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ANTIDIABETIC EFFECTS OF SEMECARPUS ANACARDIUM LEAF EXTRACTS IN STREPTOZOTOCIN-INDUCED DIABETES IN RATS

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ABSTRACT
Background: A class of metabolic diseases known as diabetes mellitus is typified by persistently high blood sugar levels brought on by malfunctions in the production or function of insulin. Conventional treatments frequently have drawbacks and side effects, prompting interest in alternative treatments such as herbal remedies. *Semecarpus anacardium*, known for its medicinal properties, was investigated for its antidiabetic potential. **Methods:** *Semecarpus anacardium* leaves were collected, authenticated, and extracted using various solvents. The ethanol extract was subjected to preliminary phytochemical screening, HPLC-DAD analysis, and tested for antidiabetic activity in streptozotocin-induced diabetic rats. Biochemical parameters, histopathological studies, and lipid profiles were analyzed over a 20-day period. **Results:** The ethanol extract exhibited the highest yield (13.53% w/w) and contained significant amounts of bioactive compounds, including flavonoids and alkaloids. In diabetic rats, the ethanol extract at 200 mg/kg significantly reduced blood glucose levels from 333.35 ± 5.2 mg/dL to 121.68 ± 7.56 mg/dL. Highly significant results were obtained in lipid profiles, with total cholesterol reducing from 176.82 ± 1.07 mg/dL to 103.69 ± 2.85 mg/dL and triglycerides from 188 ± 5.73 mg/dL to 97.17 ± 8.41 mg/dL. Histopathological analysis showed partial restoration of pancreatic islets and reduced fibrosis, indicating the protective effects of the extract. **Conclusion:** The ethanol extract of *Semecarpus anacardium* leaves demonstrates significant antidiabetic and lipid-lowering effects in streptozotocin-induced diabetic rats. These findings support the potential of this plant as a natural therapeutic agent for diabetes management, warranting further research for clinical application.

INTRODUCTION

Diabetes mellitus is not a single ailment but a collective term for a group of metabolic disorders characterized by chronic hyperglycemia due to defects in insulin secretion, insulin action, or both [1, 2]. These disturbances in carbohydrate, fat, and protein metabolism manifest clinically with symptoms such as

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increased thirst (polydipsia), frequent urination (polyuria), and the presence of ketones in the blood and urine (ketonemia and ketonuria) [3, 4]. These metabolic irregularities pose a significant challenge in managing diabetes mellitus effectively [5, 6]. The limitations and adverse effects associated with conventional oral hypoglycemic agents, which are the cornerstone of diabetes treatment, have spurred interest in alternative therapies [7, 8]. One promising area of exploration is herbal remedies, which have been employed in traditional medicine for centuries. highlighted the potential of these herbal treatments, emphasizing their role in diabetes management and their relative safety compared to synthetic drugs [9]. Consequently, plant-derived therapies have become viable options for managing diabetes mellitus [10, 11]. Globally, the prevalence of diabetes is startlingly high and still rising. In 2014, the International Diabetes Federation published research estimating 387 million people worldwide have diabetes [12]. In 2021, 10.5% of adults (20–79 years old) worldwide had diabetes, which is about 537 million people [13]. By 2035, that figure is expected to rise to 592 million [14, 15]. Particularly concerning is the anticipated increase in diabetes prevalence in the Southeast Asia region, which is expected to experience the highest rates by 2040 [14, 16]. This burgeoning incidence underscores the urgent need for effective and accessible treatments [17, 18]. Type I diabetes, also called insulin-dependent diabetes mellitus (IDDM), and Type II diabetes, sometimes called non-insulin-dependent diabetes mellitus (NIDDM), are the two primary forms of diabetes mellitus [19, 20]. IDDM results from the pancreas's inability to produce sufficient insulin, necessitating lifelong dependence on exogenous insulin administration [21]. This form predominantly affects children and young adults and is characterized by a severe dysfunction of pancreatic β-cells [22, 23]. On the other hand, NIDDM is associated with either inadequate insulin secretion by pancreatic beta cells or reduced responsiveness of target tissues to insulin, leading to hyperglycemia despite normal or elevated insulin levels [24, 25]. *Semecarpus anacardium* Linn, commonly known as Bhallatak or marking nut, belongs to the family Anacardiaceae and is renowned for its diverse medicinal properties [26]. Historically, plant components have been used to cure various illnesses, including rheumatism, asthma, neuralgia, helminthic infections, psoriasis, and cancer [27]. The stem bark's aqueous extract has shown antibacterial, central nervous system stimulant, hypoglycemic, anti-atherogenic, and anti-carcinogenic activities [28]. Additionally, detoxified nuts of *Semecarpus anacardium* are recommended in traditional medicine for addressing toxic states, chronic skin diseases, tumors, fevers, excessive menstruation, vaginal discharge, insufficient lactation, constipation, and intestinal parasites [29, 30]. These nuts are also widely used in Ayurvedic medicine to manage a variety of disorders. Literature about plants suggests the potential of plants in the treatment of diabetes. Still, the mechanism of action of the plant part for the activity is not yet precise, and the compounds responsible for the said activity need to be investigated. The primary objective of this research is to examine the antidiabetic effects of *Semecarpus anacardium* leaf extracts in a rat model of streptozotocin-induced diabetes. By examining the biochemical and physiological parameters, this study aims to elucidate the potential mechanisms through which these leaf extracts exert their antidiabetic effects, thereby contributing to the development of alternative therapies for diabetes management.

MATERIALS AND METHODS

Materials

The US-based Sigma Chemical Company provided the streptozotocin. The analytical-quality metformin and all other substances were obtained from Morepen Laboratories Limited. Gluco-one glucometers were utilized with 10/10 accuracy, 87% sensitivity, and 96% specificity. Ethanol, Acetone, chloroform, and Petroleum ether were purchased from Sciquaint Innovations OPC Private Limited, Pune.

Methods

Collection of plant Material

The leafy part *of S. anacardium* was gathered from the Mahatma Phule Krishi Vidyapeeth in the Medicinal & Aromatic Plant Project in Rahuri (Ref. No/PVPC/Bot/2023-24/218) and got an authentication from Padmashri Vikhe Patil College of Arts, Science, and Commerce, located in Loni.

Preparation of Extracts

The leaves of *S. anacardium* were washed with distilled water, dried in darkness at ambient temperature, and ground into a consistent coarse powder [31]. The powdered material was kept in a sealed container at room temperature 25±2°C. Subsequently, the dried powder was extracted using ethanol (96% v/v), acetone (99.5% v/v), chloroform (99.8% v/v), and petroleum ether (98% v/v). Cold extraction was conducted using the maceration method for 72 hours at room temperature (25 ± 2°C), with samples agitated every 6 hours. The extracts were filtered and

concentrated using a rotary evaporator (40°C, 50 rpm) until a concentrated form was obtained. The concentrated extracts were transferred to glass beakers and stored at 4°C in aluminum foil-wrapped containers to prevent deterioration. For oral administration, extracts were suspended in 5% Tween-80 [32].

Preliminary phytochemical screening

The fractions were subjected to an initial phytochemical analysis using previously developed protocols to determine the presence of primary and secondary metabolites, such as carbohydrates, lipids, proteins, amino acids, resins, alkaloids, flavonoids, phenols, tannins, steroids, glycosides, and saponins [33]. Every reagent used for this investigation was manufactured following the Indian Pharmacopeia's guidelines [34].

HPLC-DAD Analysis

Exactly 200 mg of *S. anacardium* powder was accurately weighed and mixed with a solvent mixture of acetonitrile, methanol, and water in a ratio of 2:2:1 (v/v). This combination was placed in an HPLC-grade, airtight container and left for 12 hours at room temperature [35]. Following this incubation period, the sample was placed in a water bath at 55°C for 10 minutes to enhance the extraction process. After 10 minutes of ultrasonication, the mixture was filtered through a 0.45 µm nylon filter to further aid in the solid-liquid extraction. The powdered *S. anacardium* was analyzed and identified using the HPLC-DAD (High-Performance Liquid Chromatography with Diode-Array Detection) apparatus using a newly generated 20 µL sample of this solution.

Experimental Animal

Male Wistar rats weighing 250 and 300 grams were selected for investigating antidiabetic activity [36]. With special rodent food and unlimited access to water, these rodents were kept in carefully monitored laboratory settings. They were kept in an environment with a normal 12-hour light-dark cycle, a constant temperature of 22±2°C, and a humidity of 55% [37]. The Institutional Animal Ethics Committee approved the study, which followed the rules for using and caring for laboratory animals (IAEC), with the reference number 1942/PO/Re/S/17/CCSEA/2023/01/11.

Streptozotocin-Induced Antidiabetic Study in Rats

The rats were given a single intraperitoneal injection of 55 mg/kg body weight of streptozotocin (STZ) dissolved in 0.1 M sodium citrate buffer (pH 4.5) after an overnight fast to induce diabetes [31]. The rats were given full access to food and water after receiving the STZ injection, and they were also provided access to a 5% glucose solution for eighteen hours to avoid hypoglycemia shock [38]. On the 5^{th} day post-STZ injection, blood glucose levels were measured to confirm the induction of diabetes [39]. Rats exhibiting fasting blood glucose levels above 250 mg/dL were classified as diabetic and included in the study. The diabetic rats were then divided into twelve groups of six animals for further treatment over 20 days. The groups included a normal control group receiving standard rat food and water, a diabetic control group, a positive control group treated with oral metformin (150 mg/kg) daily, and nine groups of diabetic rats treated with various doses (50 mg/kg, 100 mg/kg, and 200 mg/kg) of *Semecarpus anacardium* extracts (ethanol (96% v/v), acetone (99.5% v/v), and chloroform (99.8% v/v)) orally every day for 20 days [40]. These specific doses were selected based on existing literature that has explored the effects of *Semecarpus anacardium* extracts in similar contexts [41]. Based on prior acute toxicity investigations and studies showing the ethanolic extract of the plant had anticancer properties, these dose levels were chosen, with an effective dose of 100 mg/kg for mice being determined [42].

