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RESEARCH ARTICLE

Effect of *Sapindus trifoliatus* Ethanol Extracts in Diabetic Associated Complication

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Abstract

Background: Diabetes mellitus is a long-term metabolic disease marked by persistently high blood sugar levels. It can be the result of deficiencies in either the action or production of insulin or both. Diabetes mellitus is a major global health issue since it may lead to retinopathy, nephropathy, cardiovascular diseases and many more. *Sapindus trifoliates* plant extract may help to control hyperglycemia and prevent complications in Diabetes mellitus. **Aim:** The study aimed to evaluate the ability of ethanol extracts of the leaves (STL) and fruit (STF) extract of *S. trifoliatus* to control hyperglycemic conditions in alloxan-induced diabetic rats. **Methods:** Alloxan-induced diabetic rat models were employed to study the antidiabetic potential of ethanol plant extract (leaves and fruit) of *S. trifoliatus.* The effect of *S. trifoliatus* extract on the rat models and associated complications, including diabetic retinopathy, nephropathy and cardiac complications were assessed. In Diabetic rat models, hypoglycemic activity, antidiabetic activity, and kidney function were determined. **Results:** Treatment with plant extract showed that it has a significant antihyperglycemic effect in the experimental model of Diabetes mellitus. Additionally, the extract effectively mitigated the Diabetes-induced complications in the eye, kidney, and heart of the animal. **Conclusions:** *S. trifoliatus* extract can be used as a promising therapeutic and preventive agent in the treatment of diabetes and associated complications.

Major Findings: The study found that ethanol extracts of *Sapindus trifoliatus* leaves and fruit part effectively manage diabetes in alloxan-induced diabetic rats. The extracts lowered blood glucose levels and protected against diabetes-related complications in the eyes, kidneys, and heart, suggesting the plant's potential as a natural remedy for both hyperglycemia and its associated complications.

Keywords: Alloxan, Diabetic Complications, Diabetes Mellitus, Glibenclamide, *S. trifoliatus*

1. Introduction

A large number of people with Diabetes die from complications, even though their blood glucose levels are well managed. When looking for new medications, animal models must play an essential role. While several animal models have been utilized to examine the effects of diabetes, including those that are genetically modified and others that are chemically produced, none of these models have been demonstrated to mimic the human pathophysiology. Therefore, it is of the utmost importance to locate and identify the appropriate animal model. Unhealthy levels of the pancreatic hormone insulin, which lead to consistently elevated blood sugar levels, characterize diabetes, a kind of Chronic Noncommunicable Disease (CNCD)[1-4]. It is the most pressing problem impacting health around the world and belongs to a group of metabolic diseases. Type 1, Type 2, and Gestational Diabetes are the three main classifications of Diabetes mellitus, which are based on the causes and symptoms. T lymphocytes destroy pancreatic β-cells, resulting in a total absence of insulin, in Type 1 Diabetes, a disorder now referred to as insulin-subordinate mellitus diabetes or teenage diabetes. Approximately 5% to 10% of individuals with diabetes also have Type 1 Diabetes[5-10]. The most

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prevalent form of diabetes is Type 2, which comprises at least 90% of all cases. Adult-onset diabetes, non-insulin-dependent diabetes, and others use this term to describe the same condition. Insulin resistance and relative insulin deficiency are the hallmarks of this condition.

Two likely routes of chemically induced diabetes mellitus are utilized by the deadly glucose analogue (5,5-dihydroxyl pyrimi-dine-2,4,6-trione), an organic compound known as alloxan. The pancreatic beta-cell glucose sensor glucokinase is inhibited by alloxan, which preferentially reduces insulin secretion in response to glucose, according to one study. Furthermore, it triggers the generation of superoxide radicals through a redox cycle that begins with the creation of Reactive Oxygen Species (ROS). Following its reduction to dialuric corrosive and subsequent reoxidation to alloxan, the latter generates superoxide radicals. These radicals are then dismutase (by superoxide dismutase) into hydrogen peroxide. Additionally, hydroxyl radicals may be produced as a result of side reactions[11-14]. Apoptotic cell death could be the outcome of β-cell DNA fragmentation caused by these highly reactive oxygen species. The liver has several systems for biotransformation and clearance of xenobiotics and better defensive mechanisms against ROS than β-cells; hence, the hepatotoxicity produced by alloxan, even though it is absorbed by the liver as well, is minimal or false. Furthermore, alloxan enhances basic oxidation, which includes SH classes and proteins and disrupts intracellular calcium homeostasis, leading to accumulation of supraphysiological calcium and cell injury[15-17]. Depending on the strain and route of administration, the dosages of alloxan range from 40 to 200 mg/kg for rodents and 50 to 200 mg/kg for mice. For example, administering alloxan intraperitoneally or subcutaneously requires doses that are several times higher than intravenous administration.

2. Materials and Methods

All chemicals required for all assays were analytical grade and were obtained from Sigma-Aldrich Chemicals. The *S. trifoliatus* leaves and fruit parts were harvested in the Himalayan region of Uttarakhand between June and August. The materials were collected, cleaned, and then dried in the shade. A mixer was used to grind or blend dried materials into fine powders. The Soxhlet apparatus was used to extract the plant material from the powder.

The animals were divided into 7 groups of 6 each. The standard group received 1 ml of the reference drug Glibenclamide (2 mg/kg), the 1st Group received only the vehicle (1 ml of normal saline), the 2nd Group received Alloxan (70 mg/kg dissolved in citrate buffer pH 4.5) intraperitoneal route, the 3rd and 4th Groups received an ethanol extract of leaves of *S. trifoliatus* (STL 250mg and 500mg/kg body weight per oral) and the 5th and 6th Groups received an ethanol extract of fruits of *S. trifoliatus* (STF 250mg and 500mg/kg body weight per oral).

2.1 Preliminary Phytochemical Studies

A preliminary phytochemical analysis of the aqueous, methanol, and ethanol extracts of *S. trifoliatus* leaves (STL) and fruit (STF), obtained using the Soxhlet apparatus, identified bioactive compounds like alkaloids, flavonoids, saponins, tannins, and glycosides. For further studies, ethanol extracts were found best of all three recommended as they efficiently extract a wide range of compounds, making them ideal for further pharmacological research.

