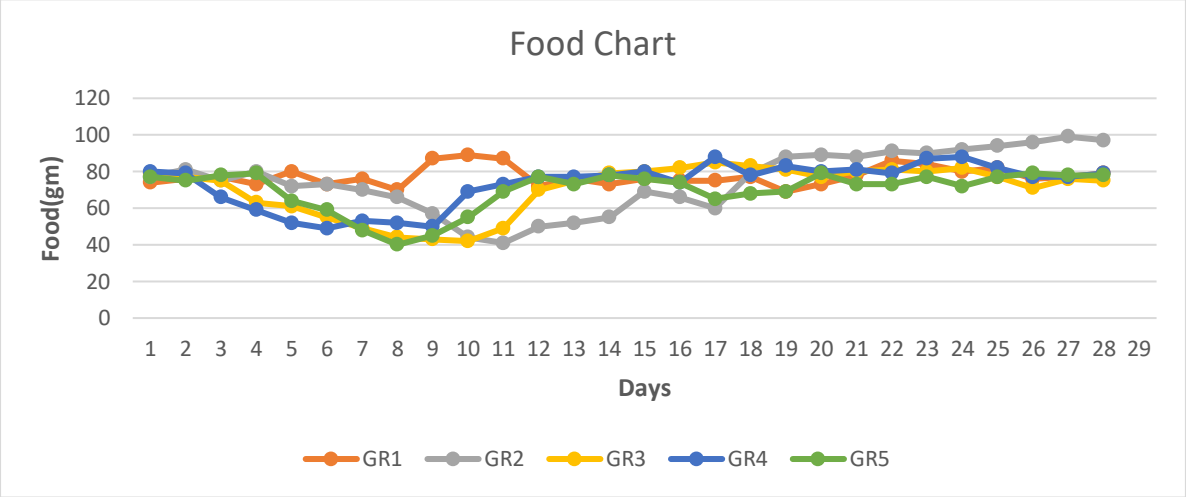



Figure 3. Food chart

We looked at each group's food intake in this parameter. Standard metformin was utilized, and Nutraceutical Powder low dose and high dose were present in the test 1 and test 2 groups,

respectively. In Test 1, an extract of 200 mg/kg is used; in Test 2, an extract of 500 mg/kg is utilized; distilled water is used as a normal control, and the disease control group is left untreated. In comparison to the normal, standard, and both test groups, the above chart demonstrated a considerable and gradual decrease in food intake in the illness control group, it's one of signs of diabetes type1.

***Impact of Nutraceutical Powder on Water intake in a Model of Diabetes Induced by Alloxan***

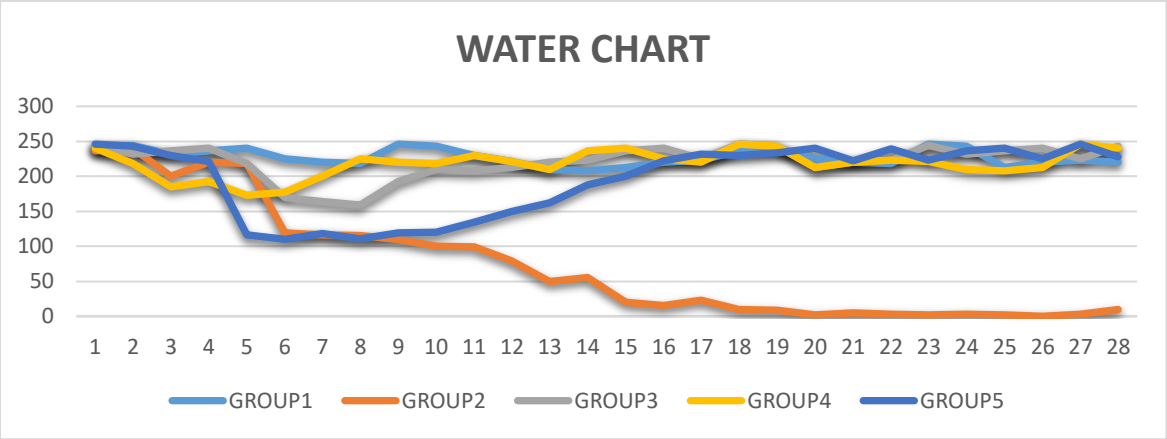



Figure 4. water chart

We examined the water intake of every group in this particular parameter. Standard metformin was used, while the test 1 and test 2 groups received low dose and high dose of Nutraceutical Powder, respectively. A 200 mg/kg extract is used in Test 1, and a 500 mg/kg extract is used in Test 2. Distilled water is used as a normal control, and the disease control group is not given any medication. The preceding data showed a significant and progressive increase in the amount of water consumed by the sickness control group when compared to the normal, standard, and both test groups.