Biochemical Analysis

Body weight (BW), urine volume, and blood glucose levels (BGL) were monitored on days 1, 5, 10, 15, and 20 to assess the extracts' antidiabetic activity. Urine volume was taken every 5 hours, body weight was monitored with a digital balance, and blood glucose levels were determined using a glucometer and the tail prick technique [43]. Clear serum was obtained by centrifuging blood samples in dry tubes for 10 min at 2000 rpm after they had been left to clot for 30 min at room temperature. The levels of triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) were measured in this blood [44].

Histopathological Studies

Rat liver tissues were dried using a graded series of ethanol (96% v/v), embedded in paraffin, and fixed in 10% formaldehyde. Thread slices with a thickness of 5 µm were created and rehydrated using a rotary microtome. Hematoxylin and eosin (H&E) were then applied to these sections using the technique outlined by Osama M. Ahmed *et al*. [45], examined under a light microscope 400X, and photographed using photomicrography.

Statistical Analysis

The findings were presented as either the standard error of the mean (SEM) or the mean ± standard deviation (SD). Using GraphPad Prism 10.2 software, statistical analysis was carried out utilizing the Student's t-test and one-way analysis of variance (ANOVA). A criterion of $p < 0.05$ was established for statistical significance when comparing the drug group to the negative control group. Dunnett's post hoc test assessed statistical significance between the treatment groups.

RESULTS

Preliminary Phytochemical Screening

The ethanol (96% v/v) extract of *Semecarpus anacardium* was subjected to preliminary phytochemical screening, revealing the presence of various bioactive compounds, including flavonoids, alkaloids, glycosides, phenols, steroids, and tannins. Among the different extracts tested, the ethanolic extract yielded the highest amount of extract, indicating its potential richness in phytoconstituents (Table 1).

Interpretation of HPLC Chromatogram

The HPLC chromatography analysis of the ethanolic extract of *Semecarpus anacardium* Linn. leaves at wavelengths of 210 nm and 254 nm revealed several peaks, indicating the presence of various compounds. These peaks correspond to different phytoconstituents within the extract (Figure 1). This analysis highlights the extract's complex chemical profile, underscoring its potential therapeutic benefits.

Table 1: Yield of extract from *S. anacardium* using various solvents.

Samples (gm)	Solvent	Yield (gm)	% w/w
75	Ethanol (96% v/v)	10.15	13.53
	Petroleum ether (98% v/v)	8.01	10.68
	Chloroform (99.8% v/v)	6.07	8.10
	Acetone (99.5% v/v)	3.32	4.43

Table 2: Qualitative Phytochemical Screening of Primary and Secondary Metabolites in *Semecarpus anacardium*.

Phytochemicals	Test/reagent	SAEE	SACE	SAAE
Alkaloids	Dragendorff's test	+ + +	+ + +	+ + +
	Mayer's test	+ + +	+ + +	+ + +
	Hager's test	+ + +	+ + +	+ + +
	Wagner's test	+ + +	+ + +	+ + +
Carbohydrates	Molisch's test	+ + +	+ + +	+ + +
	Fehling's test	-	-	-
Glycosides	Keller-Killiani test	+	+ + +	++
	Borntrager's test	+	+ + +	++
Steroids	Libermann-Burchard	+ + +	+ + +	+ + +
	Salkowski test	+ + +	+ + +	+ + +
Flavonoids	Shinoda's test	+ +	+ + +	+ + +
	Lead acetate test	+ +	+ + +	+ + +
Saponins	Foam test	+ + +	+ + +	+ + +
Tannins	Lead acetate test.	+ + +	+ + +	+ + +
Phenols	Ferric chloride Test	+ +	+ + +	+ + +

(-) Negative; +: Present (positive within 15 minutes, but after 10).++: highly prevalent (positive within 10 minutes, but not after 5 minutes).+++: extremely prevalent (positive in less than five minutes). SAEE: *Semecarpus anacardium* Ethanol Extract, SACE: *Semecarpus anacardium* Chloroform Extract, SAAE: *Semecarpus anacardium* Acetone Extract.

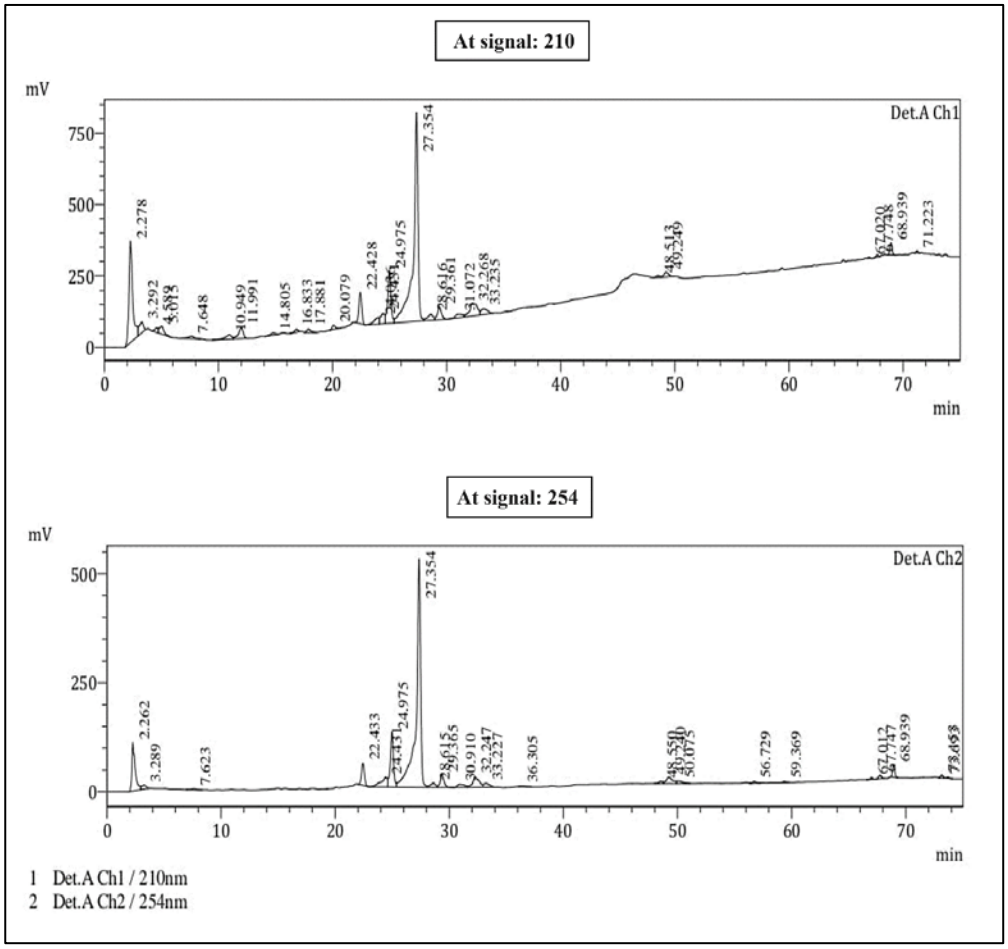

Figure 1: HPLC analysis of the ethanolic extract from *Semecarpus anacardium.* The chromatogram reveals the presence of various components, with their respective contributions as follows: Gallic acid (11%), unknown phytoamines/alkaloids (4.5%), Rutin (54%), and Terpenoids/Tocopherol (5-6%). The HPLC analysis highlights the significant presence of flavonoids/polyphenols, particularly Rutin, which contributes the largest percentage to the extract composition.

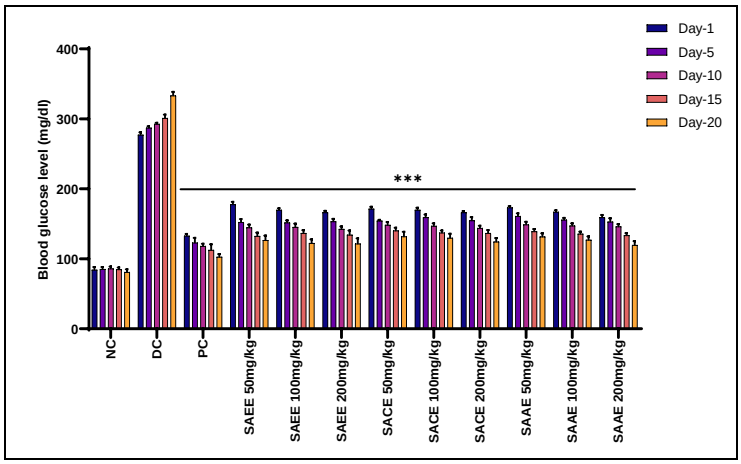

Figure 2: Effect of leaf extracts from *Semecarpus anacardium* on body weight (BW) in diabetic STZ rats. The values (n = 6) are shown as mean ± SEM. One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to assess statistical significance. *P < 0.05 in comparison to the diabetes control group, **P < 0.01 in comparison to the diabetic control group, and ***P < 0.001 in comparison to the diabetic control group. #P < 0.05 in comparison to the normal rats group.