2.2 Acute Toxicity Studies

Animals used for experiments- Animal house in Delhi sold male inbred Swiss albino mice weighing between 20 and 35 grams, animals were purchased for various research and laboratory purposes. The animals were housed in well-ventilated polypropylene cages with a 12-hour light or dark cycle. The animals were provided with regular pellet feed and unrestricted access to drinking water during the trial (Hindustan Lever Limited, Bangalore). The animals were acclimated to the laboratory setting a week before the start of the experiments. The Institutional Animal Ethics Committee (IAEC) Suresh Gyan Vihar University Jaipur Rajasthan, under the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), grants approval for ethical use of animals in experiments.

For the toxicity study, every stage of the acute toxic class technique uses four mice of the same sex. Depending on the mortality or morbidity state of the rats, an average of two to three phases are required to

assess the test drugs acute toxicity. A valid scientific conclusion based on the data was possible because so many mice were employed in this method. The predefined dosage technique (2000, 1000, 500, 50, and 5 mg/kg body weight) was used to study and classify medications according to the Globally Harmonized System (GHS) for chemicals that induced acute toxicity.

2.3 Experiment Methodology

Male Albino mice weighing between 20 and 35 g were used in this study. Oral administration of plant parts of *S. trifoliatus* at 500, 1500, 2500, or 5000 mg/kg body weight was possible. The animals were given dose volume ad libitum following an overnight fast. After administering *S. trifoliatus*, food was delayed for an additional three to four hours while toxicity symptoms were monitored. The autonomic nervous system, respiratory system, circulatory system, eyes, skin, fur, mucous membranes, motor activity, and behavior pattern of the mice were all affected. We measured the mice's body weight both before and after the medication were given. Additionally, observations included convulsions, tremors, salivation, diarrhea, unconsciousness, and lethargy. The signs and the onset of toxicity are also highlighted (OECD 2002).

2.4 Ethanol Extract's Antidiabetic Activity

Diabetes induction - For both sexes, Albino Wistar rats weighing between 190 and 230 grams were utilized. Rats were deprived all night long before receiving an intraperitoneal injection of alloxan (70 mg/kg diluted in citrate buffer pH 4.5). Accu-check was used to measure the Blood Glucose Level (BGL) after 72 hours, confirming the induction of diabetes. Rats having Blood Glucose Levels (BGL) more than 300 mg/dl were employed in this study.

After diabetes induction,the animals were divided into 7 groups of 6 each. The Standard Group received 1 ml of the reference drug Glibenclamide (2 mg/kg), the 1st Group received only the vehicle (1 ml of normal saline), the 2nd Group received Alloxan (70 mg/kg dissolved in citrate buffer pH 4.5) intraperitoneal route, the 3rd and 4th Groups received an ethanol extract of leaves of *S. trifoliatus* (STL 250mg and 500mg/kg body weight per oral), and the 5th and 6th Groups received an ethanol extract of fruits of *S. trifoliatus* (STF 250mg and 500mg/kg body weight per oral).

2.5 Measuring Blood Glucose Level

For a continuous 20 days, 7 Groups (n = 6) of specifically selected diabetic rodents were given the same treatment. At 0, 5, 10, 15, and 20 days, the fasting blood glucose levels were evaluated using the GOD-POD (glucose oxidase-peroxidase) unit technique.

2.6 Glycosylated Hemoglobin Estimation (%HbA1c)

The glycosylated hemoglobin was estimated using the Drabinski method. Drabeck's reagent (5 milliliters) should be added after 0.02 milliliters of serum have been separated. Following 5 minutes of blending the mixture, the absorbance at 540 nm was measured[17-19]. The concentration of glycosylated hemoglobin was computed using the following equation.

$$GHb = \frac{At}{As} * Cs(g/dl)$$

At= test absorbance

As= standard absorbance

Cs= Standard Lipid Profile Concentration (g/dl)

2.7 Effect on Diabetic Rat's Eye

2.7.1 Lens Preparation

Rats were sacrificed before the ocular globes were removed. There were lens dissections done. A pre-chilled 0.2 M potassium phosphate buffer, pH 7.0, was used to homogenise the pair of lenses. To determine the protein content, this homogenate was used.

2.7.2 Protein Estimation in Lens

Proteins create a coloured complex when they react with the Folin-ciocalteau reagent. The current colour is caused by phosphomolybdate, which is reduced by tryptophan and tyrosine in the protein. Alkaline copper which is soluble also reacts with the protein. The intensity of the colour is determined by the amount of aromatic amino acids present.

2.7.3 Procedure

Tissue homogenate (0.1 ml) was diluted with 10 ml of saline. After being separated, 1 ml of 10% TCA was combined with 1 ml of diluted tissue homogenate. The mixture was centrifuged for 15 minutes at 300 rpm. The residue was suspended in 1.0 millilitre of 0.5N sodium hydroxide after the supernatant was discarded. After

removing 0.1 ml of the solution from the above and diluting it to 1 ml, 4.5 ml of alkaline copper reagent was combined. The mixture was allowed to stand at 37° C for ten minutes. 0.5 ml of phenolphthalein was added, and then the reagents were mixed. After giving the tubes a thorough shake, they were left at room temperature for half an hour. In addition, a blank and a series of concentration standards ranging from 20 to 100 µg were carried out. In a UV spectrophotometer, the blue shading shape was resolved at 620 nm in comparison to a reagent blank. The standard graph was created using Bovine serum albumin (Sigma Chemicals Co.). The protein concentration is given in milligrams per gram of tissue's moist weight.

2.7.4 Sodium and Potassium Contents of the Lens

Using flame photometry, the Na+ and K^+ contents of the lens supernatant were ascertained. The concentrations of Na+ and K^+ were reported as mg/100g wt. of lens using standards.

2.7.5 Water Contents

The precipitates from the centrifugation process above were maintained at 110° C in an oven until a steady dry weight was attained. By figuring out the lens's changes in wet and dry weight, the water content was calculated. The average values are expressed in mg/100g of the lens's wet weight.

2.8 Effect of Ethanol Extract in Diabetic Rat's Kidney

In-vitro determination of plasma creatinine, urine creatinine, Blood Urea Nitrogen (BUN) and urine protein were performed by using commercially available ERBA kits (Biochemistry test kits).

2.8.1 Relative Kidney Weight

Relative Kidney wt. (gm/100gm) has been measured by Formula[20,21].

2.9 Effect of Ethanol Extract in Diabetic Rat's Heart

2.9.1 Hemodynamic Measurement

The animals were trained to measure blood pressure and heart rate for 3 weeks, using a non-invasive technique called rat tail-cuff plethysmography, the hemodynamic parameters. Such as heart rate and blood pressure (both systolic and diastolic) were recorded on the 0^{th} and 20^{th} day of each group of rats. To calculate the heart weight or body weight ratio, animals were put to sleep, their hearts removed, and their blood separated before the hearts were weighed. The heart-to-body weight ratio was then calculated using the given formula[22].