***Effect of Nutraceutical Powder on Cholesterol parameter in a Model of Diabetes Induced by Alloxan***

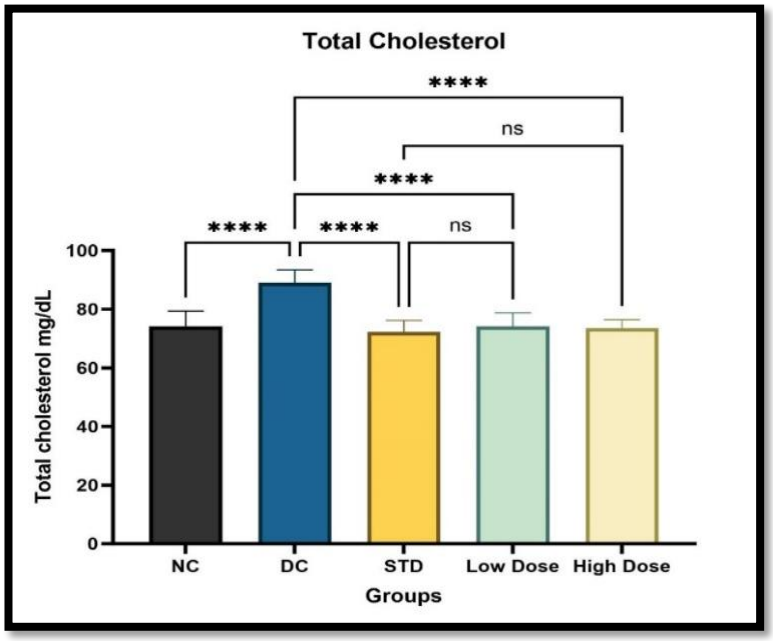



Figure 5. Cholesterol level in rat

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control.

Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the cholesterol level in the disease control group (89.01, ±3.079) was significantly higher than the cholesterol level in the normal control group (74.22, ±2.350). Comparing Standard (72.24, ± 1.924), Test 1 (74.13, ± 3.192), and Test (73.5**,** ±2.924) to that of Disease control, it was shown to be much lower.

Here, the results were displayed as mean + SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on Triglyceride parameter in a Model of Diabetes Induced by Alloxan***

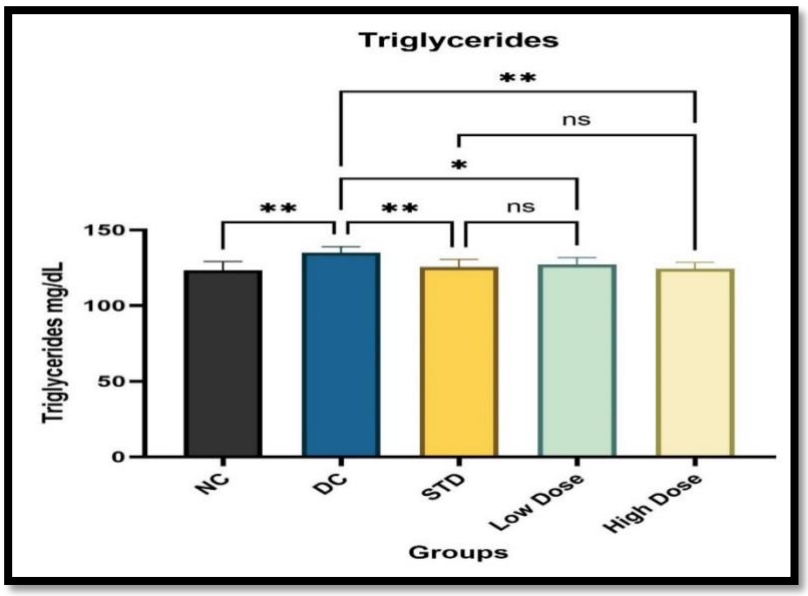



Figure 6. Triglycerides level in rat

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control.

Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the Triglyceride level in the disease control group (135.16, ±3.014) was significantly higher than the Triglyceride level in the normal control group (123.49 ±2.760). Comparing Standard (125.5, ± 2.924), Test 1 (127.04, ± 3.192), and Test2 (124.38, ±2.924) to that of Disease control, it was shown to be much lower.

Here, the results were displayed as mean ±SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on HDLparameter in a Model of Diabetes Induced by Alloxan***

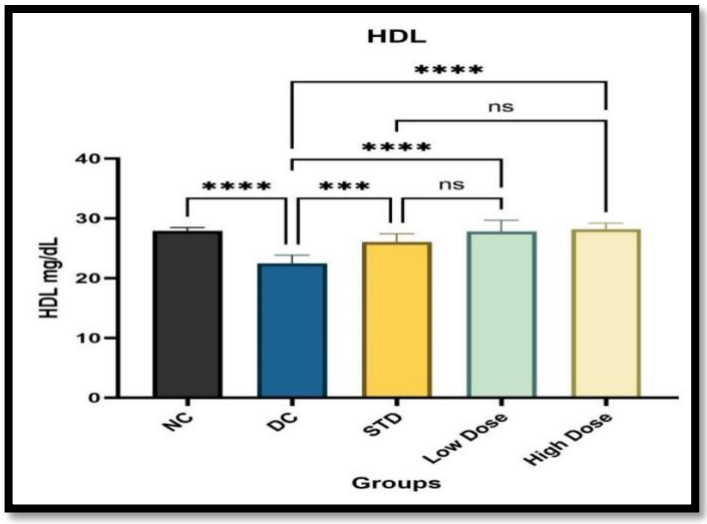



Figure 7. HDL level in rat

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control.

Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the HDL level in the disease control group (22.46, ±3.04) was significantly less than the HDL level in the normal control group (27.9, ±2.90). It was found significantly higher Comparing Standard (27.07, ± 2.24), Test 1 (28.79, ± 2.19), and Test2 (28.16, ±2.10) to that of Disease control.

Here, the results were displayed as mean ±SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on LDL parameter in a Model of Diabetes Induced by Alloxan***

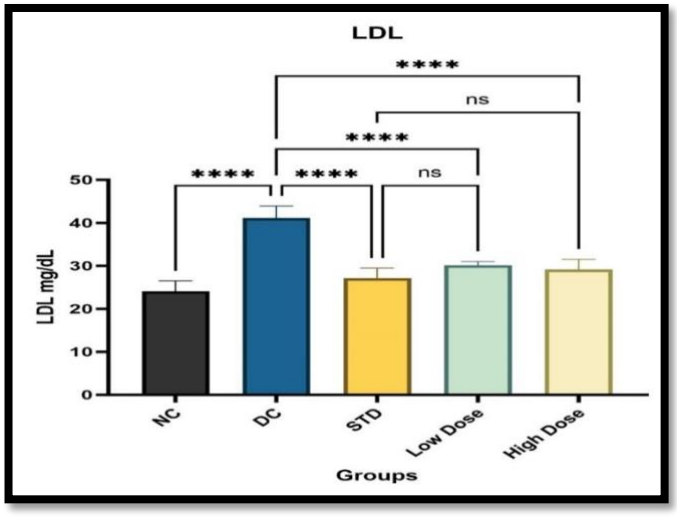



Figure 8. LDL level in rat

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control.

Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the LDL level in the disease control group (38.18, ±2.224) was significantly higher than the LDL level in the normal control group (24.08, ±2.130). Comparing Standard (18.24, ± 2.924), Test 1 (23.27, ± 3.061), and Test2 (22.85, ±2.347) to that of Disease control, it was shown to be much lower.

Here, the results were displayed as mean ±SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on VLDLparameter in a Model of Diabetes Induced by Alloxan***

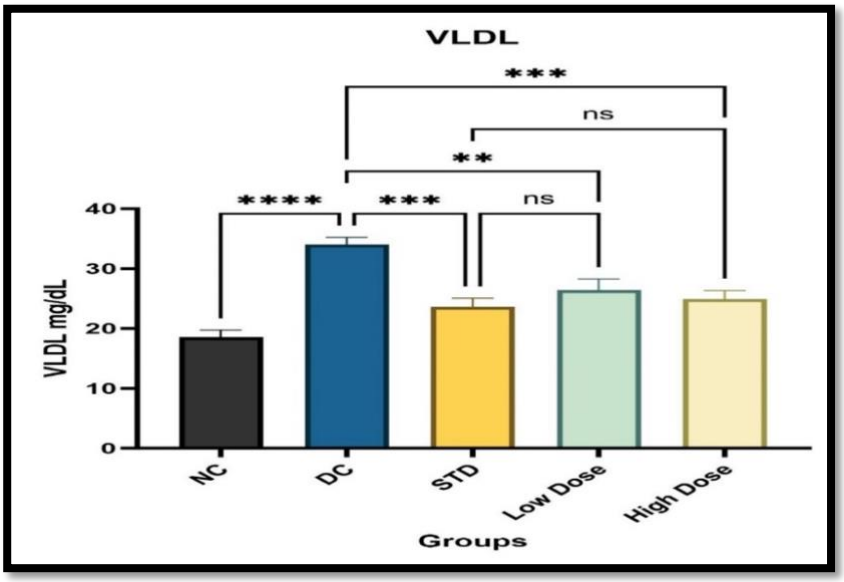



Figure 8. VLDL level in Rat

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control.

Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the VLDL level in the disease control group (27.03, ±3.036) was significantly higher than the VLDL level in the normal control group (18.54, ±2.931). Comparing Standard (21.34, ± 2.974), Test 1 (23.43, ± 2.961), and Test2 (22.56, ±2.387) to that of Disease control, it was shown to be much lower.

