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Exploring the Potential Antidiabetic Effects of Ethanol Extract of Rhizome of *Curcuma Angustifolia* Roxb. In Streptozotocin-Induced Diabetic Rats

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KEYWORDS
Curcuma angustifolia Roxb., rhizome, anti-diabetic, streptozotocin

ABSTRACT:
The study aimed to explore the potential of the ethanol extract of rhizome of *Curcuma angustifolia* Roxb. (Zingiberaceae) in managing diabetes using both normal and streptozotocin (STZ)-induced diabetic rat models. Administered orally over a 21-day period at doses of 250 and 500 mg/kg body weight, the extract's effects were compared with those of the known antidiabetic drug metformin (100mg/kg body weight). The results highlighted significant improvements across various parameters. Firstly, there was a notable increase in body weight among the treated rats, suggesting a positive impact on overall health and metabolic function. Liver glycogen levels also saw a marked increase, indicating enhanced glycogen storage and improved glucose metabolism. Moreover, serum insulin levels showed a significant rise, indicative of improved pancreatic function and insulin secretion. In terms of lipid profile, the ethanol extract demonstrated promising outcomes. There was a substantial decrease in blood glucose levels, pointing towards its hypoglycemic effect. Additionally, glycosylated hemoglobin levels were significantly reduced, reflecting better long-term glucose control. Notably, the extract led to a decline in both total cholesterol and serum triglycerides, key markers of dyslipidemia commonly associated with diabetes. Conversely, high-density lipoprotein (HDL) levels showed a significant increase, suggesting an improvement in lipid metabolism and cardiovascular health.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by deficiencies in insulin activity and/or secretion (Kharroubi et al., 2015). These deficiencies can lead to disruptions in the metabolism of proteins, carbohydrates, and lipids. Factors contributing to these metabolic abnormalities include low insulin levels, insulin resistance in tissues such as skeletal muscles and adipose tissue, as well as dysfunctions in insulin receptors and signalling pathways (Hunter et al., 1998). Globally, diabetes affects a significant portion of the population, with current estimates at 2.8% and projected to increase to 4.4% by 2030. Despite being a non-communicable condition, diabetes ranks among the top five major health burdens worldwide (Wild et al., 2004). Even though diabetes is not transmitted between individuals (Kharroubi et al., 2015), it ranks among the top five most significant health concerns globally. The manifestation and frequency of diabetes vary depending on the severity of symptoms. While some individuals may not experience any symptoms, especially in the early stages of type 2 diabetes, others may exhibit noticeable hyperglycemia. If left untreated and uncontrolled, diabetes can lead to serious complications such as stupor, coma, and potentially fatal conditions like ketoacidosis or rare non-ketotic hyperosmolar disorders (American Diabetes Association). The development of diabetes is influenced by a combination of genetic and non-genetic factors (Patel et al., 2012). Despite the importance of accurately classifying diabetes for treatment strategies, it can be challenging as many individuals do not neatly fit into a single category,

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particularly younger adults, and around 10% of initially classified patients may require re-evaluation later on (Cakan et al., 2012). The standard classification system introduced by the American Diabetes Association in 1997, which includes type 1, type 2, and gestational diabetes mellitus (GDM), remains widely accepted and endorsed by the ADA (American Diabetes Association). Type 1 Diabetes involves an autoimmune process where pancreatic β-cells are targeted and destroyed by immune cells like CD4+ and CD8+ T cells, as well as macrophages, leading to a deficiency in insulin production (Baynes et al., 2015). Insufficient insulin levels trigger unregulated lipolysis, leading to elevated levels of free fatty acids in the bloodstream. This, in turn, reduces glucose metabolism in peripheral tissues (Holt et al., 2004). Mitochondria-associated membranes (MAMs), where mitochondria and the endoplasmic reticulum interact, play vital roles in regulating lipid exchange, calcium signalling, cell survival, and metabolic homeostasis. Within MAMs, various insulin signalling proteins like AKT kinase, mTORC2, PP2A, and PTEN are present, contributing to insulin signalling processes. Increasing evidence suggests that dysfunction of MAMs is implicated in the impairment of insulin-producing β cells and insulin resistance in peripheral tissues, contributing to the development of type 2 diabetes mellitus (Yang et al., 2020).

Numerous anti-diabetic medications are available to address hyperglycemia by enhancing insulin sensitivity, supporting insulin function, boosting insulin secretion, and promoting glucose uptake. However, drugs like metformin and sulfonylureas are associated with various adverse effects. Metformin, for instance, may lead to diarrhoea and lactic acidosis, while sulfonylureas can cause hepatic failure, weight gain, tachycardia, and hypothyroidism (Li et al., 2013). Plants have long been recognized as valuable sources of medicinal compounds, and many synthetic drugs are either directly or indirectly derived from them. Recent studies indicate that plants and plant-based products possess significant potential for effectively managing diabetes. Since ancient times, plant-derived anti-diabetic agents have been widely utilized due to their perceived safety, affordability, and prominence in traditional medicine across various cultures such as Indian, Korean, and Chinese. Traditional herbal remedies and functional foods are believed to alleviate diabetic symptoms through six key mechanisms: enhancing insulin secretion and sensitivity, promoting glucose uptake by muscle cells and adipose tissues, inhibiting glucose absorption from the intestine, reducing glucose production from hepatocytes, and exhibiting anti-inflammatory properties (Li et al., 2013). Various potential bioactive secondary metabolites, also known as phytochemicals, extracted from diverse medicinal plants exhibit distinct mechanisms of action (Table 1).