2.9.2 Determination of AST (Aspartate Aminotransferase)

The substrate (0.5 ml) was added to the test tubes for both the test and the blank, and they were incubated for a short while at 27° C in a hot water bath. Transfer 0.3 ml of serum into each test tube and incubate for 30 minutes at 37° C. Following thorough mixing and a 20 minute room-temperature incubation period, 0.5 ml of DNPH reagent was added to each tube. Both tubes were filled with 5.0 ml of NaOH (0.4N), and 10 minutes were allowed to pass. Note the absorption at 540 nm.

2.9.3 Determination of Superoxide Dismutase (SOD)

The reaction sample contained 0.2 ml of 0.1 mm EDTA and 1 ml of 50 nm sodium carbonate, and 0.5 ml of serum, 0.4 millilitres of 1 mm hydroxylamine-hydrochloride, were added to start the reaction. The absorbance variation was measured at 560 nm. The control was conducted concurrently without homogenate. SOD (Superoxide Dismutase) activity was expressed as the enzyme's % inhibition of the NBT (Nitro Blue Tetrazolium) reaction.

2.10 Statistical Analysis

For a sample of 6 rodents, all values were expressed as mean ± SD. After performing an analysis of variance (ANOVA) test, each subject was compared individually using the Newman-Keuls test with heart data. Next, the heart-to-body weight ratio was calculated using the given formula.

3. Results

3.1 Phytochemical Analysis

The Table 1 indicates the aqueous, methanol, and ethanol extracts of *S. trifoliatus* contain significant bioactive compounds, including alkaloids, phenols, glycosides, flavonoids, saponins, and steroids. Additionally, the reason why ethanol-based extracts were used in this

Table 1. Phytochemical analysis

Physicochemical Tests	STL (Aqueous Extract)	STL (Ethanol Extract)	STL (Methanol Extract)	STF (Aqueous Extract)	STF (Ethanol Extract)	STF (Methanol Extract)
Glycosides	+	++	+	+	+	-
Flavonoids	+	++	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Alkaloids	+	++	+	-	+	-
Tannins	+	+	+	+	+	+
Phenols	+	++	+	+	+	+

+: Presence of compounds
-: Absence of compounds
STL- *S. trifoliatus* leaves
STF- *S. trifoliatus* fruit

Table 2. Results of acute toxicity studies for *S. trifoliatus* leaves extract

S. No	Treatment	Dose (mg/kg b.w.)	Signs of Toxicity	Onset of Toxicity	Duration of Study
1	STL	500 mg	No	Nil	14 Days
2	STL	1500 mg			
3	STL	2500 mg			
4	STL	5000 mg			

Table 3. Results of acute toxicity studies for *S. trifoliatus* fruit extract

S. No.	Treatment	Dose (mg/kg b.w.)	Signs of Toxicity	Onset of Toxicity	Duration of Study
1	STF	500mg	No	Nil	14 Days
2	STF	1500mg			
3	STF	2500mg			
4	STF	5000mg			

research was because they effectively extract a range of these compounds while preserving their bioactivity. Ethanol is therefore a reliable and secure solvent for testing on animals. Furthermore, ethanol extracts facilitate standardization, ensuring consistency and enabling better comparisons across different studies.

3.2 Acute Toxicity Studies

In this study, the mice's body weights both before and after the drug treatment was evaluated, and the weight changes were not as significant. There were no abnormalities seen in the animal's body, and mortality was not noted. Therefore, the doses of 250 mg/kg and 500 mg/kg b.w. were selected for study (Table 2).

The mice's body weight was recorded both before and after drug treatment, with less noticeable weight changes. In addition to no signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep, or coma, there are no changes in the skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic, central nervous system, motor activity, or behavior pattern. Additionally, the initial signs and symptoms of toxicity were noted. Mortality was noted at the highest dose. Therefore, choose dose levels of 250 mg/kg and 500 mg/kg b.w. (Table 3).

3.3 Hypoglycemicactivity

3.3.1 Normoglycemic Rats (Single Dose Method)

In rats with normoglycemia, treatment of the plant extract in a single dose was observed to lower blood glucose levels, (Figure 1). The highest drop in blood glucose was observed in an hour for STL-500 mg/

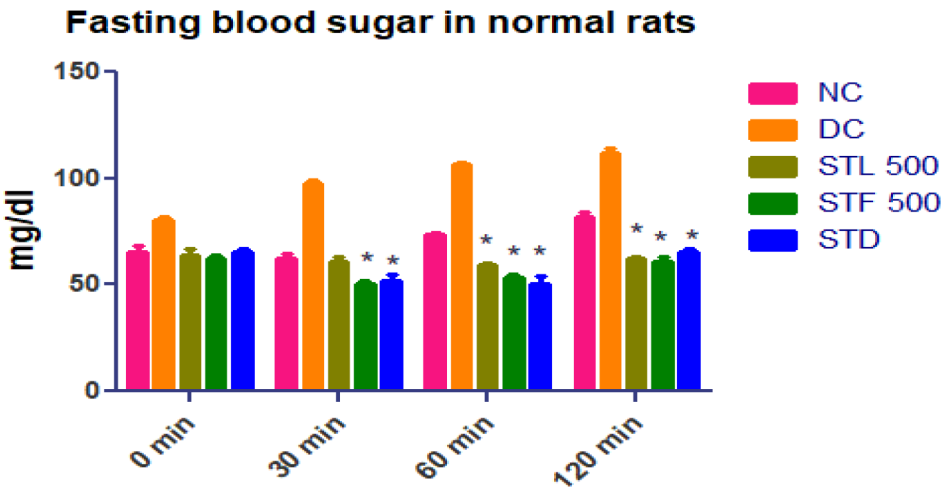


Figure 1. Effect of single-dose administration of ethanol extracts of *S. trifoliatus* extract on blood glucose levels in normal rats.

Values expressed Mean ± SEM, where #P<0.001 as compare to control, *P<0.05, **P<0.01 and ***P<0.001 compared to DC group, n=6 in each group.