Here, the results were displayed as mean ±SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on Blood glucose level parameter in a Model of Diabetes Induced by Alloxan***

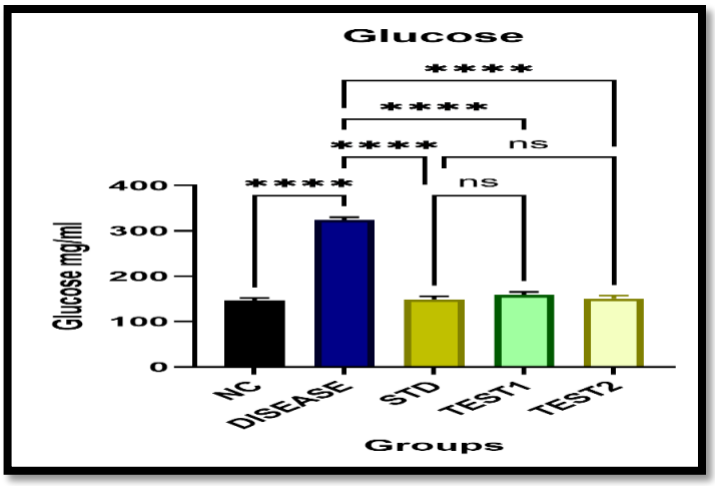



Figure 9. Blood glucose level in Rat

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control. Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the blood glucose level in the disease control group (324, ±2.076) was significantly higher than the blood glucose level in the normal control group (146, ±2.071). Comparing Standard (149, ± 2.974), Test 1 (159, ± 2.061), and Test2 (151, ±2.087) to that of Disease control, it was shown to be much lower. Here, the results were displayed as mean ±SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on Creatinine level parameter in a Model of Diabetes Induced by Alloxan***

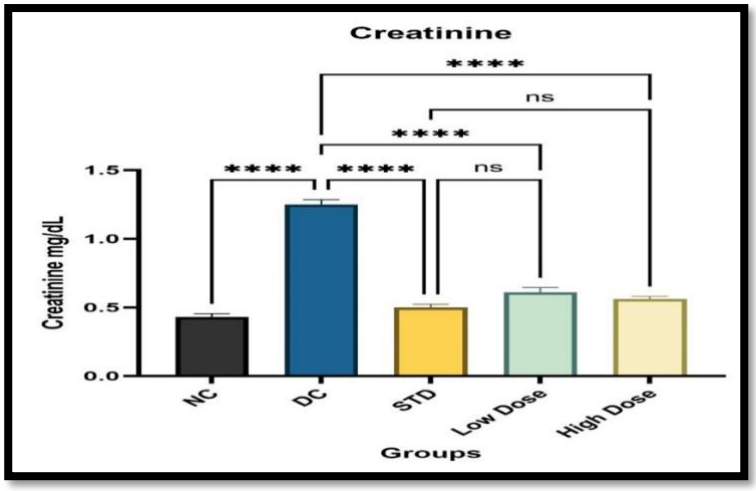



*Figure 10. Creatinine level in Rat*

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control.

Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the creatinine level in the disease control group (1.25, ±1.006) was significantly higher than the creatinine level in the normal control group (0.43, ±1.271). Comparing Standard (0.5, ± 2.974), Test 1 (0.79, ± 1.011), and Test2 (0.49, ±1.007) to that of Disease control, it was shown to be much lower. Here, the results were displayed as mean ±SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on SGPT level parameter in a Model of Diabetes***

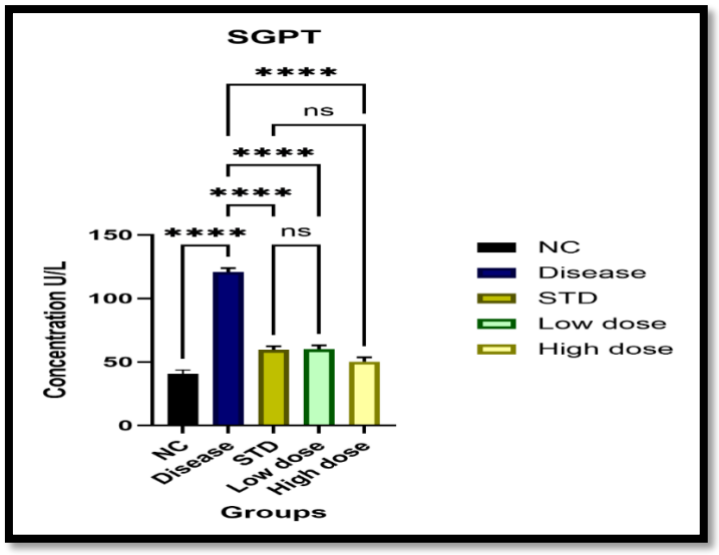



Figure 11. SGPT level in Rat

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control. Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the SGPT level in the disease control group (120.7, ±2.16) was significantly higher than the SGPT level in the normal control group (40.6, ±2.21). Comparing Standard (59.4, ± 2.17), Test 1 (60, ± 2.15), and Test2 (50.3, ±2.27) to that of Disease control, it was shown to be much lower. Here, the results were displayed as mean ±SEM.

