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Original Research Article

Anti-diabetic effect of *Eucalyptus camaldulensis* (Red gum) leaf-supplemented diet in streptozotocin-induced diabetic rats

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Abstract

Purpose: *To investigate the potential anti-diabetic effect of supplementing a diet with Eucalyptus camaldulensis leaves in streptozotocin (STZ) induced Wistar rats.*
Methods: *Methanolic extract of E. camaldulensis was screened using gas chromatography-mass spectrometric analysis. After that, eighteen animals were separated into three groups: Group A served as control group. Group B, the diabetic control group, was induced with STZ, and Group C was induced with STZ and fed a 10 % E. camaldulensis leaf-supplemented diet for 14 days. Thereafter, rats were sacrificed, and fasting blood was collected for serum glucose, enzyme and protein activity tests. Organs were excised for biochemical and histological analysis.*
Results: *The GC-MS fingerprint identified 17 constituents, with 2-hydroxy carbazole being the most abundant. Rats on the E. camaldulensis leaf-based diet for 14 days exhibited a significant decrease ($p < 0.05$) in serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, albumin, total bilirubin, urea, and creatinine concentrations compared to diabetic controls. Insulin levels significantly increased ($p < 0.05$). Additionally, the E. camaldulensis diet led to a significant decrease in serum lipid profile levels but increased high-density lipoprotein cholesterol (HDL-C). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities increased significantly, while malondialdehyde levels decreased significantly ($p < 0.05$). Histopathology revealed positive effects on hepatocytes, acini, pancreatic duodenal lymphoid activation, islets of Langerhans resurgence, and normal kidneys.*
Conclusion: *Eucalyptus camaldulensis leaf-supplemented diet demonstrates anti-diabetic, hypolipidemic and antioxidant enzyme activities. Carbazole identified by GC-MS may be the potential anti-diabetic and hypolipidemic agent in E. camaldulensis leaf-based diet.*

Keywords: *Eucalyptus camaldulensis, Diabetes mellitus, Anti-diabetic, Insulin, α-amylase*

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INTRODUCTION

Diabetes mellitus (DM) has emerged as a formidable global public health challenge in the 21st century, with an estimated 425 million people affected worldwide a figure projected to escalate to 629 million by 2045 [1]. The burden of this condition is prevalent in low and middle-income countries, which account for approximately 80 % of all diabetes cases [2].

Sub-Saharan Africa alone currently harbors around 12 million individuals with diabetes, and projections indicate a surge to 18 million by 2030, solidifying the region as one experiencing one of the world's fastest-growing rates of diabetes mellitus [3]. High blood sugar levels characterize diabetes. It can cause consequences, including frequent urination, blindness, renal failure, and nerve damage that can result in amputation, heart disease, and reduced blood supply to the limbs [4]. In addition to being irreversible, these issues place a heavy financial strain on healthcare systems, particularly in underdeveloped countries. This public health crisis's silent yet impending nature underscores the potential challenges it may pose to healthcare systems and economies soon.

Diabetes is linked to a metabolic disorder involving an imbalance between antioxidants and free radicals. Free radicals are generated during normal cell metabolism and may cause oxidative damage to DNA, proteins, carbohydrates, and lipids. This damage is typically countered by protective antioxidants, an imbalance where free radicals overwhelm antioxidant defenses, which can result in widespread pathological consequences, including cell death [5]. Despite advancements in diabetes treatment, existing synthetic drugs have limitations and adverse effects, prompting ongoing efforts to discover new and improved therapeutic options [6]. In this context, natural remedies, mainly extracts from medicinal plants, have gained attention for their safety and efficacy. One such plant is *Eucalyptus camaldulensis*, commonly referred to as river red gum. Eucalyptus serves various purposes, including windbreak, aesthetics, and diverse applications such as timber, firewood, and malaria treatment with aqueous leaf extracts [7].

Extracts and fractions from *E. camaldulensis* leaves have shown hypoglycemic effect in rats, making it a promising candidate for further investigation in diabetes treatment [8]. This study sought to investigate the anti-diabetic effect of *E. camaldulensis* leaf-supplemented diet in streptozotocin-induced diabetes in Wistar rats, contributing valuable insights to the pursuit of alternative and efficacious diabetes treatments.