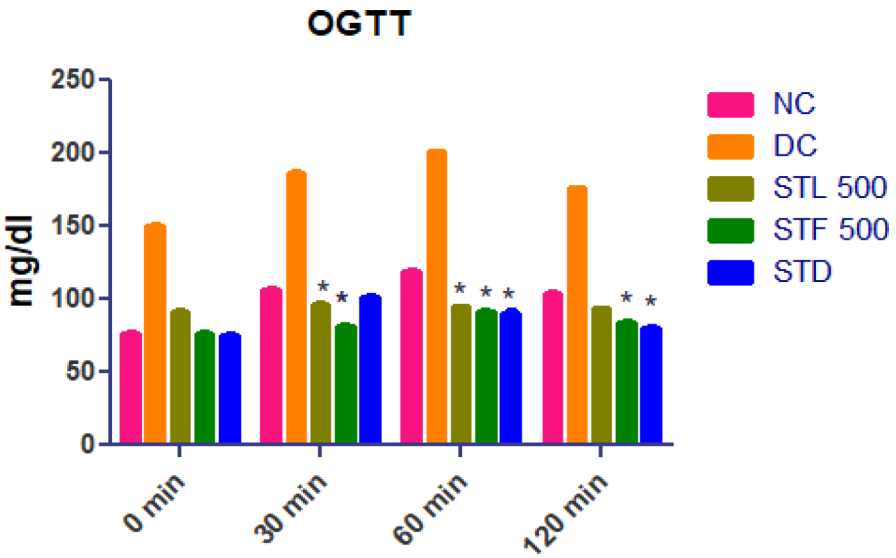


Figure 2. Effect of ethanol extracts of *S. trifoliate* on blood glucose levels in glucose-loaded hyperglycemic (OGTT) alloxan induced diabetic rats.

Values expressed Mean ± SEM, where #P<0.001 as compare to control, *P<0.05, **P<0.01 and ***P<0.001 compare to DC group, n = 6 in each group.

kg, 30 minutes for STF-500 mg/kg, and an hour for glibenclamide, the conventional medication, in a single dosage study.

3.3.2 Oral Glucose Tolerance Test (OGTT) (Single Dose Method)

Figure 2 displays the ethanol extracts effects on rats that were given glucose. When compared to normal control rats, it is shown that treatment with 500 mg/kg doses of both the leaf and fruit extract of *S. trifoliatus* dramatically lowered blood sugar at 30, 60, and 120 minutes. Rats given glibenclamide also had considerably decreased blood glucose levels during all periods.

3.4 Antidiabetic Activity

3.4.1 Blood Glucose Level

Following a 20-day course of treatment, with the help of Accuchek® we measured the Blood Glucose

Level (BGL). According to the results, every sample had significant effects on BGL (Figure 3). STL-500 (78.745%) and STF-500 (82.636%) demonstrated higher blood glucose reductions compared to DC.

3.4.2 *Glycated Hemoglobin (%HbA1c)*

Results revealed that glycated hemoglobin significantly (***p<0.001) diminished by all the samples. The order of decrease observed was STD highest followed by STF-500 and STL-500 compared to DC (Figure 4). Further, it showed its ability to halt diabetic associated complications.

3.5 Effect of Drugs in Diabetic Associated Complications

3.5.1 *Effect of Drugs in Alloxan-induced Diabetic Rat's Eye*

Results showed that every extract (except STL-250) considerably (*P<0.05, **P<0.01, and ***P<0.001) decreased the amount of water in the lens (Figure 5). A dose-dependent increase in total protein in the lens was observed with extracts (*P<0.05, **P<0.01, and ***P<0.001), which may have been caused by inhibition of the polyol pathway. STF-500 and STF-250 were found to have a stronger effect than STL 250 and STL 500 leaves floral extracts.

3.5.2 *Effect of Drugs in Alloxan-induced Diabetic Rat's Kidney*

3.5.2.1 *Effect of Drugs on Relative Kidney wt. and Biomarkers of Alloxan-induced Diabetic Rat's Kidney*

The administration of all samples, as compared to DC, was observed to decrease the relative kidney weight in diabetic rats in a dose-dependent manner, showing the extracts potential to protect the kidneys from damage (Figure 6). The current analysis revealed that DC had elevated renal indicators, and all extracts significantly (*P<0.05, **P<0.01, and ***P<0.001) decreased blood urea nitrogen levels, serum creatinine, and urea creatinine in a dose-dependent manner when compared to DC. A significant reduction in the total urine protein level (*P<0.05, **P<0.01, and ***P<0.001) was observed when extracts were given in a dose-dependent manner, suggesting enhanced renal functioning (Figure 6).

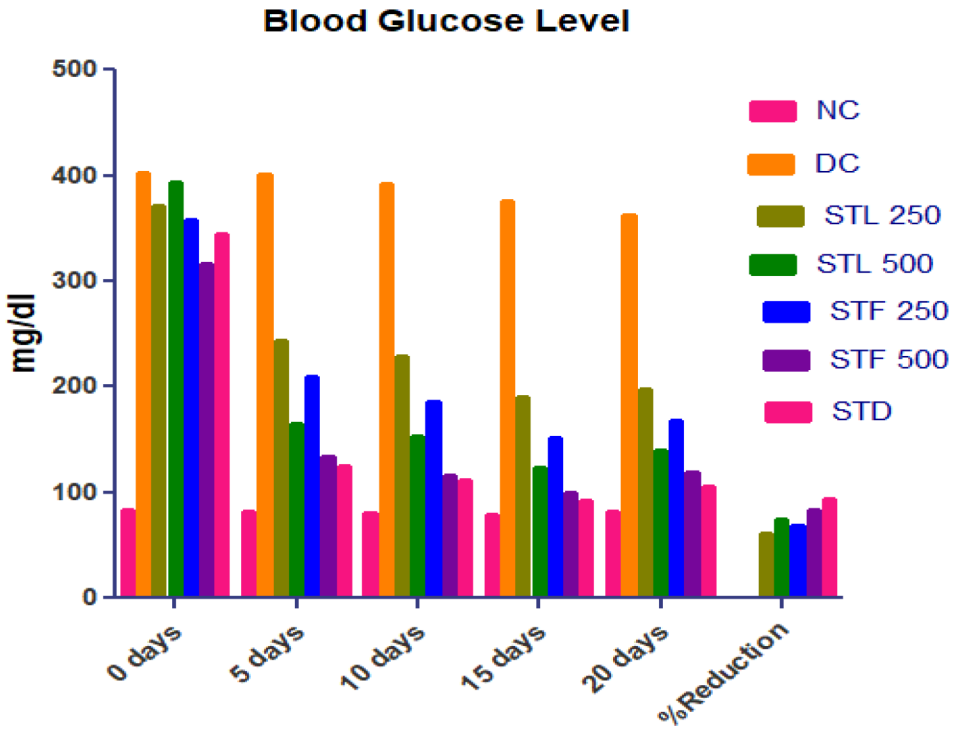


Figure 3. Effect of ethanol extracts of *S. trifoliatus* of glucose level on alloxan induced diabetic rats.
Values expressed Mean ± SEM, where #P<0.001 as compare to control, *P<0.05, **P<0.01 and ***P<0.001 compare to DC group, n = 6 in each group.