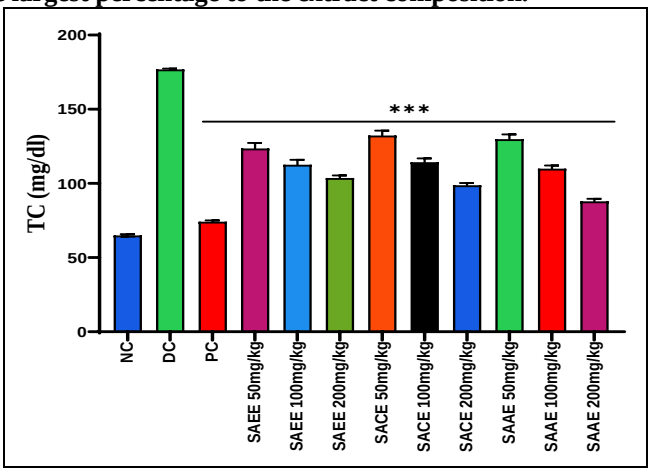

Figure 3: Effects of leaf extracts from *Semecarpus anacardium* on total cholesterol (TC) in diabetic STZ rats. The values (n = 6) are shown as mean ± SEM. One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to assess statistical significance. *P < 0.05 in comparison to the diabetes control group, **P < 0.01 in comparison to the diabetic control group, and ***P < 0.001 in comparison to the diabetic control group. #P < 0.05 in comparison to the normal rats group.

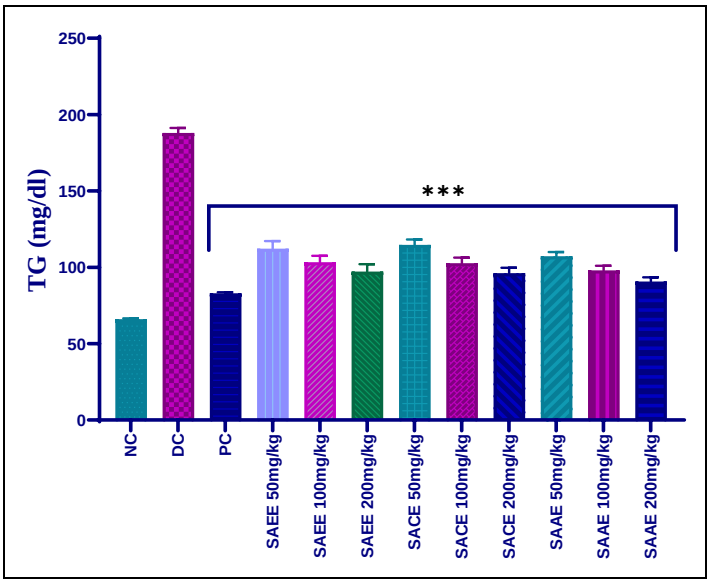

Figure 4: Effects of leaf extracts from *Semecarpus anacardium* on TG in diabetic STZ rats. The values (n = 6) are shown as mean ± SEM. One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to assess statistical significance. *P < 0.05 in comparison to the diabetes control group, **P < 0.01 in comparison to the diabetic control group, and ***P < 0.001 in comparison to the diabetic control group. #P < 0.05 in comparison to the normal rats group.

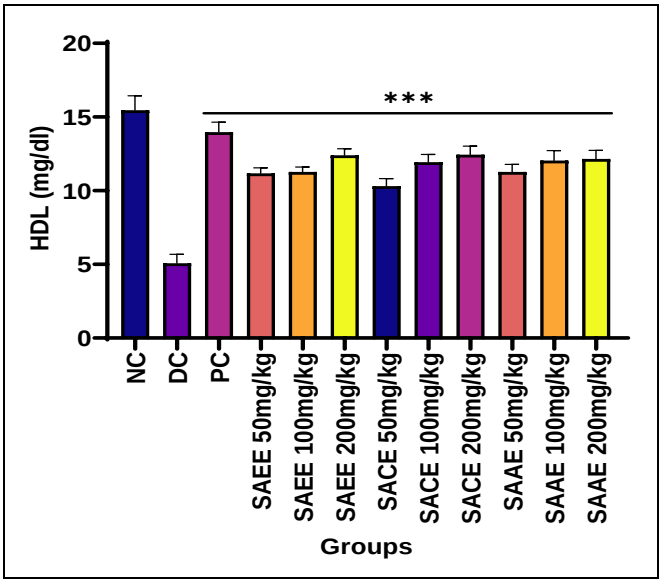

Figure 5: Effect of extract from *Semecarpus anacardium* on HDL (high-density lipoprotein) in diabetic STZ rats. The values (n = 6) are shown as mean ± SEM. One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to assess statistical significance. When compared to normal rats, #P < 0.05. *P < 0.05 in comparison to the diabetes control group; **P < 0.01 in that same group; and ***P < 0.001 in that same group.

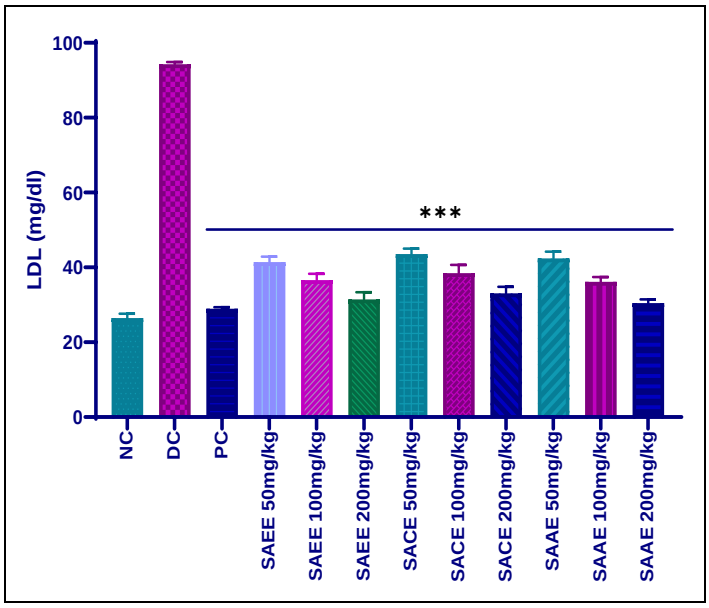

Figure 6: Effects of *Semecarpus anacardium* extract on LDL in diabetic STZ rats. Mean ± SEM (n = 6) is used to express the values. Duncan's Multiple Range Test (DMRT) and one-way analysis of variance (ANOVA) were used to assess statistical significance. In comparison to normal rats, #P < 0.05. The significance level is *P < 0.05, **P < 0.01, and ***P < 0.001 when compared to the diabetes control group

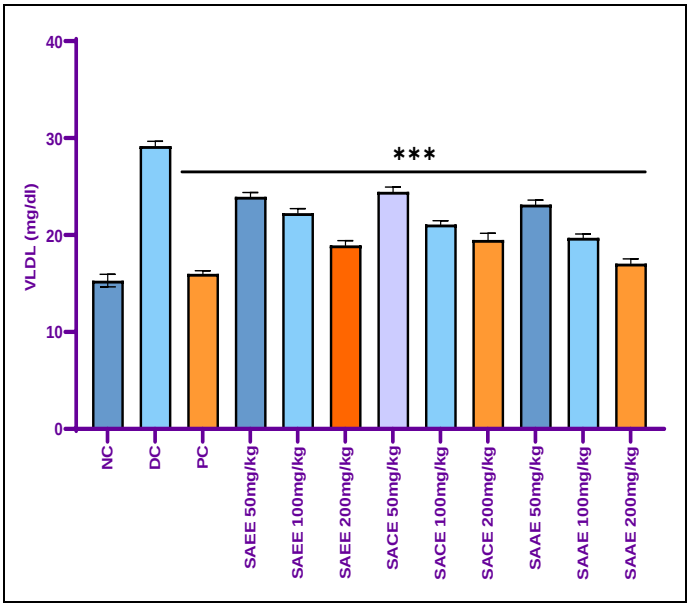

Figure 7: Effect of Very-Low-Density Lipoprotein (VLDL) in STZ diabetic rats using an extract from *Semecarpus anacardium*. The values (n = 6) are shown as mean ± SEM. One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to assess statistical significance. *P < 0.05 in comparison to the diabetes control group, **P < 0.01 in comparison to the diabetic control group, and ***P < 0.001 in comparison to the diabetic control group. #P < 0.05 in comparison to the normal rats

Histopathology study

Microscopic observation of the pancreas in the experimental tissue section is shown in Figures 8 and 9.