EXPERIMENTAL

Reagents

Analytical grade streptozotocin, tetraoxosulphate (VI) acid, hydrochloric acid, sodium chloride, sodium hydroxide, epinephrine, chromate, glutathione, and tri-sodium citrate were gotten from Sigma Aldrich (USA). Every chemical and reagent used was of analytical quality, and it was purchased from reputable commercial vendors.

Collection and Identification of *Eucalyptus camadulensis* leaf

E. camadulensis leaves were collected from the botanical garden of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. *E. camadulensis* leaf with voucher number UBH-E466 was identified by Dr. EI Aigbokhan, a taxonomist in the same Department. The plant was authenticated by Prof C Kalu in the Department of Forestry and Wild Life, Faculty of Agriculture, University of Benin, Benin City, Nigeria.

Preparation of *Eucalyptus camaldulensis* leaf

Eucalyptus camaldulensis leaves were harvested, sorted, and washed with clean water. Leaves were then air-dried for four (4) weeks at room temperature until a consistent weight was achieved. Upon completion of the drying process, the leaves were finely powdered, and the powder was pelleted and formulated into a diet (Table 1).

Preparation of methanol extract of *Eucalyptus camaldulensis* leaf

A 1,400 g of pulverized *E. camaldulensis* leave was macerated with 2,500 mL of methanol while being continuously stirred for a period of 72 h. The filtrate was freeze-dried and concentrated via rotary evaporation. The resulting extract was analyzed using gas chromatography-mass spectrometry (GC-MS; Agilent Technologies, Santa Clara, CA, USA).

Table 1: Formulation of normal and *Eucalyptus camaldulensis* leaf-based test diet

Constituent	Normal diet	Test diet
Corn starch	65.5	55.5
Casein	14.0	14.0
Sucrose	10.0	10.0
Fiber	5.0	5.0
Soybean oil	3.5	3.5
Premix vitamin	1.0	1.0
Minerals mix	1.0	1.0
E. camaldulensis	-	10.0
Total	100 g	100 g

Animals

Eighteen male Wistar rats (8 - 9 weeks old) were exposed to a controlled environmental temperature (28 ± 2 ^{o}C) with 12 h light/dark cycle. The animals were allowed to acclimatize

with the normal formulated diet for seven (7) days and water was given *ad libitum.* All animals in this study were handled according to the international natural and institutional guidelines for the care and use of laboratory animals in biomedical research. The institutional Ethical Review Committee of the Faculty of Life Sciences, University of Benin, Nigeria, approved the study (approval no. LS20014).

Induction of diabetes mellitus and group treatment

The approach reported by Uadia *et al* [9] was used to induce diabetic mellitus. Briefly, the rats were divided into three groups of six rats each. The three groups were normal group (group 1), diabetic control group (group 2) and diabetic treated group (group 3). Diabetes mellitus was induced in rats of group 2 and group 3 following an overnight fast by a single intraperitoneal injection of streptozotocin (STZ) at a single dose of 50 mg/kg body weight. Glucose solution (10 %) was given to STZ-induced rats which was withdrawn after 24 h.

Seventy-two hours (day 1) after STZ administration, the overnight fasted rats had their blood glucose level measured. Those rats in group 3 with blood glucose levels above 200 mg/dL were switched from the normal diet to 10 % *E. camadulensis* supplemented diet and fed for 14 days. Fasting blood glucose levels were monitored on days 1, 3, 7, and 14 using a glucometer (Accu-Chek Active, Roche, Indiana). Thereafter, following overnight fast, animals were sedated with chloroform and sacrificed, blood was collected from the tail vein, and biochemical parameters were analyzed. Additionally, the rats' pancreas, liver, and kidney were preserved in 10 % buffered formalin for subsequent histopathological analysis.

Biochemical analysis

Lipid profile and fasting blood glucose

Lipid profile and fasting blood glucose concentration were assessed with Randox kits. The calculation of low-density lipoprotein and very-low-density lipoprotein cholesterol (VLDL-C) was based on the method described by Friedewald *et al* [10].

Liver function tests

Liver function tests, including alanine transaminase (ALT) and aspartate transaminase (AST), were conducted using the procedure outlined by Reitman and Frankel [11].

Total protein levels

Total protein levels were determined following the method described by Tietz [12], and albumin and total bilirubin levels were assessed using the method of Doumas *et al* [13].

Antioxidant enzymes

Antioxidant enzymes, encompassing superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA), were determined using the methods outlined by Misra and Fridovich [14], Cohen *et al* [15], Ellman [16], and Buege and Aust [17], respectively.