The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on SGOT level parameter in a Model of Diabetes Induced by Alloxan***

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control.

Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the SGOT level in the disease control group (129.5, ±2.211) was significantly higher than the SGOT level in the normal control group (92.17, ±2.191). Comparing Standard

(94± 2.17), Test 1 (113.5, ± 2.153), and Test2 (103.7, ±2.174) to that of Disease control, it was shown to be much lower.

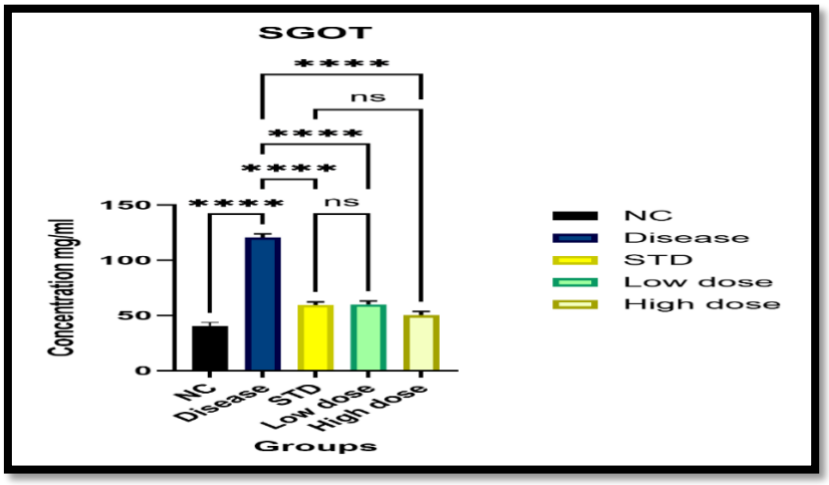



Figure 12. SGOT level in Rat

Here, the results were displayed as mean ±SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

***Effect of Nutraceutical Powder on Insulin level parameter in a Model of Diabetes Induced by Alloxan***

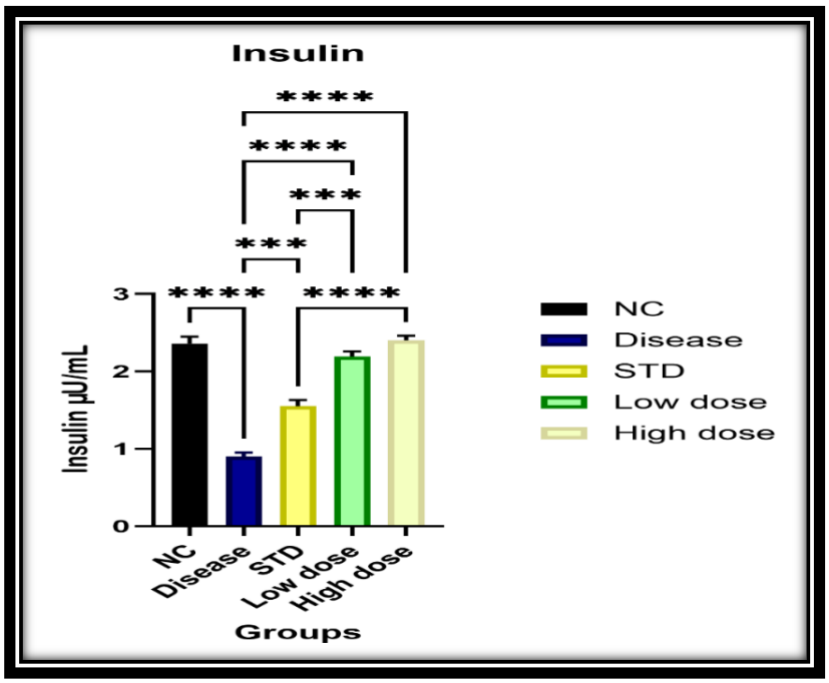



Figure 13. Insulin level in Rat

In this case, there were three controls: two different concentrations of the test extract, metformin (500 mg/kg), alloxan (120 mg/kg), and distilled water as the normal control.