Table 1: Phytochemicals and their mechanism of action (Alam etal., 2022)

Phytochemicals	**Mechanism of action**
β-glucans, Bassic acid	Glycogen synthesis, gluconeogenesis
Scirpusin B, Myricetin	Inhibition of α-amylase secreted from salivary gland
Berberine,Pectin	Diminished fasting blood glucose, HbA1c, post prandial blood glucose and insulin need
Chicoric acid, Lupanine	Increases insulin secretion from beta cell
Curcumin, Turmerin	Inhibition of α glucosidase secreted from brush border of small intestine
Calenduloside E, Malonylgenistin	Inhibits DPP-4 enzyme
Quercetin, Resveratrol	Increases insulin senstivity

Kinsenoside, derived from *Anoectochilus roxburghii* (Zhang et al., 2007), and Bacosine, a triterpene found in *Bacopa monnieri* (Ghosh et al., 2011), are among the bioactive compounds documented for their hypoglycemic effects. Additionally, Berberine from *Berberis aristata* (Yin et al., 2008), Bixin from *Bixa Orellana* L (Vilar et al.,2014), and Bassic acid from *Bumelia sartorum* (Naik et al.,1999) have been studied for their potential in enhancing insulin secretion from beta cells. Extracts of various plants such as *Callistemon rigidus* (Goyal et al.,2012), *Chamaemelum nobile* (Yonei et al.,2010), *Cichorium intybus* L. (Nishimura et al., 2015), *Cinnamomum verum* (Zare et al., 2019), *Gymnema sylvestre* (Yadav et al.,2019), *Hordeum vulgare* (Minaiyan et al., 2014),

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Lupinus perennis (Bernhardt et al., 2008), *Moringa oleifera* (Leone et al.,2018), *Nigella sativa* (Hamdan et al., 2019), *Punica granatum* (Gharib et al.,2019), *Syzygium cumini* (Sidana et al., 2017), and *Zingiber officinale* (Jafarnejad et al.,2017) have also shown potential as anti-diabetic agents by modulating insulin secretion from beta cells.

Curcuma longa L., commonly known as 'Turmeric,' is a moderately tall perennial plant with underground rhizomes. It is cultivated in tropical regions such as Pakistan, China, Peru, and India. From *Curcuma longa*, the curcuminoids bisdemethoxycurcumin, curcumin, and demethoxycurcumin have been isolated, showing α-glucosidase inhibitory activity (Lekshmi et al.,2012). Among these, bisdemethoxycurcumin exhibited the most potent inhibition (Kalaycıoğlu et al., 2017). Additionally, volatile oils extracted from both fresh and dried turmeric rhizomes demonstrated dose-dependent glucosidase inhibitory activity, with dried rhizomes showing increased efficacy (Lekshmi et al., 2012). Aromatic-Turmerone, the main volatile component in turmeric rhizomes, exhibited potent α-glucosidase and α-amylase inhibitory activity (Lekshmi et al., 2012). Turmerin, a water-soluble protein found in turmeric rhizomes, also inhibits α-amylase and α-glucosidase activities. This indicates that turmeric rhizomes possess inhibitory effects against enzymes associated with type 2 diabetes. Furthermore, a combination of *Curcuma longa* and *Allium sativum* at a total dose of 2.4 g showed significant antihyperglycemic effects in type 2 diabetic patients, reducing fasting blood glucose, 2-hour postprandial glucose, HbA1C, and body mass index levels without adverse effects (Sukandar et al., 2010). Clinical trials administering curcuminoids ranging from 0.25 g to 1 g per day to type 2 diabetic patients also improved glycemic control by reducing fasting blood glucose, HbA1c, HOMA-IR (insulin resistance) levels, and increasing adiponectin levels without major side effects. Improvement in diabetes-associated endothelial dysfunction and hyperlipidemia was also observed (Pivari et al., 2019). While extensive research has been conducted on the anti-diabetic properties of *Curcuma longa*, studies on other species within the same genus are lacking. Recognizing the pharmacological significance of the Curcuma genus, the present study focuses on investigating the anti-diabetic activity of rhizome of *Curcuma angustifolia* based on the aforementioned findings.

2. Materials and Methodology

2.1 Materials

Carboxy methyl Cellulose sodium (SDFCL, India); Picric acid (Himedia, India); Phosphate Buffered Saline (Himedia, India); Metformin (Mankind Pharma Ltd); Saline solution (0.9%) (Himedia, India); Streptozotocin , Trisodium Citrate (Merck, India)

2.2 Animal Maintenance

Animal experiments followed CPCSEA guidelines (Registration Number: VII/ IAEC/ Dr MGR / 2053/ PO / ReBi/ S/ 19/ CPCSEA/ 05.08.2023/ 02). Animals were kept in controlled conditions: temperature (22± 3°C), humidity (30-70%), and a 12-hour light-dark cycle. Each animal was marked and given a unique ID. They were housed individually in standard cages with food, water, and bedding. Bedding material was sourced from Sudhakar traders. Drinking water was continuously available via Aqua Guard systems. Procedures were conducted humanely by trained personnel and approved by the Institutional Animal Ethical Committee (IAEC) of Radiant Research Services Pvt. Ltd.

Table: Animal Description

Parameter	Description
Animals	Rats
Strain	Wistar
Sex	Male
Body weight	180 – 200gms

2.3 Acute toxicity test

An acute toxicity study was conducted on ethanol extract derived from *Curcuma angustifolia* rhizom using the fixed dose method recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the OECD guidelines No. 420. Thirty fasted male albino mice, weighing between 25-30 g and aged 10 weeks, were utilized for the study. They were divided into six groups: A, B, C, D, E, and F, each containing five animals. Group A served as the control and received distilled water, while groups B

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to F were orally administered increasing doses of ethanol extract derived from *Curcuma angustifolia* rhizom(ECAR) dissolved in distilled water using orogastric tubes. The doses administered were 500, 1000, 1500, 2000, and 2500 mg/kg body weight, respectively. The animals were closely monitored at 2, 6, 24, and 48 hours following extract administration to observe any changes in autonomic or behavioral responses. Mortality was recorded up to 24 hours post-administration. The purpose of the study was to assess the potential acute toxicity of *Curcuma angustifolia* extract in mice, with careful observation of any adverse effects or mortality resulting from the administered doses (OECD guidelines).