Kidney function tests

Kidney function tests were carried out by determining the levels of creatinine and urea following earlier described methods [18,19], while serum insulin levels were estimated using an ELISA kit (Calibiotech, USA), while serum amylase was determined using Agappe amylase diagnostic kit (Switzerland).

Histopathology studies

The collected pancreas, liver, and kidney were separated from external fasciae and fixed in 10 % buffered formalin, following the protocol outlined by Bancroft and Layton [20].

Data analysis

Statistical analysis of the results was done by one-way analysis of variance (ANOVA) using IBM-SPSS version 21.0. Duncan's comparison test was employed to determine the significant difference between means. A significance level of $p < 0.05$ was set.

RESULTS

Effect of *Eucalyptus camaldulensis* leaf-based diet on fasting blood glucose

The effect of *E. camaldulensis* leaf-based diet on fasting blood glucose is presented in Figure 1. There was a significant increase in blood glucose levels in the diabetic control while a significant decrease was observed in rats fed with a 10 % *E. camaldulensis* leaf-based diet (diabetic treated). Furthermore, the glucose levels in the diabetic control group remain significantly increased ($p < 0.05$) compared to normal control and diabetic-treated groups, even after fourteen days of the feeding period. This indicates the sustained impact of the *E. camaldulensis* leaf-

based diet in mitigating the elevated blood glucose levels associated with streptozotocin-induced diabetes in rats.

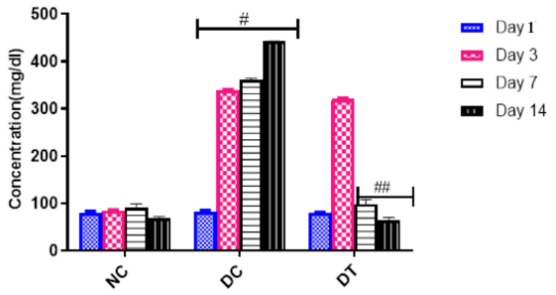

Figure 1: Effect of *Eucalyptus camaldulensis* leaf-based diet on Fasting Blood glucose in Streptozotocin-Induced Diabetic Rats. ***Key*:** Data are expressed as mean ± SEM, n=6. $^{\#}P < 0.05$ vs. normal control (NC); $^{\#\#}p < 0.05$ vs. diabetic control (DC). DT- Diabetic treated

Effect of *Eucalyptus camaldulensis* leaf based-diet on antioxidant activities

The effect of *E. camaldulensis* leaf on antioxidant enzyme activities is depicted in Figure 2. The findings indicate a substantial decline in superoxide dismutase (SOD) and catalase levels in rats challenged with STZ only (diabetic control) compared to normal control group. Conversely, the treated group showed an increase in SOD and catalase activities. Rats induced with STZ only were observed to be considerably lower in glutathione peroxidase (GPx) compared to normal control ($p < 0.05$). Following a 14-day treatment period, diabetic-treated group exhibited a statistically higher GPx level than the diabetic control groups ($p < 0.05$). Furthermore, the malondialdehyde (MDA) levels were increased in rats induced with streptozotocin. Nevertheless, after a 14-day treatment duration, rats consuming an *E. camaldulensis* leaf-based diet exhibited significantly reduced ($p < 0.05$) MDA levels in contrast to the challenged group (Figure 3).

Effect of *Eucalyptus camaldulensis* leaf-based diet on serum lipid profile

Figure 4 illustrates the lipid profile alterations. The *E. camaldulensis* leaf-based diet reduced triglycerides and total cholesterol. Also, the result showed a significant decrease in the high-density lipoprotein cholesterol (HDL-C) and an increase in the low-density lipoprotein cholesterol (LDL-C) and VLDL-C levels in diabetic rats (diabetic control group) compared to normal control.