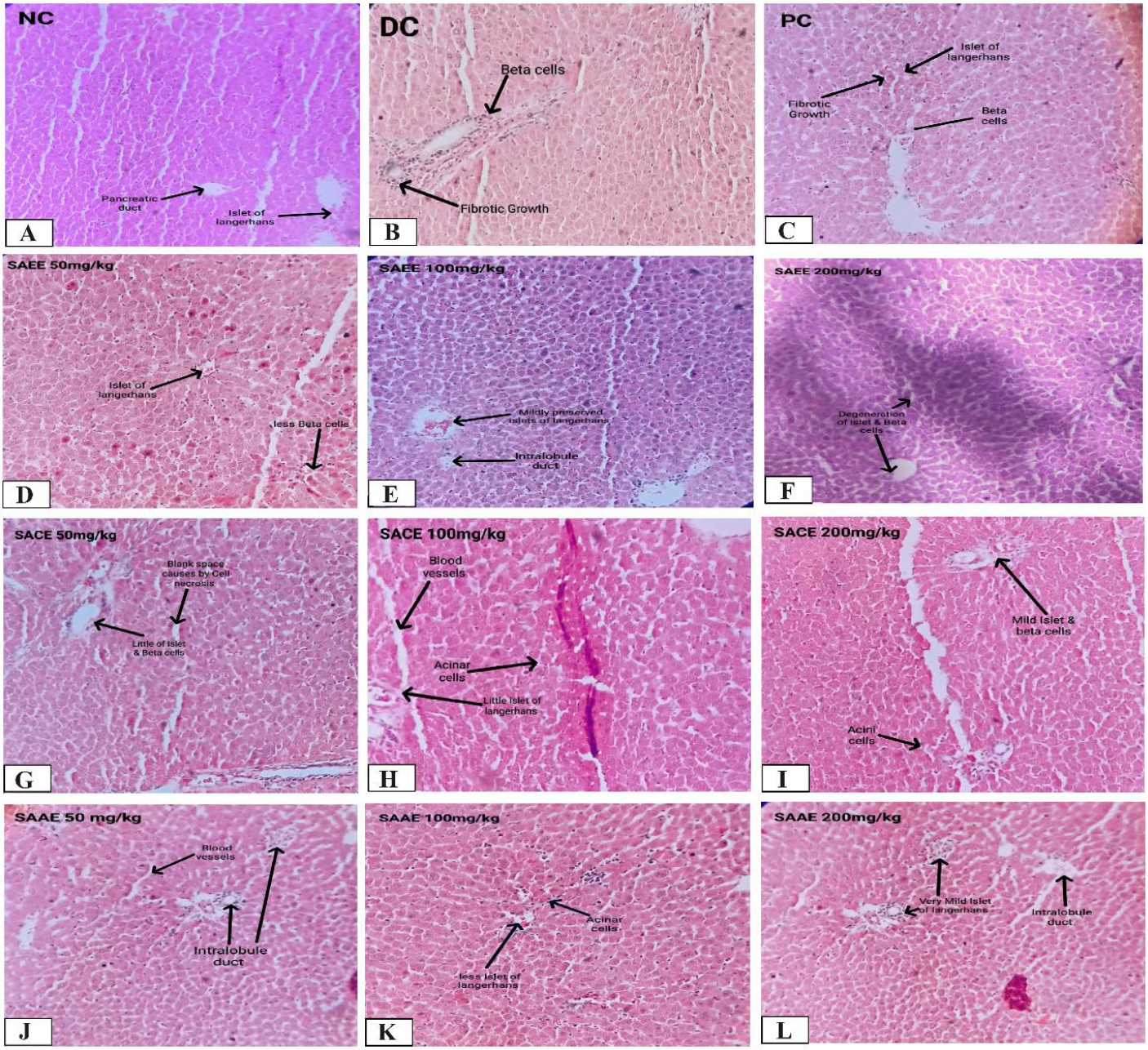

Figure 8: Histopathological examination of pancreas tissue samples from different experimental groups (Haematoxylin and Eosin stain, 400x). (A) NC (Normal Control) shows normal pancreatic architecture with well-defined islets of Langerhans and pancreatic ducts. (B) DC (Diabetic Control) exhibits significant fibrotic growth and reduced beta cells. (C) PC (Positive Control) shows partial restoration of islets and presence of beta cells with some fibrotic growth. (D) SAEE 50 mg/kg shows islets with fewer beta cells. (E) SAEE 100 mg/kg demonstrates milder fibrosis and partial inflammation. (F) SAEE 200 mg/kg shows decreased fibrosis and presence of beta cells. (G) SACE 50 mg/kg reveals mild restoration of islets and some blood vessels. (H) SACE 100 mg/kg shows acinar cells, blood vessels, and mild restoration of islets. (I) SACE 200 mg/kg demonstrates mild restoration of islets and beta cells. (J) SAAE 50 mg/kg shows mild restoration of intralobular ducts and blood vessels. (K) SAAE 100 mg/kg reveals partial restoration of islets and the presence of acinar cells. (L) SAAE 200 mg/kg shows very mild restoration of islets, intralobular ducts, and acinar cells.

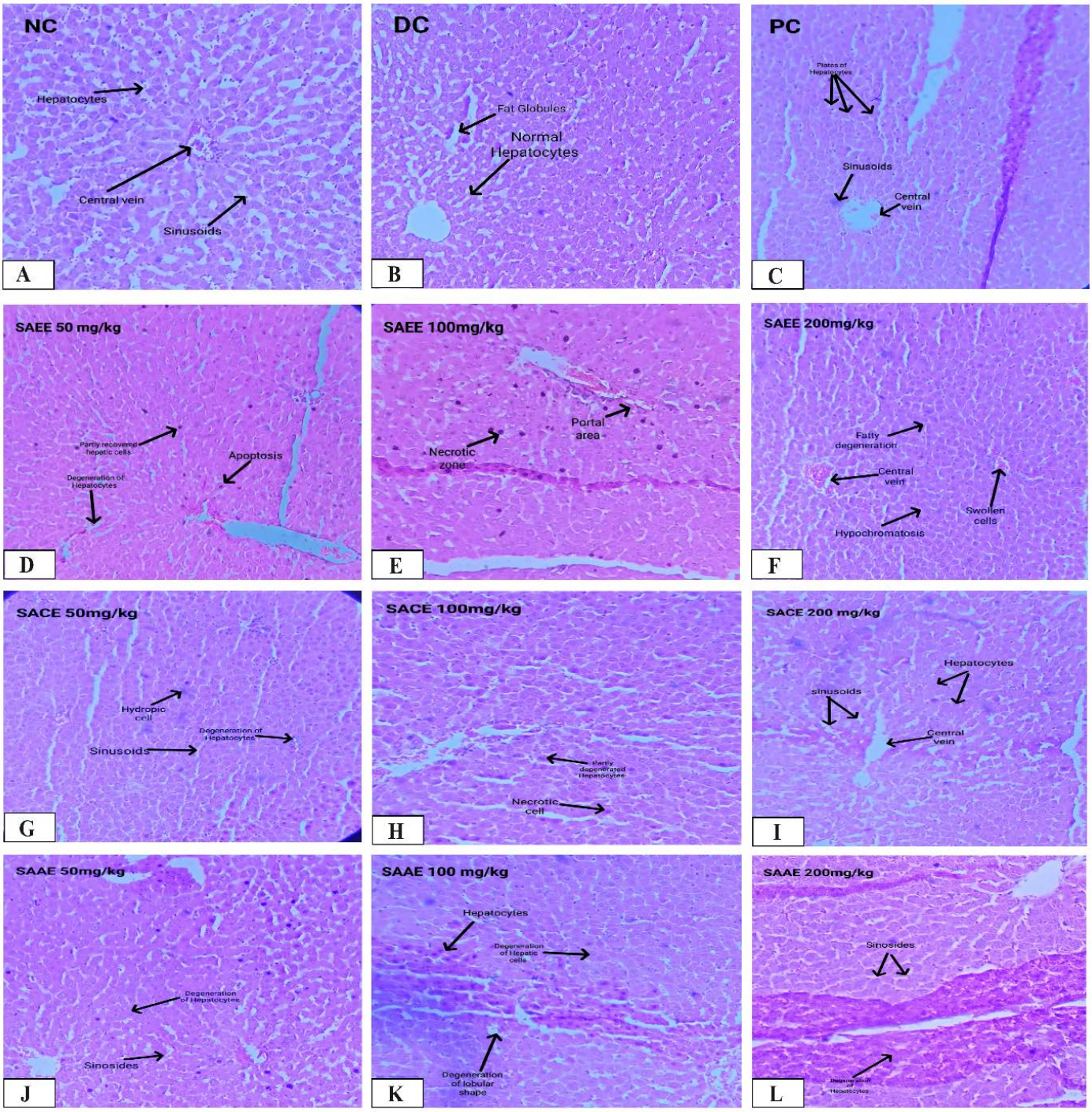

Figure 9: Histopathological examination of liver tissue samples from different experimental groups (Haematoxylin and Eosin stain, 400x). (A) NC (Normal Control) shows normal liver architecture with healthy hepatocytes, central vein, and sinusoids. (B) DC (Diabetic Control) exhibits fat globules and normal hepatocytes. (C) PC (Positive Control) reveals normal sinusoids and central vein with slight alterations. (D) SAEE 50 mg/kg shows mild degeneration and apoptosis. (E) SAEE 100 mg/kg demonstrates necrotic zones and affected portal areas. (F) SAEE 200 mg/kg shows fatty degeneration, swollen cells, and hypochromatosis. (G) SACE 50 mg/kg reveals hydropic changes and mild degeneration. (H) SACE 100 mg/kg shows necrotic cells and affected sinusoids. (I) SACE 200 mg/kg demonstrates relatively normal hepatocytes and central vein. (J) SAAE 50 mg/kg shows mild degeneration and normal sinusoids. (K) SAAE 100 mg/kg reveals hepatocytes with degeneration and swollen cells. (L) SAAE 200 mg/kg shows mild degeneration and normal sinusoids.

Table 3: Impact of *Semecarpus anacardium* leaf extracts on blood glucose levels, body weight, and urine volume as measures of antidiabetic activity in normal and experimental rats.