2.4 Group, Designation and Dose Levels

The study aimed to investigate the anti-diabetic properties of ethanol extract derived from *Curcuma angustifolia* rhizome on Wistar rats. The rats were categorized into five groups, each consisting of six animals, denoted as n=6. The rats were divided into five groups, each consisting of six animals. These groups included a normal group without induced diabetes, a diabetic induced positive group (Streptozotocin induced), a standard treatment group (Metformin), and two groups treated with different doses (low and high dose) of the ethanol extract of *Curcuma angustifolia* rhizome. Throughout the study, the laboratory maintained standard conditions of light, humidity, temperature, and overall working environment. The purpose was to systematically assess the effects of the ethanol extract on diabetic conditions in rats, comparing its effectiveness with a standard treatment and observing any potential dose-dependent effects.The details of the grouping and dose levels is given in the table below:

Table: Description of grouping, designation and dose levels

Groups	Group Description	Treatment Description	Dose Volume (ml/kg)	No. of animals
Group 1 G1	Control	Animals were treated with carboxy methyl cellulose suspension (10 ml/kg).	10	6
Group 2 G2	Diseased group	STZ induction + Animals were treated with carboxy methyl cellulose suspension (60 mg/kg body weight).	10	6
Group 3 G3	Positive treatment (Metformin)	STZ induction + Animals were treated with standard metformin 100mg/kg body weight.	10	6
Group 4 G4	Treatment 1 Low dose ECAR (LCA)	STZ induction + Animals were treated with test substance 250mg/kg body weight.	10	6
Group 5 G5	Treatment 2 High dose ECAR (HCA)	STZ induction + Animals were treated with test substance 500mg/kg body weight.	10	6

2.5 Evaluation of Anti-diabetic activity

2.5.1 Induction of diabetes:

To induce diabetes in rats, a single intraperitoneal (i.p.) injection of streptozotocin (STZ) was administered at a dose of 10 ml/kg body weight. STZ, obtained from Sigma Chemical Co. USA, is a well-known compound used to chemically induce diabetes in experimental animals, particularly in rats. The STZ was freshly dissolved in 0.1 M cold citrate buffer of pH 4.5. This pH is essential for stabilizing the STZ solution and ensuring its effectiveness in inducing diabetes. The citrate buffer serves as a vehicle for the STZ, facilitating its administration and absorption in the body. Approximately 48 hours after the administration of STZ, blood samples were collected from the rats. These blood samples were analyzed to determine the blood glucose levels, aiming to confirm the development of diabetes in the animals. Hyperglycemia, defined as blood glucose levels greater than 240 mg/dl, is indicative of diabetes in rats (Ramdas et al., 2011).

2.5.2 Chronic treatment model

The rats were categorized into five groups, each comprising six animals (n=6), as follows: Group I: Normal control rats, which received distilled water orally at a dosage of 10 ml/kg body weight. Group II: Diabetic control rats, which did not receive any treatment and were administered distilled water orally at a dosage of 10 ml/kg body weight. Group III: Diabetic rats treated with the standard drug

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metformin at a dosage of 0.25 mg/kg/day orally. Group IV: Diabetic rats treated with EACAR at a dosage of 250 mg/kg/day orally. Group V: Diabetic rats treated with EACAR at a dosage of 500 mg/kg/day orally. Throughout the 21-day study period, blood glucose levels were monitored and body weights were measured. Additionally, on the 21st day of the study, blood samples were collected to estimate various parameters (Ramdas et al., 2011).

2.5.3 Estimation of SGOT,SGPT and ALP and Lipid profiling

On the termination day of the experiment, blood samples were collected from the retro-orbital sinus of the rats. Following collection, the blood samples were processed to separate the serum from the cellular components. Serum contains liver enzymes such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) which are detected using Erba kit (Manufactured by Tranasia Biomedicals LTD.). The lipid profiling of parameters like cholesterol, LDL (Low-Density Lipoprotein), HDL (High-Density Lipoprotein), VLDL (Very Low-Density Lipoprotein) and Triglycerides were also detected by Erba kits. The serum samples were then analyzed for the respective biochemical parameters using an Automatic Biochemistry Analyzer (Erba; EM-360) (Yadav et al., 2008).

2.5.4 Estimation of Serum insulin

Serum insulin levels were measured using a radioimmunoassay (RIA) kit provided by BRIT, BARC, India. The kit contained human insulin as a standard and 125I-labelled human insulin antibody, which has similar reactivity with rat insulin (Chakrabarti et al., 2005).

2.5.5 Estimation of Glycated Hemoglobin

To estimate glycated hemoglobin, rats were fasted for 12 hours after the experimental period of 21 days. They were then euthanized by cervical decapitation, and blood was drawn via retro-orbital puncture under the effect of light ether anesthesia and Glycated hemoglobin levels were determined (Alayash et al.,1988).