3.5.3 Effect of Drugs in Alloxan-induced Diabetic Rat's Heart

3.5.3.1 Effect of Drugs in Biomarkers and ECG Parameters of Alloxan-induced Diabetic Rat's Heart

In the current study, it was found that SOD levels significantly increased in animals treated with all extracts, except STF-250 (*P<0.05, **P<0.01, and ***P<0.001), as compared to Diabetic Control (DC) mice. In addition, all of the extracts significantly (*P<0.05, **P<0.01, and ***P<0.001) reduced the levels of cardiac enzymes, AST, in comparison to the diabetic control, indicating that they offered cardioprotection and prevented heart damage (Figure 7). The animals administered with different extracts showed significant reductions in all hemodynamic parameters, including the HW/BW ratio, mean B.P., systolic B.P., and diastolic B.P. (*P<0.05, **P<0.01, and ***P<0.001), except

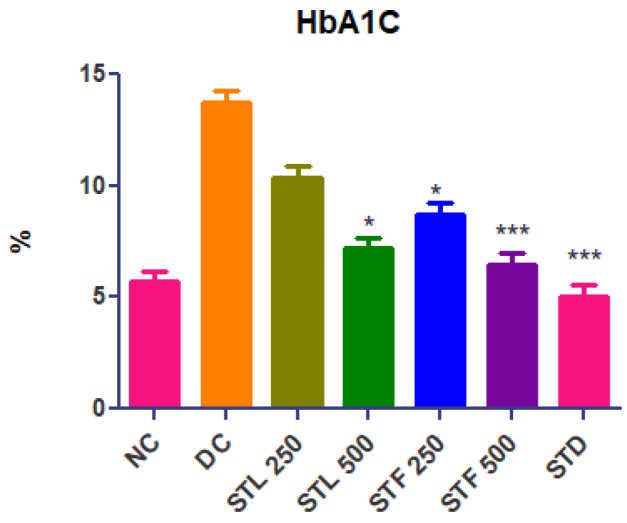


Figure 4. Effect of ethanol extracts of *S. trifoliatus* on % reduction of HbA1c in alloxan induced diabetic rats.
Values expressed Mean ± SEM, where #P<0.001 as compare to control, *P<0.05, **P<0.01 and ***P<0.001 compare to DC group, n = 6 in each group.

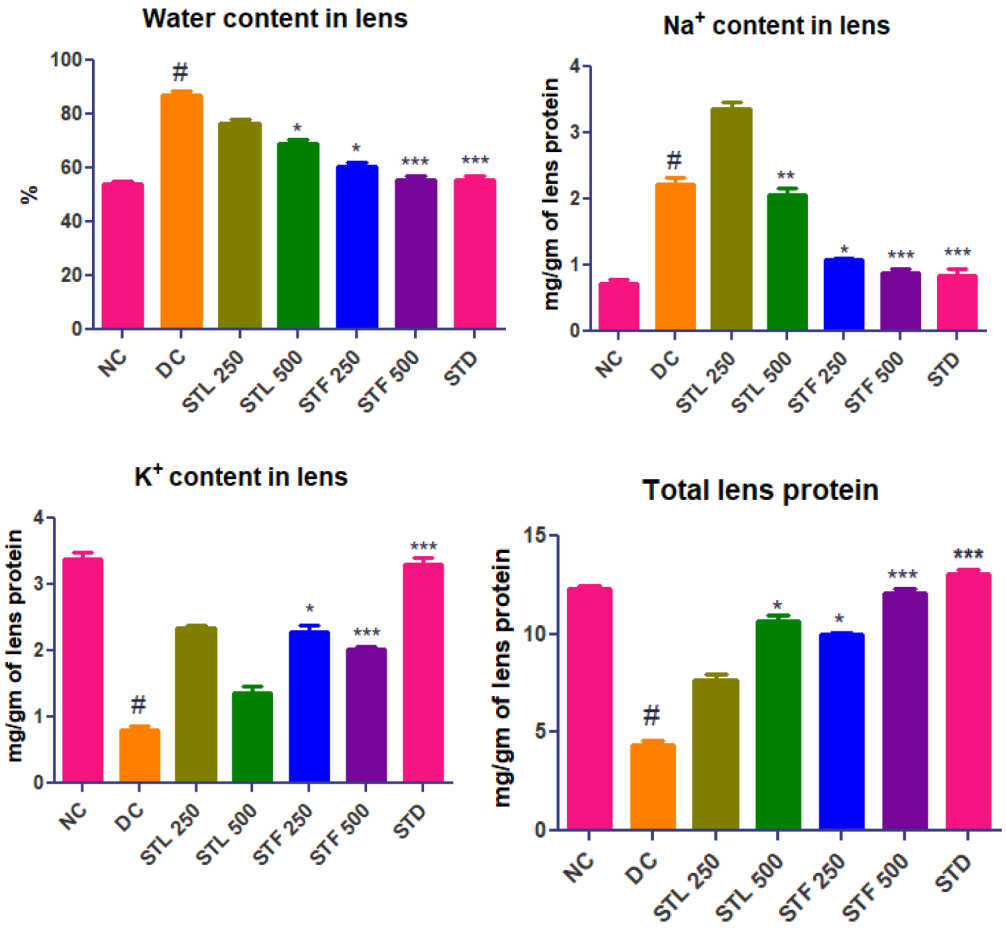


Figure 5. Effect of ethanol extracts of *S. trifoliatus* in alloxan-induced diabetic rat's eye.
Values expressed Mean ± SEM, where #P<0.001 as compare to control, *P<0.05, **P<0.01 and ***P<0.001 compare to DC group, n = 6 in each group.

STL-250 (Figure 8). Based on the findings, there is a sufficient contractile reserve in leaf, and fruit extracts to counteract the harmful impact of diabetes on cardiac contractility. Their contractility index finally showed a noticeable improvement. Various extracts significantly increased heart rate and lowered cardiac weight.