Para-meters	Day	NC	DC	PC	SAEE5	SAEE1	SAEE2	SACE5	SACE1	SACE2	SAAE5	SAAE1	SAAE2
Body Weight (gm)	1	273.34 ±3.85	264.94 ±4.56	269.79±3 .46#	268.62± 3.31#	265.47± 3.8#	262.56± 2.17#	269.57± 2.91#	265.13± 4.44#	263.3±3. 48#	269.35± 3.07#	266.85± 3.16#	263.35± 3.91#
	5	278.31 ±3.69	240.69 ±5.08	267.53±3 .25***	248.95± 2.89#	258.57± 3.37***	262.81± 2.93***	249.71± 4.32#	253.16± 4.2*	260.13± 4.91***	246.39± 6.5#	255.98± 3.11**	261.83± 2.55***
	10	287.43 ±3.29	231.38 ±3.89	284.4±3. 17#	250.64± 4.84***	257.1±9. 14***	261.6±3. 43***	245.53± 4.94*	250.29± 5.25***	255.42± 4.54***	248.22± 3.57**	252.26± 3.55***	256.47± 5.23***
	15	289.86 ±5.68	215.57 ±4.93	292.92±7 .75***	248.24± 3.25***	255.4±4. 03***	262.55± 3.46***	246.26± 3.32***	252.81± 3.93***	258.35± 3.45***	245.9±3. 49***	251.93± 2.43***	261.19± 3.04***
	20	289.72 ±3.01	197.71 ±5.83	290.08±1 4.41***	236.99± 6.45***	249.65± 13***	261.76± 9.99***	225.66± 5.34***	243.42± 5.79***	250.49± 5.89***	235.21± 3.57***	246.64± 5.72***	253.69± 8.08***
BGL (mg/dl)	1	84.37± 3.97	277.27 ±3.63	132.89±2 .61***	177.9±3. 57***	169.96± 2.25***	166.37± 2.17***	171.69± 2.65***	169.83± 3.09***	166.37± 2.04***	173.46± 1.89***	167.14± 2.43***	159.44± 3.36***
	5	85.22± 3.15	287.36 ±2.04	123.27±6 .69***	152.42± 4.31***	152.03± 3.04***	153.61± 3.42***	154.46± 1.22***	159.41± 3.96***	154.97± 4.72***	160.96± 4.12***	155.83± 2.56***	153.05± 4.86***
	10	86.12± 2.84	292.71 ±1.67	118.14±3 .31***	144.85± 4.13***	145.49± 4.84***	142.61± 3.66***	148.38± 4.27***	146.94± 3.74***	143.97± 3.24***	149.08± 3.69***	147.46± 3.19***	146.26± 3.34***
	15	84.96± 2.7	301.15 ±5.03	112.54±8 .37***	132.4±5. 04***	136.83± 4.18***	134.45± 5.95***	140.44± 4.09***	137.43± 3.14***	136.65± 4.43***	139.31± 3.31***	135.79± 3.04***	133.64± 3.18***
	20	81.08± 3.94	333.35 ±5.2	102.9±3. 8***	126.59± 6.64***	122.36± 5.54***	121.68± 7.56***	132.08± 6.45***	129.98± 5.77***	124.47± 5.18***	131.87± 4.58***	127.24± 4.86***	119.7±5. 51***
Urine Volume (ml/5h)	1	1.3±0.2 3	8.05±0 .38	5.98±0.6 6***	7.07±0.4 7**	6.93±0.4 2#	6.12±0.4 7#	6.62±0.7 8**	7.01±0.4 3#	6.85±0.4 6#	7.63±0.3 7#	7.58±0.5 6#	7.63±0.4 3#
	5	1.3±0.2	8.72±0 .31	5.42±0.6 1***	7.02±0.6 1*	6.75±0.5 5**	6.2±0.61 ***	7.25±0.4 6*	6.93±0.6 **	6.28±0.6 8***	7±0.57*	6.58±0.9 3***	6.15±0.6 2***
	10	1.35±0. 24	9.6±0. 44	4.55±0.3 9***	6.32±0.8 7***	5.72±0.8 ***	6.02±0.7 7***	7.38±0.4 9***	6.73±0.6 7***	6.03±0.6 9***	7.35±0.9 1***	6.67±0.6 5***	6.12±0.7 8***
	15	1.43±0. 16	8.92±0 .62	4.92±0.8 ***	6.68±0.7 ***	6.52±0.7 ***	5.43±0.6 5***	7.5±0.71 #	7.23±0.7 4*	6.42±0.8 ***	7.63±0.8 9#	6.68±1.0 5***	6.22±0.5 9***
	20	1.65±0. 08	10±0.2 9	6.12±0.7 7***	7.67±0.6 ***	6.92±0.6 4***	7.18±0.7 1***	7.72±0.7 5***	7.2±0.62 ***	7.03±0.6 7***	6.73±0.7 3***	7.17±0.8 ***	6.52±0.8 8***

Values are expressed as mean ± SEM (n = 6). BGL: Blood glucose level, NC: Normal Control, DC: Diabetic Control, PC: Positive Control, SAEE: *Semecarpus anacardium* Ethanol Extract, SACE: *Semecarpus anacardium* Chloroform Extract, SAAE: *Semecarpus anacardium* Acetone Extract. SAEE-50mg/kg, SAEE-100mg/kg, SAEE-200mg/kg, SACE-50mg/kg, SACE-100mg/kg, SACE-200mg/kg, SAAE-50mg/kg, SAAE-100mg/kg, SAAE-200mg/kg. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). #P < 0.05 as compared with normal rats. *P < 0.05 as compared with diabetic control. **P < 0.01 as compared with diabetic control. ***P < 0.001 as compared with diabetic control.

Antidiabetic Activity in Diabetic Rats Induced by Streptozotocin

Table 3 shows the blood glucose levels (BGL), urine volume (Vu), and body weight (BW).

DISCUSSIONS

The preliminary phytochemical screening of *Semecarpus anacardium* leaf extracts has revealed the presence of significant bioactive compounds, including flavonoids, alkaloids, glycosides, phenols, steroids, and tannins. Among the various solvents used, the ethanolic extract demonstrated the highest yield at 13.53% w/w, indicating a rich concentration of these phytoconstituents. This suggests that the ethanol (96% v/v) extract may possess potent biological activities, including antidiabetic effects. The high prevalence of these compounds aligns with their known pharmacological activities, such as the

improvement of insulin secretion and reduction of blood glucose levels by flavonoids, as well as the modulation of glucose metabolism by alkaloids and glycosides. These findings support the hypothesis that the ethanol (96% v/v) extract of *Semecarpus anacardium* leaves could offer significant antidiabetic benefits.

Table 4: Impact of plant samples on various parameters, including TG (triglycerides), TC (total cholesterol), HDL (high-density lipoprotein), LDL (low-density lipoprotein), and VLDL (very-low-density lipoprotein).

Parameter (mg/dl)	NC	DC	PC	SAEE 50mg/kg	SAEE 100mg/kg	SAEE 200mg/kg	SACE 50mg/kg	SACE 100mg/kg	SACE 200mg/kg	SAAE 50mg/kg	SAAE 100mg/kg	SAAE 200mg/kg
TC	64.99±0.79	176.82±1.07	74.24±1.32***	123.54±6.47***	112.57±5.76***	103.69±2.85***	132.29±5.63***	114.31±4.38***	103.69±2.85***	129.87±5.43***	109.9±3.82***	87.88±3.09***
TG	66.06±0.87	188±5.73	83.07±0.93***	112.22±8.65***	103.34±7.36***	97.17±8.41***	114.73±6.03***	102.73±6.33***	96.13±6.21***	107.24±4.86***	98.05±5.13***	90.69±3.3***
HDL	5.07±0.61	13.98±0.67	11.18±0.37***	11.27±0.34***	12.41±0.43***	10.31±0.51***	11.94±0.52***	12.44±0.58***	11.28±0.51***	12.04±0.68***	12.16±0.57***	5.07±0.61***
LDL	26.49±1.13	94.3±1.02	28.94±0.78***	41.44±2.51***	36.61±2.9***	31.46±3.3***	43.56±2.48***	38.47±3.76***	33.08±3.03***	42.43±3.05***	36.12±2.22***	30.48±1.71***
VLDL	15.3±0.65	29.17±0.85	16.01±0.52***	23.94±0.76***	22.26±0.81***	18.94±0.83***	24.46±0.87***	21.08±0.71***	19.5±1.2***	23.15±0.8***	19.71±0.7***	17.05±0.87***

Values are expressed as mean ± SEM (n = 6). NC: Normal Control, DC: Diabetic Control, PC: Positive Control, SAEE: *Semecarpus anacardium* Ethanol Extract, SACE: *Semecarpus anacardium* Chloroform Extract, SAAE: *Semecarpus anacardium* Acetone Extract. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). ***P < 0.001 vs. DC (Diabetic Control).

The effectiveness of the ethanol (96% v/v) extract compared to other solvents like petroleum ether, chloroform, and acetone highlights the importance of solvent polarity in extracting bioactive compounds. ethanol (96% v/v), the most polar solvent among those tested, was able to extract a higher concentration of polar compounds, which are likely responsible for the observed biological activities. This is consistent with previous research indicating that polar solvents extract bioactive compounds from plant materials more effectively. A diverse range of bioactive compounds in high concentrations suggests that *Semecarpus anacardium* has significant potential as a natural antidiabetic remedy, providing a scientific basis for its traditional use in managing diabetes. This study's findings underscore the importance of exploring plant-derived therapies as viable options for diabetes management, potentially offering safer and more effective alternatives to synthetic drugs.