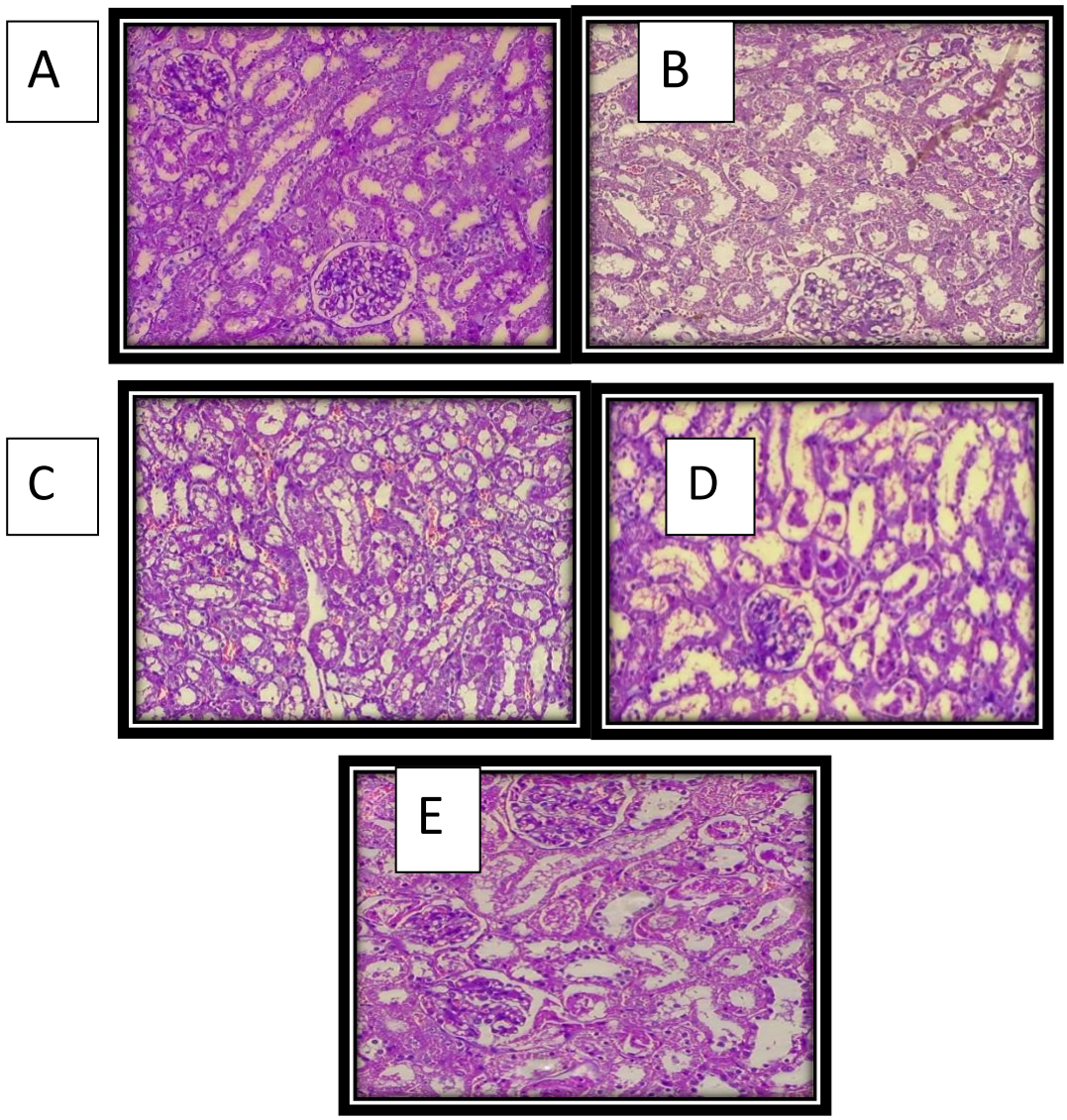
Test 2 has [500 mg/kg] of extract, while Test 1 contains [200 mg/kg]. This model's result indicates that the insulin level in the disease control group (0.9, ±3.01) was significantly less than the insulin level in the normal control group (2.36, ±2.81). It was found significantly higher Comparing Standard (1.55, ± 2.84), Test 1 (2.19, ± 2.79), and Test2 (2.4, ±2.80) to that of Disease control.

Here, the results were displayed as mean ±SEM. The graph $ shows a significant difference between the disease control and normal control groups, and the *** shows a significant difference between the test and standard groups and the disease control group! where $p < 0.001$ denoted a highly significant degree of significance. Here, as compared to the normal control, there is no discernible difference between the standard and test groups.

**Histopathology**

***Histopathology of Pancreas, Kidney, Liver***

**Histopathology of Kidney:** Figure30: Histopathology 0f Kidney: A-Normal control, B-Disease control, C-Standarad Control, D-Test1 (Low dose), E-Test2(high dose): all these images taken in 25x dimension.




Sample photograph of a group of rats' kidneys that were given normal water orally for three weeks demonstrates the normal histology of the renal tubules and glomeruli.