4. Discussion

Diabetes mellitus is prevalent in developed and developing nations. This illness may affect 25% of the world's population. Diabetes mellitus is characterized by insulin resistance and carbohydrate digesting issues. Despite the broad availability of natural and artificial hypoglycemic medicines, diabetes and its repercussions remain a serious medical concern. Diabetes can be controlled using many Indian medicinal medicines. Medical herbs are noted for their simple use and few adverse effects. Plants have historically provided medical materials; many industrial drugs come from them. Chronic diabetes damages the heart, blood vessels, kidneys, eyes, and nerves over time. Identifying

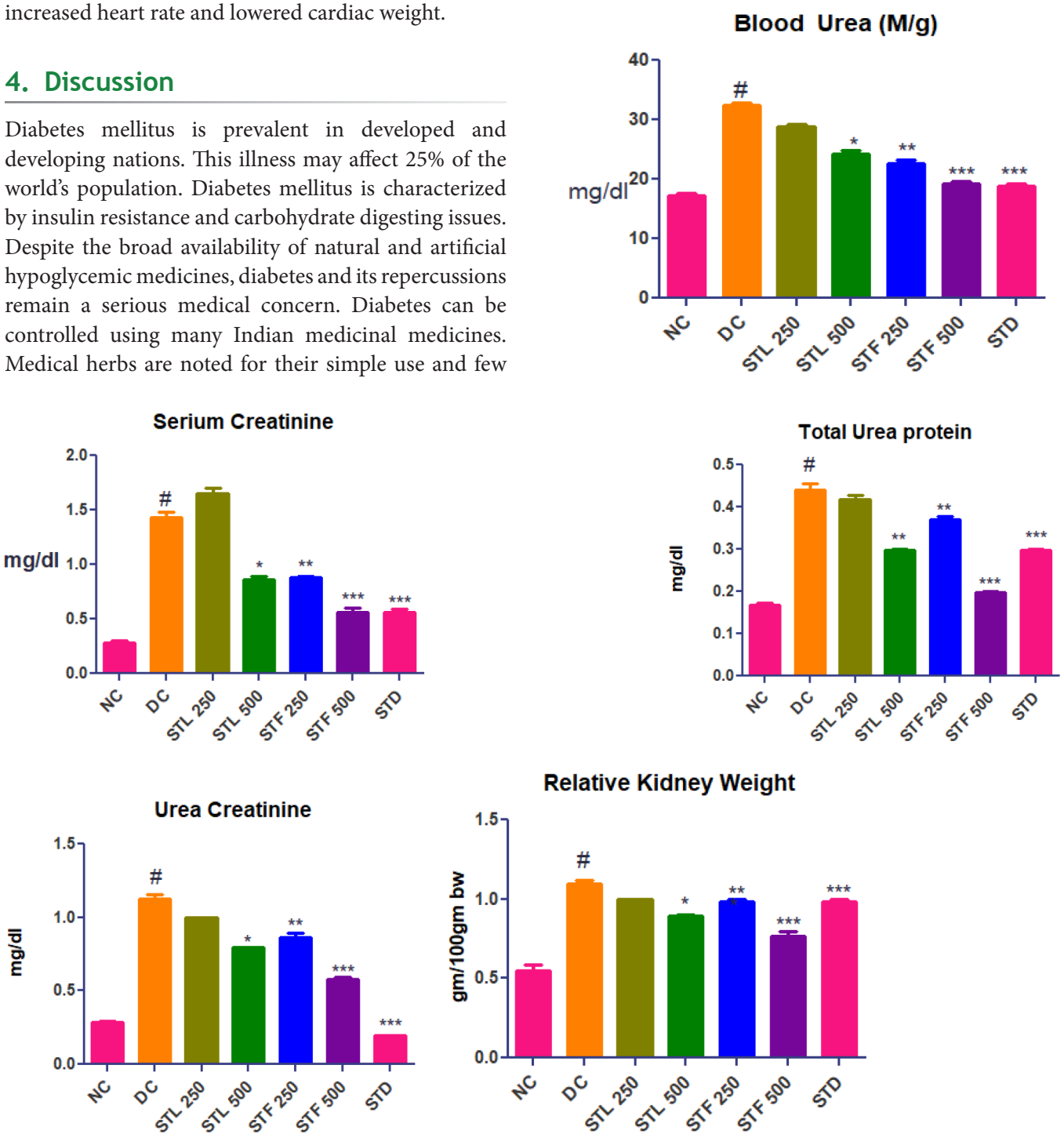


Figure 6. Effect of ethanol extracts of *S. trifoliatus* on alloxan induced diabetic rat's kidney.
Values expressed Mean ± SEM, where #P<0.001 as compare to control, *P<0.05, **P<0.01 and ***P<0.001 compare to DC group, n = 6 in each group.

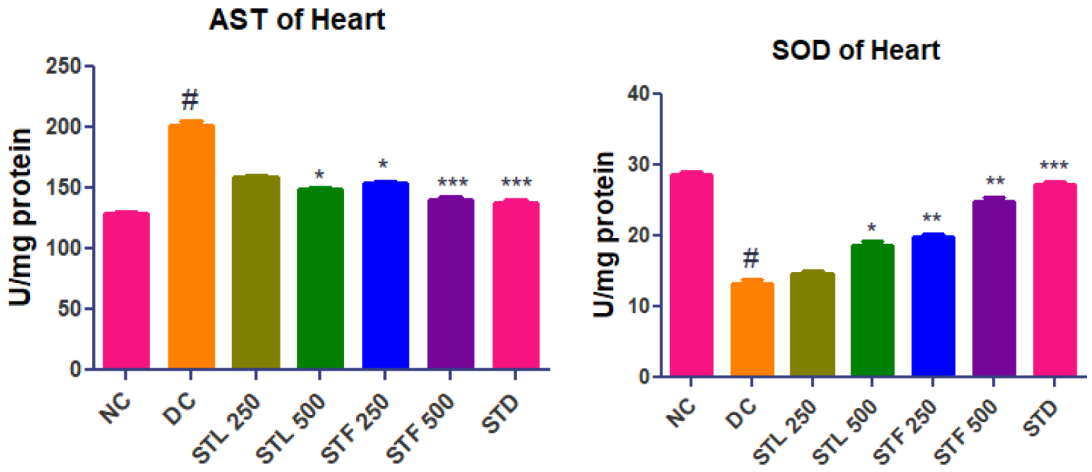


Figure 7. Effect of ethanol extracts of *S. trifoliatus* on biomarkers of alloxan induced Diabetic rat's heart.
Values expressed Mean ± SEM, where #P<0.001 as compare to control, *P<0.05, **P<0.01 and ***P<0.001 compare to DC group, n = 6 in each group.

phytoconstituents that cure or prevent diabetes is crucial.