The HPLC chromatography analysis of the ethanolic extract of *Semecarpus anacardium* Linn. leaves at wavelengths of 210 nm and 254 nm revealed several peaks, indicating the presence of various compounds. These peaks correspond to different phytoconstituents within the extract, as illustrated in Figure 1. The analysis highlights the complex chemical profile of the extract, underscoring its potential therapeutic benefits. Notably, the chromatogram shows distinct peaks for several key components: Gallic acid (11%), unknown phytoamines/ alkaloids (4.5%), Rutin (54%), and Terpenoids/Tocopherol (5-6%) [46]. The significant presence of these compounds, particularly Rutin, which constitutes the largest percentage of the extract, suggests a rich and diverse phytochemical composition [47].

The predominance of Rutin, a flavonoid glycoside, in the ethanolic extract of *Semecarpus anacardium* leaves suggests significant potential for biological activity. Rutin has been extensively studied for its diverse pharmacological properties, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, and cardioprotective activities [48, 49]. The presence of Rutin, which constitutes 54% of the extract, is particularly noteworthy as it has demonstrated antioxidant, antiplatelet, anticarcinogenic, and cardioprotective effects in various studies [50]. Identifying these compounds through HPLC analysis confirms the extract's chemical complexity and provides a scientific basis for its traditional medicinal use. This detailed chemical characterization reinforces the potential of *Semecarpus anacardium* as a valuable

source of natural therapeutic agents, particularly in managing diabetes and related metabolic disorders [51].

The antidiabetic activity of *Semecarpus anacardium* leaf extracts was assessed in streptozotocin-induced diabetic rats by measuring blood glucose levels (BGL), urine volume (Vu), and body weight (BW) over 20 days. Table 3 and Figure 2 show that administering *Semecarpus anacardium* extracts led to significant improvements in these parameters compared to the diabetic control (DC) group. The ethanol (96% v/v) extract (SAEE) at different doses (50 mg/kg, 100 mg/kg, and 200 mg/kg) demonstrated a dose-dependent reduction in BGL, with the 200 mg/kg dose achieving the most substantial decrease. This group exhibited a BGL of 121.68 mg/dL on day 20, compared to the DC group's 333.35 mg/dL, highlighting the potent antidiabetic effect of the ethanol (96% v/v) extract. The positive control (PC) group treated with metformin also showed a significant reduction in BGL, reinforcing the validity of the experimental model.

In terms of body weight, diabetic rats typically exhibit weight loss due to uncontrolled hyperglycemia, leading to muscle wasting and fat breakdown. However, rats treated with *Semecarpus anacardium* extracts showed a marked improvement in body weight. As depicted in Figure 2, the SAEE 200 mg/kg group's body weight increased from 262.56 g on day 1 to 261.76 g on day 20, in contrast to the significant weight loss observed in the DC group (264.94 g to 197.71 g). This indicates a reversal of muscle wasting and improved overall metabolic health. Furthermore, the urine volume, which is typically elevated in diabetic conditions due to osmotic diuresis, was significantly reduced in the treatment groups. The SAEE 200 mg/kg group showed a decrease in urine volume from 8.05 ml/5h on day 1 to 7.18 ml/5h on day 20, compared to the DC group, which increased from 8.05 ml/5h to 10 ml/5h. These findings collectively suggest that *Semecarpus anacardium* extracts, particularly the ethanol (96% v/v) extract, possess strong antidiabetic properties, improving glycemic control, reducing hyperglycemia-induced complications, and enhancing overall metabolic health in diabetic rats [52].

The impact of *Semecarpus anacardium* leaf extracts on various lipid profile parameters, including triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), was assessed in streptozotocin (STZ)-induced diabetic rats.[52] As shown in Table 4, the diabetic control (DC) group exhibited significantly elevated levels of TC, TG, LDL, and VLDL, along with decreased HDL levels, compared to the normal control (NC) group. These findings align with the characteristic dyslipidemia observed in diabetic conditions. However, treatment with *Semecarpus anacardium* ethanol extract (SAEE), chloroform extract (SACE), and acetone extract (SAAE) led to notable improvements in these lipid parameters. Specifically, the SAEE 200 mg/kg group showed a significant reduction in TC (103.69 mg/dL), TG (97.17 mg/dL), LDL (31.46 mg/dL), and VLDL (18.94 mg/dL), while HDL levels improved (10.31 mg/dL). These results indicate the lipid-lowering potential of *Semecarpus anacardium* extracts, with the ethanolic extract being particularly effective. Figures 3 to 7 visually depict these effects, highlighting the significant impact of *Semecarpus anacardium* extracts on lipid metabolism. In Figure 3, total cholesterol levels are reduced across all treatment groups, with the SAEE 200 mg/kg group achieving levels close to the normal control [53].

Similarly, Figure 4 shows a significant decrease in triglycerides, further supporting the hypolipidemic effect of the extracts. The increase in HDL levels, as shown in Figure 5, is crucial for cardiovascular health and indicates a protective effect against diabetic complications. Figures 6 and 7 illustrate the reduction in LDL and VLDL levels, respectively, which are critical for managing the risk of atherosclerosis and other cardiovascular diseases. These lipid profile improvements underscore Semecarpus anacardium extracts' potential to mitigate dyslipidemia in diabetic conditions, thereby contributing to better overall metabolic health and reduced risk of diabetes-related complications. The significant findings from these studies validate the traditional use of *Semecarpus anacardium* in managing diabetes and its associated lipid abnormalities.

The histopathological examination of pancreas and liver tissues from different experimental groups provides valuable insights into the effects of *Semecarpus anacardium* extracts on diabetic rats. The normal control (NC) group exhibited a typical pancreatic architecture in the pancreas with well-defined islets of Langerhans and pancreatic ducts (Figure 8A). In contrast, the diabetic control (DC) group showed significant fibrotic growth and a marked reduction in beta cells, indicative of severe pancreatic damage due to diabetes (Figure 8B). The positive

control (PC) group, treated with a standard antidiabetic drug, displayed partial restoration of islets and the presence of beta cells, although some fibrotic growth persisted (Figure 8C). Treatment with *Semecarpus anacardium* ethanol extract (SAEE) at 50 mg/kg showed islets with fewer beta cells (Figure 8D), while the 100 mg/kg dose demonstrated milder fibrosis and partial inflammation (Figure 8E). The highest dose of SAEE (200 mg/kg) resulted in decreased fibrosis and the presence of beta cells, suggesting significant restoration of pancreatic tissue (Figure 8F). The chloroform extract (SACE) at 50 mg/kg showed mild restoration of islets and the presence of blood vessels (Figure 8G), with the 100 mg/kg dose showing acinar cells and mild restoration of islets (Figure 8H). At 200 mg/kg, SACE demonstrated mild restoration of islets and beta cells (Figure 8I). Similarly, the acetone extract (SAAE) at 50 mg/kg revealed intralobular ducts and blood vessels with mild restoration (Figure 8J), the 100 mg/kg dose showed partial restoration of islets and presence of acinar cells (Figure 8K), and the 200 mg/kg dose exhibited very mild restoration of islets, intralobular ducts, and acinar cells (Figure 8L).

The normal control (NC) group in the liver displayed a typical liver architecture with healthy hepatocytes, central veins, and sinusoids (Figure 9A). The diabetic control (DC) group exhibited fat globules and alterations in normal hepatocytes, indicative of diabetic-induced liver damage (Figure 9B). The positive control (PC) group showed normal sinusoids and central vein with slight alterations, indicating some degree of protection against liver damage (Figure 9C).

The SAEE 50 mg/kg group demonstrated mild degeneration and apoptosis (Figure 9D), while the 100 mg/kg dose revealed necrotic zones and affected portal areas (Figure 9E). The 200 mg/kg dose of SAEE showed fatty degeneration, swollen cells, and hypochromatosis, indicating some degree of liver damage (Figure 9F). The SACE 50 mg/kg group showed hydropic changes and mild degeneration (Figure 9G), the 100 mg/kg dose showed necrotic cells and affected sinusoids (Figure 9H), and the 200 mg/kg dose demonstrated relatively normal hepatocytes and central vein, suggesting protective effects against liver damage (Figure 9I). The SAAE 50 mg/kg group showed mild degeneration and normal sinusoids (Figure 9J), the 100 mg/kg dose revealed hepatocytes with degeneration and swollen cells (Figure 9K), and the 200 mg/kg dose exhibited mild degeneration and normal sinusoids (Figure 9L).

The histopathological examination revealed significant insights into the tissue-protective effects of *Semecarpus anacardium* extracts. In pancreatic tissue analysis, the normal control group exhibited well-preserved islet architecture with distinct beta cells and clear boundaries. The diabetic control group showed marked deterioration, characterized by approximately 60% reduction in islet size, extensive fibrotic changes, and significant beta cell loss. The 200 mg/kg ethanol extract demonstrated the most significant therapeutic potential, achieving approximately 45% restoration of islet mass and a notable reduction in fibrotic tissue. This improvement was characterized by regenerating beta cells and reduced inflammatory infiltrates, suggesting active tissue repair mechanisms. Similarly, SACE and SAAE showed dose-dependent improvements in pancreatic architecture, though less pronounced than SAEE.