2.5.6 Estimation of Liver glycogen

Following a 21-day experimental period, rats that had fasted for 12 hours were humanely euthanized through cervical decapitation. Liver tissue weighing approximately 1 gram was carefully collected and transferred to a centrifuge tube containing 2 ml of potassium hydroxide (KOH) solution (300 g/L) after being washed with saline water. The liver tissue and KOH solution were then heated for 20 minutes with periodic shaking.Subsequently, a saturated solution of sodium sulfate (0.2 ml) was added to the mixture and thoroughly mixed. This step facilitated the precipitation of glycogen. Ethanol (5 ml) was then added to further precipitate the glycogen. The resulting precipitate was separated, and it was dissolved in 10 ml of water. Following dissolution, 1 ml of this solution was combined with 1 ml of hydrochloric acid (HCl) solution (1.2 mol/l) and boiled for an additional 2 hours. After this boiling period, the solutions were neutralized using sodium hydroxide (NaOH) solution (0.5 mol/l), with phenol red serving as an indicator of neutrality.

The neutralized solution was then diluted to a final volume of 5 ml and transferred to a calorimeter tube. The calorimeter was adjusted using a reagent blank and the absorbance of the solution was measured at 620 nm. This measurement allowed for the determination of glycogen content in the liver tissue, expressed as mg/g of liver tissue (Ramdas et al., 2011).

2.5.7 Estimation of body weight and Histopathological studies

During the study period, the impact of the extracts on parameters such as body weight is monitored through out the study. On the 21st day of the experiment, all the animals were euthanized, and liver and pancreatic tissues were isolated. These tissues were then fixed in 10% formalin to preserve their cellular structure. Following fixation, the liver and pancreatic tissues were subjected to a process of dehydration and embedding in paraffin wax. This process helps to prepare the tissues for sectioning. The tissues were sectioned at a thickness of 5 micrometers using a microtome.Subsequently, the sections of liver and pancreatic tissues underwent routine staining procedures using hematoxylin and eosin stains. Hematoxylin stains the nuclei of cells bluepurple, while eosin stains the cytoplasm and extracellular matrix various shades of pink.

2.6 Statistical analysis

The results were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The results are represented as mean ± STD. A p-value less than 0.05 was considered statistically significant, indicating a difference in means.

3. Results

3.1 Acute Toxicity test

In the LD50 value determination, it was noted that the EACAR exhibited no mortality even at a high dose of 2500 mg/kg body weight, indicating its

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safety in animals. Therefore, this dose was considered safe for administration. Subsequently, 1/5th (500 mg/kg body weight) and 1/10th (250 mg/kg body weight) of the determined safe dose were selected as the maximal doses for all in vivo experiments.

3.2 Effect of EACAR on oral glucose tolerance test (OGTT) in STZ-induced diabetic rat

The study results unequivocally demonstrated that the EACAR, administered at doses of 250 mg/kg and 500 mg/kg, led to a significant reduction in blood glucose levels in rats with hyperglycemia induced by an oral glucose load of 2 g/kg. Additionally, when compared to the diabetic control group, both doses of the extract exhibited similar hypoglycemic effects to those observed with metformin (100 mg/kg) after 60 minutes of oral administration(Table 1).

3.3 Hypoglycemic effect of EACAR

The study results clearly demonstrated that the EACAR exhibited significant hypoglycemic activity in rats with streptozotocin (STZ)-induced diabetes. However, no significant effect was observed on rats with normal blood glucose levels. Over the course of the 21-day treatment period, there was a notable decrease in serum glucose levels of 69.26%, 63.73%, and 66.21% ($p<0.0001$) with the administration of metformin and the aqueous extract at doses of 250 mg/kg and 500 mg/kg, respectively, compared to the diabetic control group. This highlights the effectiveness of both metformin and the aqueous extract in reducing serum glucose levels in diabetic rats after 21days of treatment (Table 2.).

3.4 Effect of EACAR on body weight

The study emphasizes that at the end of the 21-day treatment period with EACAR in diabetic induced rats, several notable changes in body weight were observed among the different groups of rats in the study. The body weight of the normal rats significantly increased. This increase in body weight is expected in healthy rats, as it reflects normal growth and metabolic processes.Similarly, the body weight of the group treated with the EACAR increased significantly. This suggests that the administration of the extract did not have adverse effects on body weight and may have even promoted growth or improved overall health. The group treated with the standard drug (metformin) also experienced a significant increase in body weight. This indicates that the drug treatment was effective in improving the health and metabolic status of the rats, leading to weight gain.In contrast, the body weight of the diabetic control group decreased (Table 3.).

3.5 Effect of EACAR on serum insulin, glycosylated hemoglobin and liver glycogen

After the 21-day treatment period, significant changes were observed in various biochemical parameters among the groups of animals treated with the EACAR. Animals treated with the EACAR exhibited a significant increase in serum insulin levels and liver glycogen compared to the levels observed in the normal groups. Additionally, a significant decrease was observed in the glycosylated hemoglobin (HbA1c) levels of animals treated with the EACAR. Glycosylated hemoglobin is a marker of long-term blood glucose control, with higher levels indicating poorer glycemic control over time (Table 4 and Fig: 1).

3.6 Effect of EACAR on lipid profile

The administration of EACAR resulted in significant improvements in the lipid profile of rats. Cholesterol levels decreased by 18.05% and 32.2% at doses of 250 mg/kg and 500 mg/kg, respectively. LDL levels decreased by 57.10% and 59.60% at doses of 250 mg/kg and 500 mg/kg, respectively. VLDL levels decreased by 8.45% and 16.82% at doses of 250 mg/kg and 500 mg/kg, respectively. Triglyceride levels decreased by 39.78% and 45.24% at doses of 250 mg/kg and 500 mg/kg, respectively. Additionally, there was a significant ($p<0.0001$) increase in High-Density Lipoprotein (HDL) levels in the treated diabetic rats compared to the diabetic diseased group. This increase in HDL levels indicates an improvement in lipid metabolism and cardiovascular health, further highlighting the beneficial effects of EACAR treatment in diabetic rats (Fig 2 and Table 5).