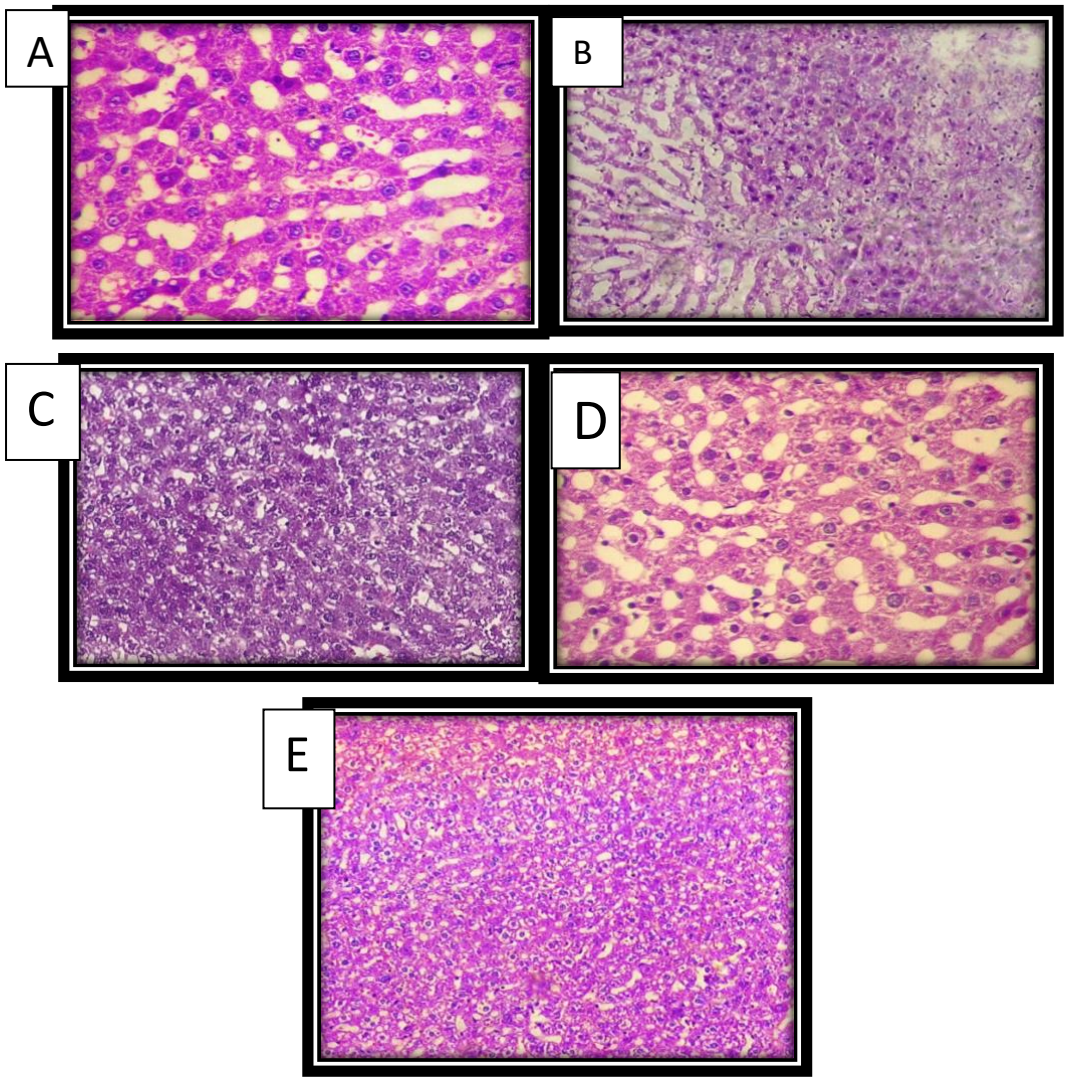
The group that was pretreated with Alloxan (120 mg/kg) showed normal to decreased glomerular cellularity in the IP, and the renal tubules showed swelling of the cells with an increase Glomerulus inflammation

Group that received oral metformin (500 mg/kg) and intraperitoneal Alloxan (120 mg/kg) for three weeks, demonstrating the maintenance of normal renal tissue histoarchitecture.

Group that received oral Nutraceutical powder (200 mg/kg) and Alloxan (120 mg/kg) for three weeks before to treatment had normal glomerular and tubule shape.

Group given oral Nutraceutical powder (500 mg/kg) and Alloxan (120 mg/kg) for three weeks prior to treatment demonstrated normal renal tissue histology in comparison to the disease control group.

**Histopathology of Liver:** Figure: Histopathology 0f Liver: A-Normal control, B-Disease control, C-Standarad Control, D-Test1 (Low dose), E-Test2(high dose): all these images taken in 25x dimension.