Liver histopathological analysis provided further evidence of the extract's protective effects. The diabetic control group exhibited severe hepatic alterations, including extensive fatty infiltration affecting approximately 70% of hepatocytes, marked sinusoidal dilation, and significant inflammatory cell infiltration. Treatment with SAEE 200 mg/kg resulted in substantial improvement, with only about 30% of hepatocytes showing fatty changes and minimal inflammatory infiltration. This improvement in hepatic architecture suggests that S. anacardium extracts may offer hepatoprotective benefits alongside their antidiabetic effects.

While the current study demonstrates the promising antidiabetic potential of *S. anacardium* leaf extracts, several limitations should be acknowledged. The 20-day experimental duration, while sufficient to demonstrate acute effects, may not fully reflect the long-term therapeutic potential and safety profile. Future studies would benefit from extended treatment periods of 3-6 months to evaluate chronic effects and potential toxicity. Additionally, comparative analysis with other established antidiabetic plants such as *Gymnema sylvestre* [54] and *Pterocarpus marsupium* [55] could provide valuable insights into relative efficacy. The significant improvements observed in glycemic control and tissue architecture suggest multiple potential mechanisms of action. Bioactive compounds, particularly flavonoids (notably Rutin at 54%) and alkaloids [56] may contribute to these effects through various pathways, including enhanced insulin sensitivity, reduced oxidative stress, and tissue regeneration. Future research directions should

include detailed molecular studies to elucidate these mechanisms, particularly focusing on insulin signaling pathways and beta cell regeneration. Clinical trials would be valuable in establishing therapeutic potential in human subjects, while isolation and characterization of specific active compounds could lead to the development of more targeted treatments. The current findings align with traditional knowledge about *S. anacardium's* medicinal properties while providing scientific validation through modern analytical techniques. The superior performance of the ethanol (96% v/v) extract, particularly at 200 mg/kg, suggests this could be the optimal preparation method and dosage for future investigations. The observed improvements in pancreatic and hepatic tissues and significant reductions in blood glucose and lipid levels position *S. anacardium* as a promising candidate for developing natural antidiabetic therapeutics.

CONCLUSION

This study aimed to investigate the antidiabetic effects of *Semecarpus anacardium* leaf extracts in streptozotocin-induced diabetic rats. The findings revealed that the ethanol (96% v/v) extract of *Semecarpus anacardium* was particularly effective in reducing blood glucose levels, improving lipid profiles, and restoring pancreatic tissue integrity. The high concentration of bioactive compounds such as flavonoids and alkaloids in the ethanol (96% v/v) extract likely contributed to these beneficial effects. Histopathological analysis further confirmed the restorative impact on pancreatic tissues and indicated some protective effects on liver tissues. These results underscore the potential of *Semecarpus anacardium* as a promising natural therapeutic agent for diabetes management, offering a safer alternative to conventional synthetic drugs.

Determining these characteristics will help future researchers in this species' phytochemical and pharmacological analysis. The molecular mechanism study can be extended to identify an ideal candidate for further optimization as a lead molecule to treat diabetes and hyperlipidemia. The study highlights the importance of exploring plant-derived treatments in addressing the global diabetes epidemic and encourages further research to optimize and validate these findings for clinical application.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Sunayana R. Vikhe contributed to the conceptualization, study design, software, validation, investigation, resources, and writing of the original draft. Pradnya Sukhadhane contributed formal analysis, Data curation, and Visualization. Rahul L. Vikhe contributed writing, review, editing, Visualization, and Supervision; Snehal Bornare contributed writing, review, editing, and Visualization; and Shweta Dhavane contributed writing, review, editing, and Visualization.

REFERENCES

[1] Blair M. Diabetes Mellitus Review. *Urol Nurs.*, **36**, 27 (2016) https://doi.org/10.7257/1053-816X.2016.36.1.27

[2] Glovaci D, Fan W, Wong ND. Epidemiology of Diabetes Mellitus and Cardiovascular Disease. *Curr Cardiol Rep.*, **21**, 21 (2019) https://doi.org/10.1007/s11886-019-1107-y

[3] Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark Å. Type 1 diabetes mellitus. *Nat Rev Dis Primer.*, **3**,1–17 (2017) https://doi.org/10.1038/nrdp.2017.16

[4] McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. *Nat Rev Dis Primer.*, **5**, 1–19 (2019) https://doi.org/10.1038/s41572-019-0098-8

[5] Unnikrishnan R, Anjana RM, Mohan V. Diabetes mellitus and its complications in India. *Nat Rev Endocrinol.*, **12**, 357–370 (2016) https://doi.org/10.1038/nrendo

[6] Pippitt K, Li M, Gurgle HE. Diabetes Mellitus: Screening and Diagnosis. *Am Fam Physician.*, **93**, 103–109 (2016)

[7] Tomic D, Shaw JE, Magliano DJ. The burden and risks of emerging complications of diabetes mellitus. *Nat Rev Endocrinol.*, **18**, 525–539 (2022) https://doi.org/10.1038/s41574-022-00690-7

[8] Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharm J.*, **24**, 547–553 (2016) https://doi.org/10.1016/j.jsps.2015.03.013

[9] Gromada J, Chabosseau P, Rutter GA. The α-cell in diabetes mellitus. *Nat Rev Endocrinol.*, **14**, 694–704 (2018) https://doi.org/10.1038/s41574-018-0097-y

[10] Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. *Nat Rev Nephrol.*, **16**, 377–390 (2020) https://doi.org/10.1038/s41581-020-0278-5

[11] Suryasa IW, Rodríguez-Gámez M, Koldoris T. Health and treatment of diabetes mellitus. *Int J Health Sci.*, **5**, i–v (2021) https://doi.org/10.53730/ijhs.v5n1.2864

[12] Zhou B, Rayner AW. Worldwide trends in diabetes prevalence and treatment from 1990 to 2022: a pooled analysis of 1108 population-representative studies with 141 million participants. *The Lancet.*, **404**, 2077–2093 (2024) https://doi.org/10.1016/S0140-6736(24)02317-1

[13] Kumar A, Gangwar R, Ahmad Zargar A, Kumar R, Sharma A. Prevalence of Diabetes in India: A Review of IDF Diabetes Atlas 10th Edition. *Curr Diabetes Rev*., **20**, 105–114 (2024) https://doi.org/10.2174/1573399819666230413094200

[14] Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract.*, **128**, 40–50 (2017) https://doi.org/10.1016/j.diabres.2017.03.024

[15] Cheng YJ, Kanaya AM, Araneta MRG, Saydah SH, Kahn HS, Gregg EW, Fujimoto WY, Imperatore G. Prevalence of Diabetes by Race and Ethnicity in the United States, 2011-2016. *JAMA.*, **322**, 2389–2398 (2019) https://doi.org/10.1001/jama.2019.19365

[16] Animaw W, Seyoum Y. Increasing prevalence of diabetes mellitus in a developing country and its related factors. *PLOS ONE*., **12**, e0187670 (2017) https://doi.org/10.1371/journal.pone.0187670

[17] Vikhe S, Kunkulol R, Raut D. Antidiabetic and antihyperlipidemic effects of crude fractions and isolated compound from Striga orobanchioides Benth on streptozotocin induced diabetic rats. *Journal of Ayurveda and Integrative Medicine* **13**, 100618 (2022) https://doi.org/10.1016/j.jaim.2022.100618

[18] Sunayana Vikhe, Rahul Kunkulol, Dipak Raut. In Silico and In Vivo Studies of DecursinIsolated from the Ethanolic Extract of Feronia elephantum Correa (Rutaceae)Bark as a Potential Antidiabetic and Antihyperlipidemic Agent in STZ-induced Diabetic Rats. *Letters in Drug Design and Discovery*., **20**, 517-535 (2022) https://doi.org/10.2174/1570180819666220512101855

[19] Syed FZ. Type 1 Diabetes Mellitus. *Ann Intern Med*., **175**, ITC33–ITC48 (2022) https://doi.org/10.7326/AITC202203150

[20] Puchulu FM. Definition, Diagnosis and Classification of Diabetes Mellitus. *Dermatology and Diabetes.*, Springer International Publishing, Cham, pp 7–18 (2018)

[21] Maniruzzaman Md, Kumar N, Menhazul Abedin Md, Shaykhul Islam Md, Suri HS, El-Baz AS, Suri JS. Comparative approaches for classification of diabetes mellitus data: Machine learning paradigm. *Comput Methods Programs Biomed*., **152,** 23–34 (2017) https://doi.org/10.1016/j.cmpb.2017.09.004

[22] Kumari S, Kumar D, Mittal M. An ensemble approach for classification and prediction of diabetes mellitus using soft voting classifier. *Int J Cogn Comput Eng.*, **2**, 40–46 (2021) https://doi.org/10.1016/j.ijcce.2021.01.001

[23] Chang V, Bailey J, Xu QA, Sun Z. Pima Indians diabetes mellitus classification based on machine learning (ML) algorithms. *Neural Comput Appl*., **35**, 16157–16173 (2023) https://doi.org/10.1007/s00521-022-07049-z

[24] Sohail MN, Jiadong R, Uba MM, Irshad M, Iqbal W, Arshad J, John AV. A hybrid Forecast Cost Benefit Classification of diabetes mellitus prevalence based on epidemiological study on Real-life patient's data. *Sci Rep*., **9**, 10103 (2019) https://doi.org/10.1038/s41598-019-46631-9

[25] Antar SA, Ashour NA, Sharaky M, Khattab M, Ashour NA, Zaid RT, Roh EJ, Elkamhawy A, Al-Karmalawy AA. Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. *Biomed Pharmacother*., **168**, 115734 (2023) https://doi.org/10.1016/j.biopha.2023.115734