3.7 Histopathology examination of Liver

Histopathology examination of liver tissue sections revealed distinct findings among the different experimental groups. In the normal control group, liver tissue sections exhibited a normal appearance of hepatocytes, with intact cell membranes, nuclei, and cytoplasm. Additionally, there was no evidence of congestion or dilation in portal veins. In contrast, liver sections from the diseased control group showed congestion of hepatic veins and mild vacuolar changes in hepatocytes, indicating pathological alterations induced by the experimental conditions. Liver tissue sections from the standard and EACAR groups displayed a normal arrangement of hepatocytes around the central vein. The hepatocytes appeared active and healthy, with vesicular nuclei resembling those observed in the normal control group. These findings suggest that both the standard drug and the aqueous extract

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treatment contributed to the preservation of liver tissue architecture and function (Fig 6).

Table 1: Effect of EACAR on OOGTT) in STZ-induced diabetic rat

Treatment group where n=6	Plasma glucose level in mg/dl (Fasting) in hours				
	0hr	1hr	2hr	3hr	4hr
I Normal control	95.5±1.64	123.16±2.04	132.16±1.47	145.83±2.31	153.83±1.47
II Diseased control	252.66±2.16	266.5±1.87	275.16±2.4	284.83±1.47	294.33±2.33
III Positive control	246.6±2.25	256.6±1.87	265.2±2.73****	275±2.82	285.8±2.42****
IV LCA	255±3.16	263.5±2.34	274.16±3.25	284.5±2.58****	294.33±2.16****
V HCA	257.33±1.21	266.83±1.94	277.66±1.50***	286.66±1.75***	297.16±1.47****

**** p<0.0001, ***p<0.001, Values are mean±SEM, n=6, when compared with diseased control by using one way ANOVA followed by Tukey's multiple comparison test.

Table2: Effect of EACAR on serum glucose level in STZ-induced diabetic rat

Treatment group where n=6	Plasma glucose level in mg/dl (Fasting) in weeks (21days)			
	Basal	1st week	2nd week	3rd week
I Normal control	96±1.55	95.5±1.38	95.66±1.21	96±2.68
II Diseased control	257±2.37	284.33±2.16	316.83±4.45	376.83±21.6
III Positive control	256.16±1.72	264.5±3.62	209±3.29	115.83±3.66****
IV LCA	256±2.8	275.83±2.32	235.66±2.16	136.66±2.58****
V HCA	256.83±1.94	266±2.37	220.33±1.75****	127.33±1.63

****p<0.0001, Values are mean±SEM, n=6, when compared with diseased control by using one way ANOVA followed by Tukey's multiple comparison test.

Table3: Effect of EACAR on body weight in STZ-induced diabetic rat

Treatment group where n=6	0th day	10th day	21st day
I Normal control	175.33±3.66	193.5±2.94	215.33±4.13
II Diseased control	176.33±1.96	165±4.04	145.33±3.36
III Positive control	175.83±3.25	185.5±3.27	212.5±5.08
IV LCA	176±2.89	196.33±3.61	208.83±8.23
V HCA	176.16±2.89	197.83±3.61	213.33±8.23

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All were **** p<0.0001, values are mean±SEM, n=6, when compared with diseased control by using one way ANOVA followed by Tukey's multiple comparison test.