In this study, Diabetic rats were used to examine the effects of *S. trifoliatus* part on diabetes and its complications. The water, ethanol, and methanol-soluble leaves have extractive values of 9.54%, 11.33%, and 5.67%, respectively. The fruits principal constituents include mucilage (11-11.5%), sugars (11%), and saponins. Fruit contains 85% triglycerides, 30% fatty acids, and sterol. These statistics may help standardize fruit. According to phytochemical screening, *S. trifoliatus* fruit and leaf extracts in aqueous, methanol, and ethanol forms contain sugars, glycosides, tannins, alkaloids, carbohydrates, flavonoids, saponins, steroids, gums, fixed oil and fats, and phenolic combinations. Since phytochemical screening shows that ethanol fruit and leaf extract contain more phytoconstituents than methanol and aqueous extracts, an additional animal study is done. Since 2000 mg/kg did not kill albino mice once acute toxicity resolved, all in vivo analyses in rats used 250 and 500 mg/kg.

The OGTT and normoglycemic rat models were used for hypoglycemic action at 250 and 500 mg/kg body weight. In both models, STF reduced blood glucose levels the most. Compared to glibenclamide, all extracts except STL-250 demonstrated significant hypoglycemic effects at 250 and 500 mg/kg body weight in the OGTT paradigm. The rats given STF had lower blood glucose than those given STL. Ethanol extract demonstrated strong hypoglycemic and antioxidant properties. Thus, an ethanol *S. trifoliatus* extract was added to the study to investigate its possible impact on diabetes-related issues that developed. Loss of insulin-releasing β-cells is the primary cause of diabetes mellitus. Alloxan induces insulin-dependent diabetes by selectively inhibiting glucokinase, the beta cells glucose sensor, and producing Reactive Oxygen Species (ROS), which kill beta cells. It also inhibits glucose-induced insulin synthesis.

The antidiabetic study gave alloxan-induced diabetes control rats STL-250, STL-500, STF-250, and STF-500. Plasma HbA1C and blood glucose were monitored. STF-250 and STF-500 reduce blood glucose better than STL-250 and STL-500. Chronic hyperglycemia produces hemoglobinemia, raising diabetic rodents HbA1C. Renal, brain, and diabetic retinopathy are linked to HbA1C convergence. It is commonly used to diagnose and evaluate diabetes issues. Rats with diabetes have less haemoglobin. *S. trifoliatus* extract significantly decreased HbA1C in diabetic rats dose-dependently. Thus, reducing HbA1C has improved diabetic rats ability to avoid diabetes issues. Diabetes-induced lens oxidative stress is mediated by the polyol pathway. Finally, sorbitol buildup causes osmotic pressure, which stresses the Endoplasmic Reticulum (ER), a protein synthesis site, and releases free radicals. Changes in glucose levels can also cause an Unfolded Protein Response (UPR) that damages lens filaments by generating oxidative pressure and ROS. This may cause ER stress. High glucose levels in the aqueous humour can glycate lens proteins, generating

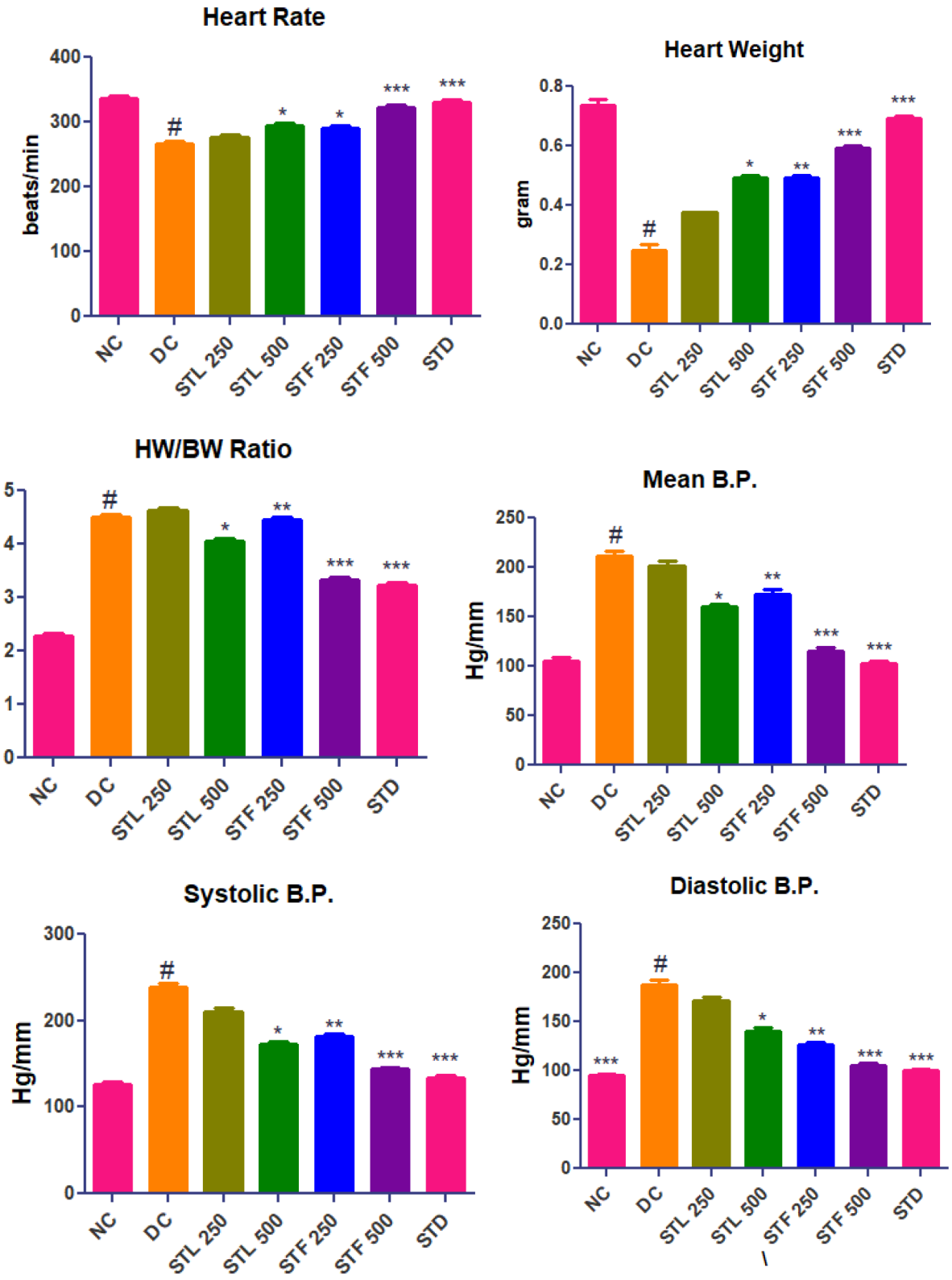


Figure 8. Effect of ethanol extracts of *S. trifoliatus* on hemodynamic measurement of alloxan-induced diabetic rat's heart.