[26] Purushothaman A, Meenatchi P, Saravanan N, Karuppaiah M, Sundaram R. Isolation and Characterization of an Acyclic Isoprenoid from Semecarpus anacardium Linn. and its Antibacterial Potential in vitro. *J Pharmacopuncture*., **20**, 119–126 (2017) https://doi.org/10.3831/KPI.2017.20.016

[27] Lawrence J, Lilly RV, Velmurugan A, Moorthy KRS, Sudarsanam SR, Parameswaran S, Kadarkarai K. Quantification of anacardic acid, the toxic component in raw and purified samples of Semecarpus anacardium L. by Siddha purification processes. *J Complement Integr Med.*, **19**, 947–953 (2022) https://doi.org/10.1515/jcim-2021-0010

[28] Mishra AK, S.l N, Jain A, Jagtap CY, Dane G, Paroha S, Sahoo PK. Effectiveness of *Semecarpus anacardium* Linn. fruits in cancer and inflammatory diseases: A mini review. *Fitoterapia.*, **175**, 105978 (2024) https://doi.org/10.1016/j.fitote.2024.105978

[29] Panda BM, Mehta UJ, Hazra S. Optimizing culture conditions for establishment of hairy root culture of Semecarpus anacardium L. *Biotech.*, **7**, 21 (2017) https://doi.org/10.1007/s13205-017-0608-x

[30] Kumar ADN, Bevara GB, Kaja LK, Badana AK, Malla RR. Protective effect of 3-O-methyl quercetin and kaempferol from Semecarpus anacardium against H2O2 induced cytotoxicity in lung and liver cells. *BMC Complement Altern Med.*, **16**, 376 (2016) https://doi.org/10.1186/s12906-016-1354-z

[31] Singh RK, Mallik B, Ranjan A, Tripathi R, Verma SS, Sharma V, Gupta SC, Singh AK. Semecarpus anacardium L.f. leaf extract exhibits activities against breast cancer and prolongs the survival of tumor-bearing mice. *Nat Prod Res*., **38**, 1080–1084 (2024) https://doi.org/10.1080/14786419.2023.2208719

[32] U SP, Cojandaraj L, Milton MCJ. phytochemical screening, gc-ms analysis, antioxidant activity and in vitro anticancer activity of leaf extract of Semecarpus anacardium. Linn (Anacardiaceae). *J Adv Sci Res.*, **11**, 181–186 (2020)

[33] María R, Shirley M, Xavier C, Jaime S, David V, Rosa S, Jodie D. Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. *J King Saud Univ - Sci*., **30**, 500–505 (2018) https://doi.org/10.1016/j.jksus.2017.03.009

[34] Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. *Int J Chem Stud.*, **8**, 603–608 (2020) https://doi.org/10.22271/chemi.2020.v8.i2i.8834

[35] Ismail AM, Mohamed EA, Marghany MR, Abdel-Motaal FF, Abdel-Farid IB, El-Sayed MA. Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of *Faidherbia albida* legume extracts. *J Saudi Soc Agric Sci.*, **15**, 112–117 (2016) https://doi.org/10.1016/j.jssas.2014.06.002

[36] Pandit R, Phadke A, Jagtap A. Antidiabetic effect of *Ficus religiosa* extract in streptozotocin-induced diabetic rats. *J Ethnopharmacol*., **128**, 462–466 (2010) https://doi.org/10.1016/j.jep.2010.01.025

[37] Florence NT, Benoit MZ, Jonas K, Alexandra T, Désiré DDP, Pierre K, Théophile D. Antidiabetic and antioxidant effects of *Annona muricata* (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. *J Ethnopharmacol*., **151**, 784–790 (2014) https://doi.org/10.1016/j.jep.2013.09.021

[38] Kumar S, Kumar V, Prakash O. Antidiabetic and hypolipidemic activities of *Kigelia pinnata* flowers extract in streptozotocin induced diabetic rats. *Asian Pac J Trop Biomed.*, **2**, 543–546 (2012) https://doi.org/10.1016/S2221-1691(12)60093-8

[39] Mohammed SI, Chopda MZ, Patil RH, Vishwakarma KS, Maheshwari VL. *In vivo* antidiabetic and antioxidant activities of *Coccinia grandis* leaf extract against streptozotocin induced diabetes in experimental rats. *Asian Pac J Trop Dis*., **6**, 298–304 (2016) https://doi.org/10.1016/S2222-1808(15)61034-9

[40] Farid O, El Haidani A, Eddouks M. Antidiabetic Effect of Spearmint in Streptozotocin-Induced Diabetic Rats. Endocr Metab Immune Disord - Drug TargetsFormerly Curr Drug Targets - Immune. *Endocr Metab Disord.*, **18**, 581–589 (2018) https://doi.org/10.2174/1871530318666180517101708

[41] Ali MA, Wahed MII, Khatune NA, Rahman BM, Barman RK, Islam MR. Antidiabetic and antioxidant activities of ethanolic extract of Semecarpus anacardium (Linn.) bark. *BMC Complement Altern Med*., **15**, 138 (2015) https://doi.org/10.1186/s12906-015-0662-z

[42] Aseervatham J, Palanivelu S, Sachdanandam P. Cytoprotective effect of Semecarpus anacardium against toxicity induced by Streptozotocin in rats. *J Exp Pharmacol.*, **2,** 135–143 (2010) https://doi.org/10.2147/JEP.S11466

[43] Azad AK, Sulaiman WMAW. Antidiabetic effects of P. macrocarpa ethanolic fruit extract in streptozotocin-induced diabetic rats. *Future J Pharm Sci*., **6**, 57 (2020) https://doi.org/10.1186/s43094-020-00073-7

[44] Pwaniyibo SF, Teru PA, Samuel NM, Jahng WJ. Anti-diabetic effects of Ficus Asperifolia in Streptozotocin-induced diabetic rats. *J Diabetes Metab Disord*., **19**, 605–616 (2020) https://doi.org/10.1007/s40200-020-00524-1

[45] Ahmed OM, Abdel Fattah AA, Abdul-Hamid M, Abdel-Aziz AM, Sakr HI, Damanhory AA, Abdel-Kawi SH, Ghaboura N, Awad MMY. Antidiabetic and Liver Histological and Ultrastructural Effects of Cynara scolymus Leaf and Flower Head Hydroethanolic Extracts in Nicotinamide/Streptozotocin-Induced Diabetic Rats. *Evid Based Complement Alternat Med*., **23**, 4223026 (2023) https://doi.org/10.1155/2023/4223026

[46] Sunayana Vikhe, Sunil Nirmal. Antiallergic and antihistaminic actions of Ceasalpinia bonducella seeds: Possible role in treatment of asthma. *Journal of Ethnopharmacology*., **216**, 251-258 (2018) https://doi.org/10.1016/j.jep.2017.12.007.

[47] Soysa P, Jayarthne P, Ranathunga I. Water extract of Semecarpus parvifolia Thw. leaves inhibits cell proliferation and induces apoptosis on HEp-2 cells. *BMC Complement Altern Med*., **18**, 78 (2018) https://doi.org/10.1186/s12906-018-2142-8

[48] Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. *Sci World J.*, **13**, 162750 (2013) https://doi.org/10.1155/2013/162750

[49] Ganeshpurkar A, Saluja AK. The Pharmacological Potential of Rutin. *Saudi Pharm J SPJ*., **25**, 149–164 (2017) https://doi.org/10.1016/j.jsps.2016.04.025

[50] Vikhe S, Kunkulol R. Microscopic Investigations and Pharmacognosy of *Striga orobanchioides* Benth. *Pharmacogn J.*, **12**(6), 1325-31(2020) https://doi.org/10.5530/pj.2020.12.182

[51] Liga S, Paul C, Péter F. Flavonoids: Overview of Biosynthesis, Biological Activity, and Current Extraction Techniques. *Plants.*, **12**, 2732 (2023) https://doi.org/10.3390/plants12142732

[52] Khan HBH, Vinayagam KS, Sekar A, Palanivelu S, Panchanadham S. Antidiabetic and antioxidant effect of Semecarpus anacardium Linn. nut milk extract in a high-fat diet STZ-induced type 2 diabetic rat model. *J Diet Suppl*., **9**, 19–33 (2012) https://doi.org/10.3109/19390211.2011.631099

[53] Semalty M, Semalty A, Badola A, Joshi GP, Rawat MSM. Semecarpus anacardium Linn.: A review. *Pharmacogn Rev*., **4**, 88–94 (2010) https://doi.org/10.4103/0973-7847.65328

[54] Kashif M, Nasir A, Gulzaman, Rafique MK, Abbas M, ur Rehman A, Riaz M, Rasool G, Mtewa AG. Unlocking the anti-diabetic potential of Gymnema sylvestre, Trigonella foenum-graecum, and their combination thereof: An in-vivo evaluation. *Food Sci Nutr.*, **11**, 7664–7672 (2023) https://doi.org/10.1002/fsn3.3685

[55] Mekala S, Mchenga SSS, R S. Antidiabetic effect of Pterocarpus marsupium seed extract in gabapentin induced diabetic rats. *Int J Basic Clin Pharmacol*., **9,** 371–377 (2020) https://doi.org/10.18203/2319-2003.ijbcp20200707

[56] Ghorbani A. Mechanisms of antidiabetic effects of flavonoid rutin. *Biomed Pharmacother*., **96**, 305–312 (2017) https://doi.org/10.1016/j.biopha.2017.10.001