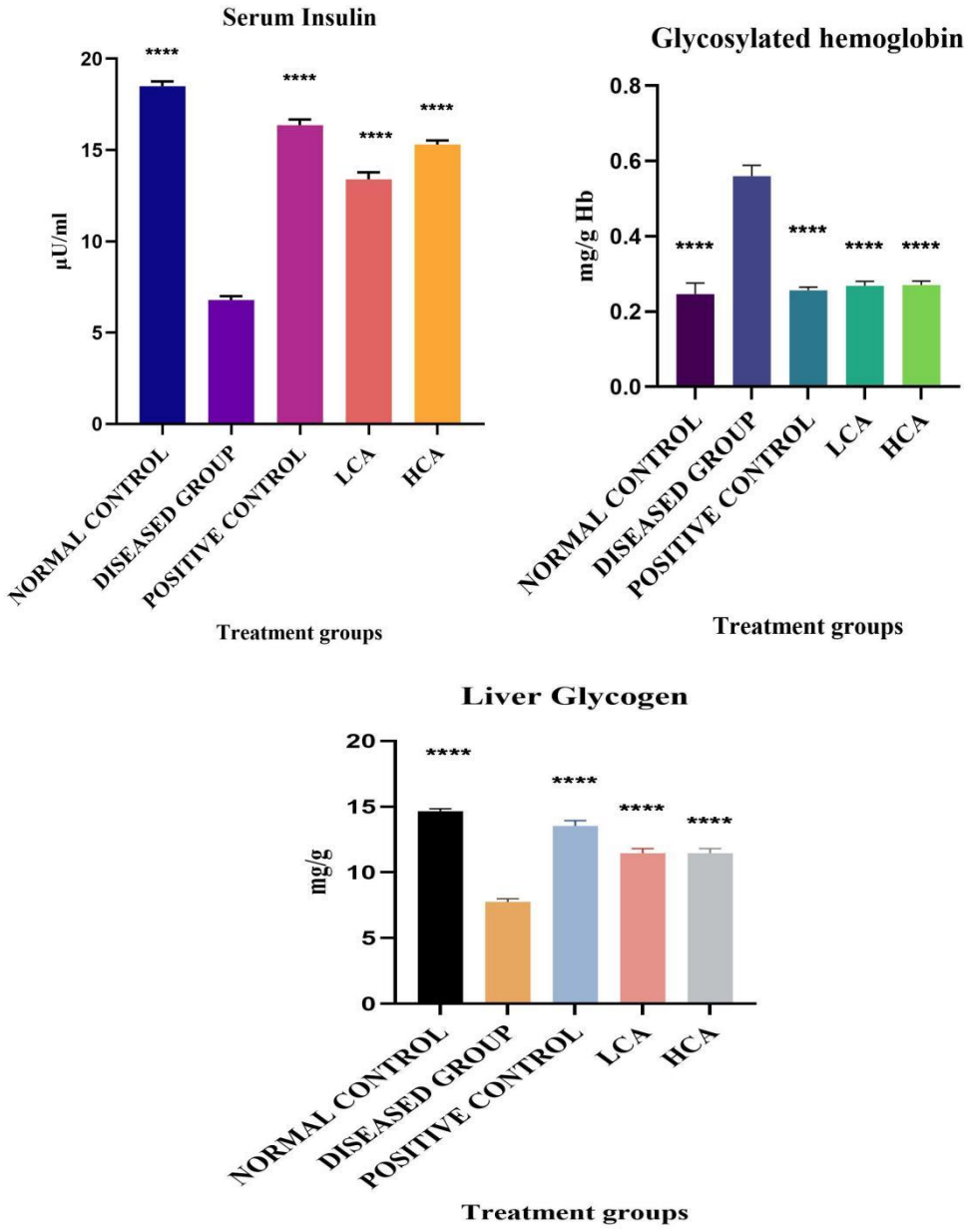

Fig 1: Effect of EACAR on serum insulin, glycosylated hemoglobin and liver glycogen

All were **** p<0.0001, values are mean±SEM, n=6, when compared with diseased control by using one way ANOVA followed by Tukey's multiple comparison test.

Table 4: Effect of ECAR on serum insulin, glycosylated hemoglobin and liver glycogen

Treatment group where n=6	Serum Insulin µU/ml	Glycosylated hemoglobin mg/g Hb	Liver Glycogen mg/g
I Normal control	18.49±0.26	0.24±0.02	14.65±0.19
II Diseased control	6.78±0.21	0.56±0.28	7.75±0.24

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III Positive control	16.36±0.31	0.25±0.008	13.53±0.41
IV LCA	13.4±0.37	0.26±0.01	11.45±0.35
V HCA	15.3±0.22	0.27±0.01	12.4±0.28

All were **** p<0.0001, values are mean±SEM, n=6, when compared with diseased control by using one way ANOVA followed by Tukey's multiple comparison test.