Values expressed Mean ± SEM, where #P<0.001 as compare to control, *P<0.05, **P<0.01 and ***P<0.001 compared to DC group, n = 6 in each group.

AGE and superoxide radicals. H_2O_2 is generated when AGE interacts with cell surface receptors like the lens epitheliums advanced glycation end product receptor. All study extracts significantly reduced lens water content[16-18]. Sorbitol may build up intracellularly and alter osmotic pressure, or the endoplasmic reticulum may be less stressed. The addition of *S. trifoliatus* extracts dramatically raised the lens protein level (***P<0.001). Suppressing the polyol pathway, which generates oxidative stress and AGEs, may have caused this surge. Fruit impact is bigger than leaves.

Most lens metabolic substrates reach the lens through the aqueous humour, which contains high amounts of small molecules like lactate, urea, and glucose and electrolytes like sodium, chloride, and bicarbonate. Regulation of calcium, sodium, and potassium levels controls lens function. The sodium-potassium pump ($Na+K^+$ ATPase) controls ion concentration and osmotic pressures. Regulation of calcium, sodium, and potassium levels controls lens function. The lens needs Na^+K^+ ATPase to stay clear, and its change is a major cause of cataracts. Between K+/Na+ impedance lens fibers develop and accumulate Na^+ and K^+ due to ATPase activity, causing cataractogenesis[17-25]. In this study, the lens K^+ level increased dose-dependently, but the *S. trifoliatus* extract dramatically lowered Na+ levels (***P <0.001). STF-500 performs better on K^+ and Na^+ content in this study, maybe due to regulating sodium-potassium pump failure.

Diabetic rats had heavier kidneys than controls. Tubular cell lining, fat or lymphocyte infiltration, or a large hemorrhagic zone in hyperglycemic rodents may cause this. *S. trifoliatus* extract reduced kidney damage in diabetic rats by keeping or restoring renal cell histoarchitecture and lowering kidney weight to almost normal[21,22-27]. Alloxan raised blood urea nitrogen, serum creatinine, and urine creatinine. Renal function, maintenance, and glomerular filtration rate affect these parameters. Diabetic rats had increased renal markers in this study. Treatment with *S. trifoliatus* extract significantly (***P<0.001) reduced serum plasma urea creatinine and blood urea nitrogen levels in a dose-dependent manner. Free radicals, Advanced Oxidative Protein Products (AOPP), Reactive Oxygen Species (ROS), and Protein Carbonyl items (PCO) may indicate oxygen-induced protein damage. Serum protein levels and membrane permeability increase due to glomerular filtration alterations. Following dose-dependent treatment of *S. trifoliatus* extract, total urine protein levels significantly decreased (***P<0.001), indicating improved renal function[28-33]. After 20 days of *S. trifoliatus* extract use, the kidney's histology showed improved renal tubules, decreased proximal convoluted tubule damage and necrosis, and enhanced renal mesangial cells and tubular epithelial cell regeneration.

Hyperglycemia generates reactive oxygen and nitrogen species, which harm the heart. In hyperglycemia, reactive oxygen and nitrogen species cause oxidative stress. This causes abnormal gene expression, signal transduction, and a cascade of events that produce programmed cardiac cell death. Therefore, cardiac cell loss is a primary cause of diabetic cardiomyopathy. Superoxide dismutase is vital because it immediately removes reactive oxygen radicals. Superoxide dismutase is a defensive catalyst that breaks down superoxide radicals. The study found that *S. trifoliatus* extract significantly increased SOD levels (***p<0.001) compared to Diabetic Control (DC), potentially reducing oxidative stress and cardiac cell death. As a serum catalyst, AST forms strong bonds with cardiovascular muscle contractile proteins. Release of AST compounds into the serum indicates heart muscle damage. Diabetes raised AST levels in rats. Animals with diabetes showed increased AST. In this investigation, *S. trifoliatus* extract significantly (***P<0.001) reduced cardiac enzyme levels, particularly AST, compared to the diabetic control group, indicating cardio-protection and heart damage prevention. Also, *S. trifoliatus* extract reduced heart injury[22,33-35].

According to research, alloxan causes the most prevalent form of diabetic cardiomyopathy. Early symptoms include endothelial dysfunction, higher free unsaturated fat levels, insulin resistance, altered Ca_2+ homeostasis, and hyperglycemia. Systolic and diastolic capacity fluctuates greatly in the intermediate period, but anomalies characterize the late stage. Alloxan-induced diabetes in rodents has long-term consequences on the ECG, systolic performance, ventricular pressure increase rate, mean blood pressure, and QT values. Our study found that *S. trifoliatus* significantly reduced all ECG parameters, including HW/BW ratio, systolic, diastolic, and mean B.P. (***P<0.001). This study found that *S. trifoliatus* extract had a substantial contractile reserve to offset diabetes effects on cardiovascular contractility. *S. trifoliatus* extract enhanced contractility. Moreover,

the *S. trifoliatus* extract also promotes cardiac weight loss.

5. Conclusion

Moreover, all of the aforementioned issues in diabetic rats are likely associated with the oxidative stress induced by elevated blood glucose levels. Oxidative stress can lead to two potential outcomes *i.e.*, harm to cellular membranes and alterations to the structural and functional integrity of sub-cellular organelles. Both of these outcomes can contribute to the development of difficulties associated with diabetes. This is mostly caused by consistently elevated levels of blood glucose, leading to tissue damage and often resulting in severe illness. According to our studies, *S. trifoliatus* ethanol extract can lower blood glucose levels and minimize the risk of diabetes-related complications. However, further significant research is required.

6. Author Contribution

Conceptualization: Sarita Rawat, Prashant Kumar Dhakad; Investigation and Methodology: Gaurav Gupta, Ritu Gilhotra, Prashant Kumar Dhakad; Project Administration and Resources: Gaurav Gupta, Prashant Kumar Dhakad; Software and Validation: Prashant Kumar Dhakad, Sarita Rawat; Writing – Original Draft: Sarita Rawat; Writing – Review and Editing: Ritu Gilhotra, Gaurav Gupta, Prashant Kumar Dhakad. All authors have read and agreed to the published version of the manuscript.

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