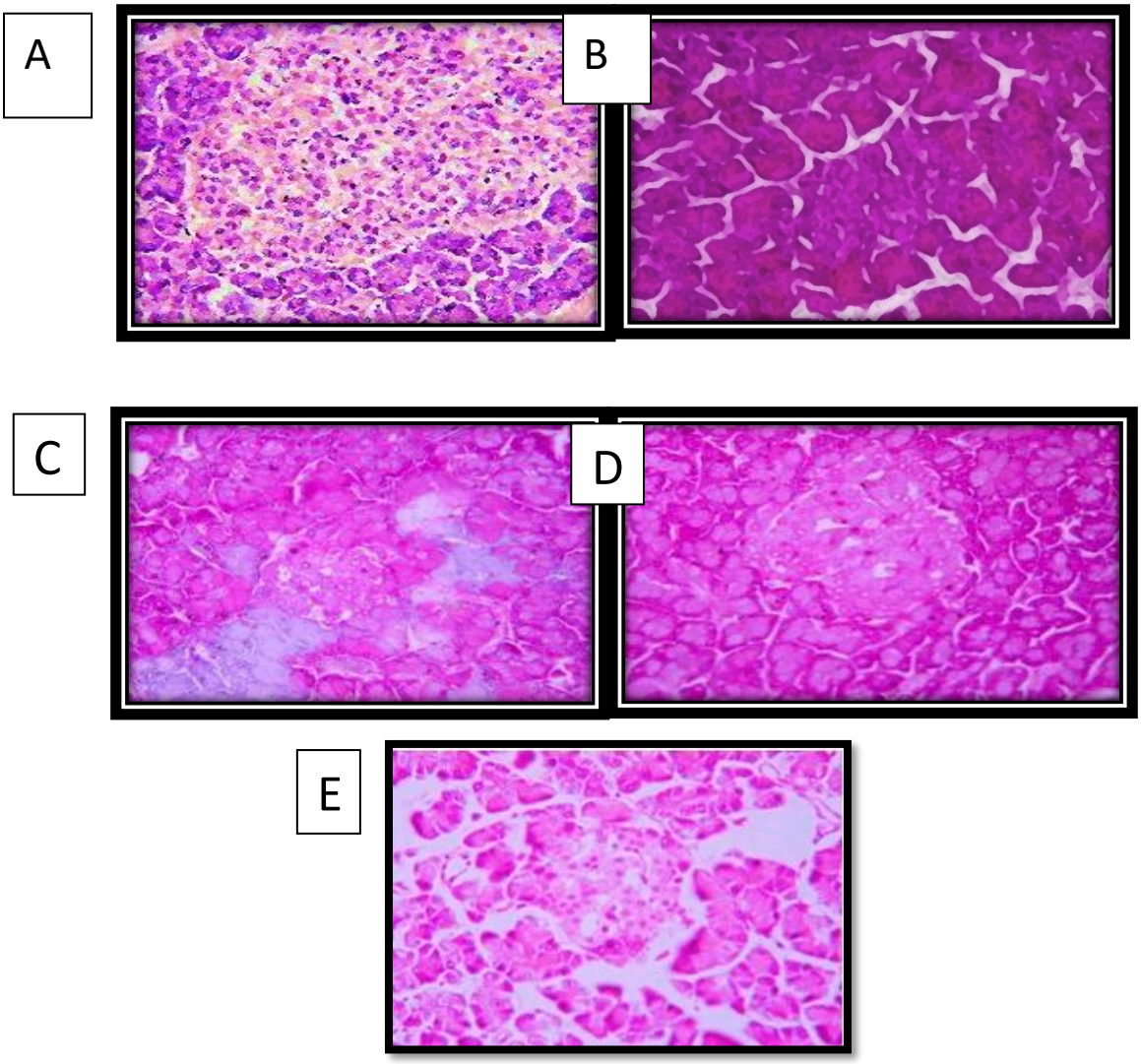



The histology of the liver tissue revealed that the normal control group had a normal liver architecture with radiating hepatocytes.

However, hepatocyte degradation and a discernible infiltration of inflammatory cells were observed in the disease control group, suggesting a minor liver injury caused by alloxan. Treatment with our formulation at two doses (200 mg/kg and 500 mg/kg) with metformin.

However, significantly improved the histology of the liver by reducing hepatocyte degradation and inflammatory cell infiltration. These results suggest the potential benefits of our formulation in preventing liver damage in individuals with type 1 diabetes.

**Histopathology of Pancreas:** Figure32: Histopathology 0f Pancreas: A-Normal control, B-Disease control, C-Standarad Control, D-Test1 (Low dose), E-Test2(high dose): all these images taken in 45x dimension.




Sample photograph of a group of rats' kidneys that were given normal water orally for three weeks demonstrates the normal histology of the renal tubules and glomeruli.

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Group that received oral Nutraceutical powder (200 mg/kg) and Alloxan (120 mg/kg) for three weeks before to treatment had normal glomerular and tubule shape.

Group given oral Nutraceutical powder (500 mg/kg) and Alloxan (120 mg/kg) for three weeks prior to treatment demonstrated normal renal tissue histology in comparison to the disease control group.

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