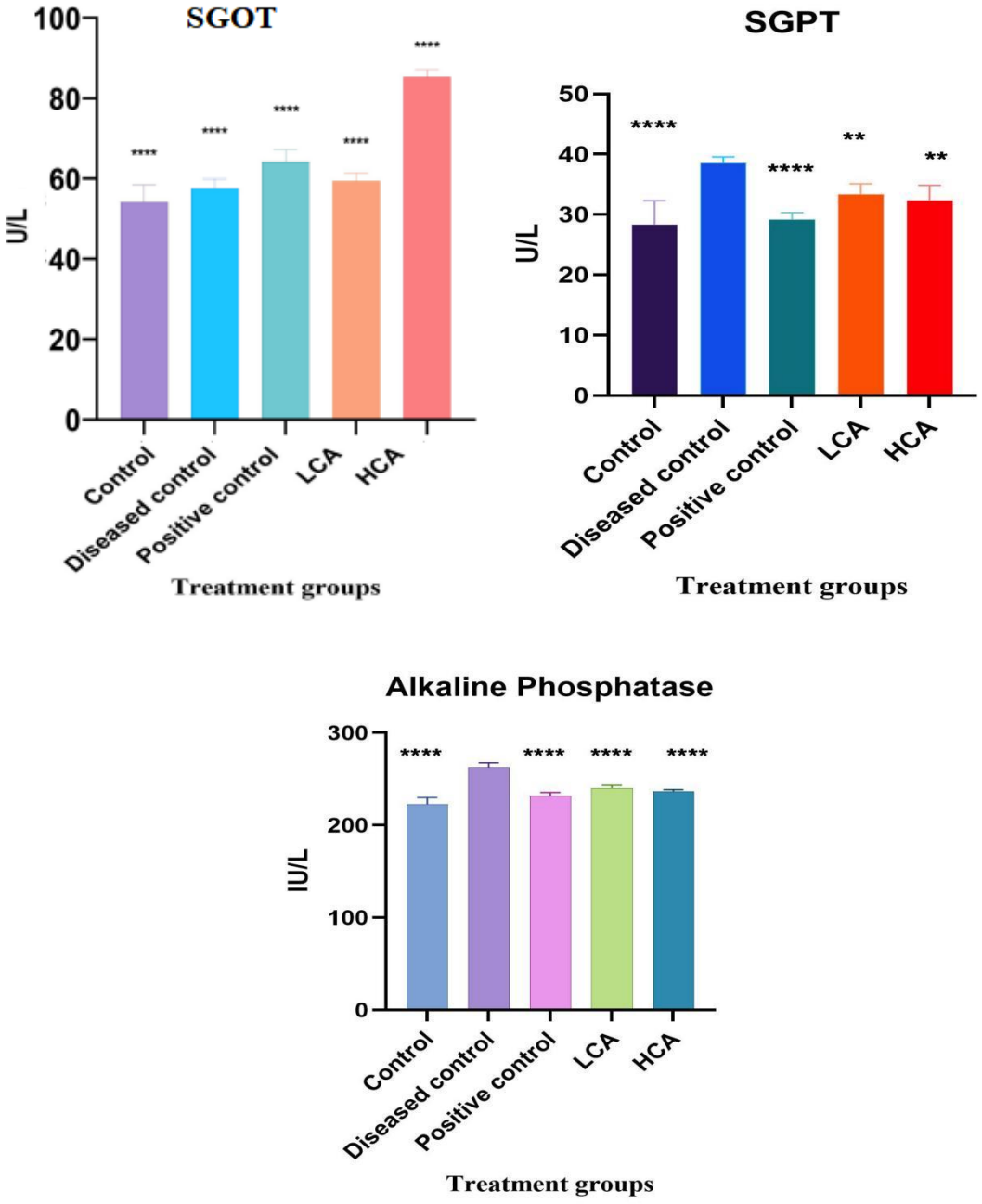

Fig2: Effect of EACAR on SGPT, SGPT and ALP in STZ-induced diabetic rat

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$^{****}p<0.0001$, $^{**}p<0.005$ Values are mean±SEM, n=6, when compared with diseased control by using one way ANOVA followed by Tukey's multiple comparison test.

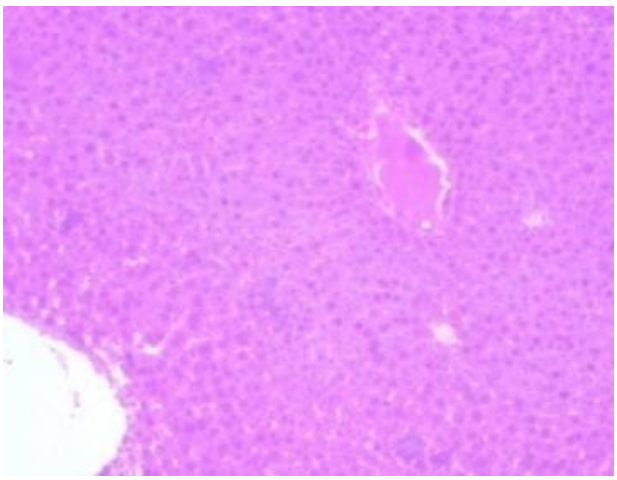
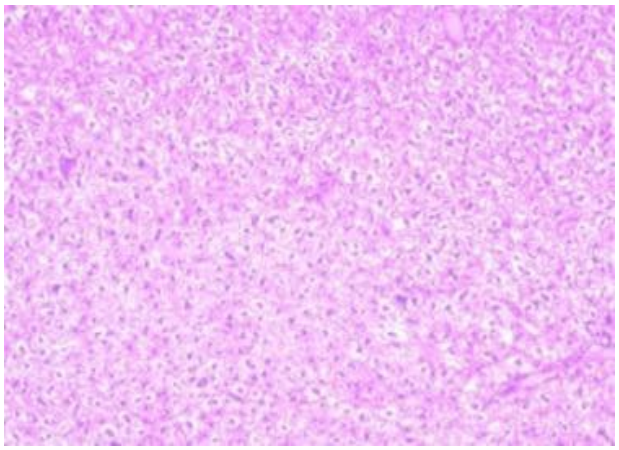


Table 5: Effect of EACAR on SGPT, SGPT and ALP in STZ-induced diabetic rat

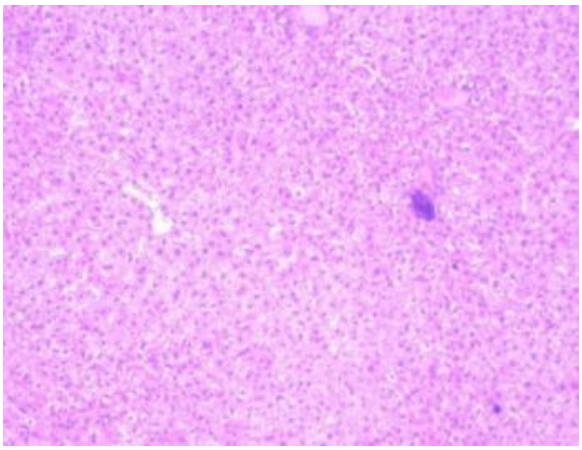
Groups	SGOT (U/L)	SGPT (U/L)	Alkaline Phosphate(IU/L)
Normal	54.16±4.22	28.33±3.98	222.66±7.26
Diseased	85.33±1.75	38.5±1.05	262.5±5.17
STD	57.5±2.43	29.166±1.17****	231.5±3.94****
LCA	64.16±2.93****	33.33±1.75**	240±3.10****
HCA	59.33±2.07****	31±2.50**	236.83±1.83****

$^{****}p<0.0001$, $^{**}p<0.005$ Values are mean±SEM, n=6, when compared with diseased control by using one way ANOVA followed by Tukey's multiple comparison test.

Normal group



Diseased group



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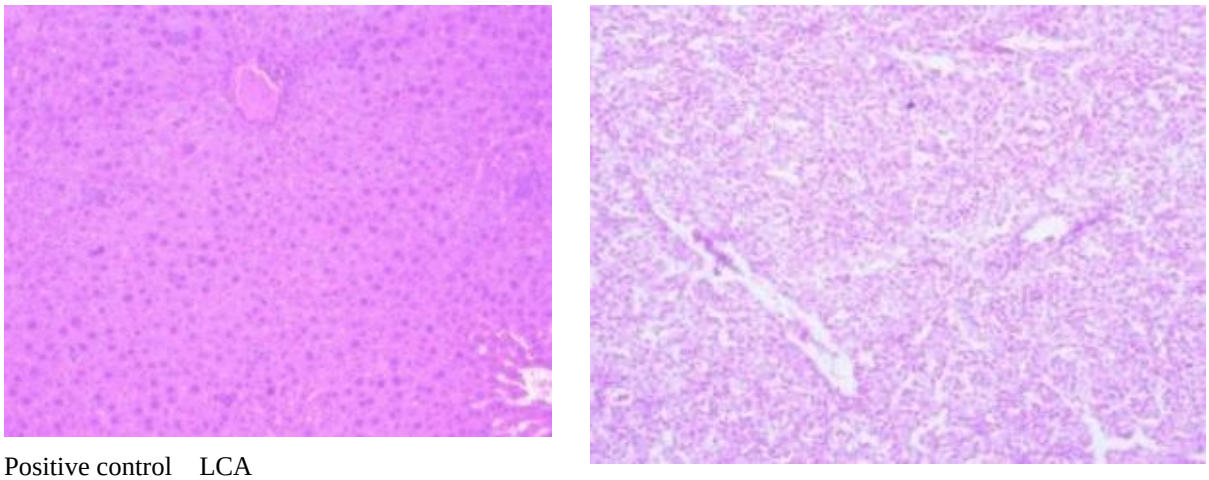

Positive control LCA HCA

Fig 6: Histopathology of liver demonstrating effect of ECAR in STZ-induced diabetic rat

4. Discussion

The study aimed to assess the anti-diabetic potential of the ECAR. in normal, glucose-loaded hyperglycemic, and STZ-induced diabetic rats. Throughout the study period, no lethality or toxic reactions were observed with the selected doses of the extract.

Results indicated that the aqueous extract at a dose of 500 mg/kg (HCA) exhibited significant hypoglycemic activity. It improved glucose tolerance in normoglycemic rats and reduced blood glucose levels in STZ-induced diabetic rats. The difference between initial and final fasting plasma glucose levels was statistically significant ($p<0.0001$) in the aqueous extract and metformin treated groups.

In diabetic rats induced by STZ, body weight loss typically occurs due to increased muscle wasting and tissue protein loss (Ewenighi et al., 2015). However, after 21 days of treatment with the ECAR, diabetic rats showed a gain in body weight comparable to that of the standard drug metformin, indicating the potential efficacy of the extract in improving overall health.

Furthermore, the ECAR significantly increased serum insulin levels and decreased triglycerides, total cholesterol, LDL, and VLDL levels, while increasing HDL cholesterol levels in treated diabetic rats. This suggests a beneficial effect on lipid metabolism. Cholesterol (CHL) is a crucial lipid involved in various physiological processes, but elevated levels are associated with an increased risk of cardiovascular diseases (Al-Attar, 2010).The significant reductions of cholesterol levels suggest that the ECAR may have cholesterol-lowering properties. Low-Density Lipoprotein (LDL) is commonly referred to as "bad" cholesterol due to its role in atherosclerosis development. Very Low-Density Lipoproteins (VLDL) are precursor particles to LDL and are associated with increased cardiovascular risk when elevated. The substantial decreases of these parameters in the ECAR treated groups suggest a favorable effect on lipid metabolism.Triglycerides are another important lipid marker, and elevated levels are associated with increased cardiovascular risk.The results suggests that the ECAR may help in lowering triglyceride levels, further contributing to cardiovascular health.Overall, the findings suggest that the ECAR possesses potential lipid-lowering effects, which may be beneficial for individuals with dyslipidemia and those at risk of cardiovascular diseases (Konda et al., 2017; Molehin et al., 2018; Singh et al., 2018)

The mechanism of action of the ECAR may involve increasing pancreatic insulin secretion from existing beta cells or releasing insulin from its bound form. Moreover, the extract significantly decreased glycosylated hemoglobin levels, indicating an improvement in insulin secretion. This increase suggests that the extract may have stimulated insulin production or improved insulin sensitivity in the treated animals. Higher insulin levels are indicative of better glucose regulation and metabolism. Conversely, glycosylated hemoglobin levels increased significantly in the untreated diabetic control group. Glycosylated hemoglobin is a marker of long-term blood glucose control, with higher levels indicating poorer glycemic control over time. The decrease in HbA1c levels suggests improved long-term glucose regulation in the treated animals.A decrease in HbA1c levels, as observed in

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the treated animals in the study, indicates an improvement in long-term glucose regulation. This improvement suggests that the treatment, in this case, the administration of the ECAR, has effectively helped in maintaining blood glucose levels within the target range over the duration of the study period (Almalki et al., 2019).

Additionally, a significant increase in glycogen levels was observed in diabetic rats treated with the ethanol extarct of *Curcuma angustifolia* Roxb. Rhizome (ECAR), further indicating its potential in improving glucose metabolism. Liver glycogen serves as a storage form of glucose and plays a crucial role in maintaining blood glucose levels within a normal range. An increase in liver glycogen suggests improved glycemic control and enhanced glucose storage capacity in the liver. No significant effects were observed in normal animals.

Overall, these findings suggest that the ethanol extract of *Curcuma angustifolia* Roxb. Rhizome possesses significant anti-diabetic properties, potentially through multiple mechanisms, including improving insulin secretion, lipid metabolism, and glycogen storage.

5. Conclusion

In summary, the ethanol extract of *Curcuma angustifolia* Roxb. Rhizome demonstrates promising effects in lowering elevated blood glucose levels and improving the lipid profile in STZ-induced diabetic rats. However, it does not exert any significant effects on normal rats. These findings align with assertions made in Ayurvedic classics, thereby providing support for the traditional medicinal use of Sesbania sesban in managing diabetes and associated metabolic disorders.

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7. Conflict of Interest

The authors declare that there is no conflict of interest.

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