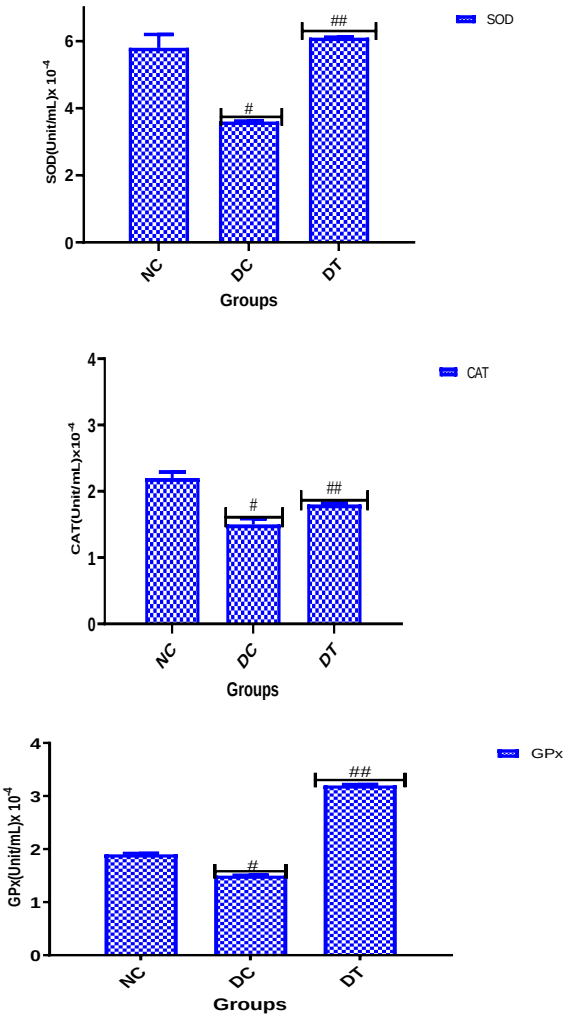

Figure 2: Effect of *Eucalyptus camaldulensis* Leaf-Based Diet on (A) Superoxide dismutase (SOD); (B) Catalase (CAT); and (C) Glutathione peroxidase (GPx) Activities in Streptozotocin-Induced Diabetic Rats. ***Key:*** N=6; $^{\#}P < 0.05$ vs. normal control (NC); $^{\#\#}P < 0.05$ vs. diabetic control (DC). Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GPx); DT- Diabetic treated

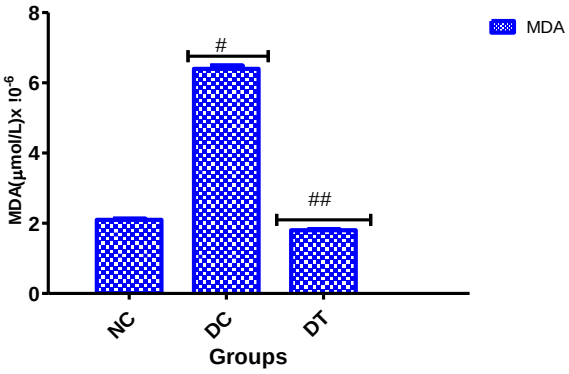

Figure 3: Effect of *Eucalyptus camaldulensis* Leaf-Based Diet on Malondialdehyde Levels in Streptozotocin-Induced Diabetic Rats. ***Key:*** N=6. $^{\#}P < 0.05$ vs. normal control (NC); $^{\#\#}P < 0.05$ vs. diabetic control (DC). DT- Diabetic treated

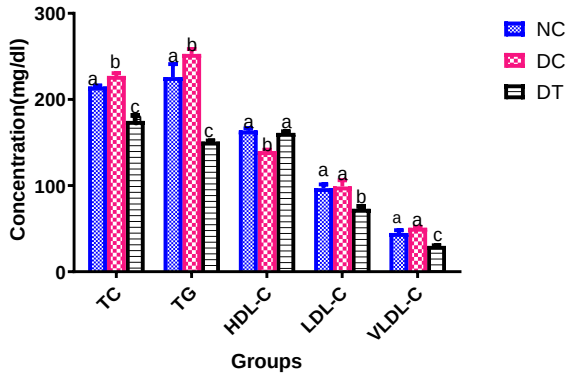

Figure 4: Effect of *Eucalyptus camaldulensis* leaf-based diet on serum lipid profile of streptozotocin-induced diabetic rats. Values with the same alphabets in different treatment groups are statistically significant ($p < 0.05$) while value with the same alphabets in the same treatment groups are not statistically significant ($p > 0.05$). TC-Total cholesterol; TG-Triglycerides; HDL-C-High density lipoprotein cholesterol; LDL-C-Low density lipoprotein cholesterol; VLDL-C- Very low-density lipoprotein; NC- Normal control; DC-Diabetic control; DT-Diabetic treated

Effect of *Eucalyptus camaldulensis* leaf-based diet on serum liver function test

Figure 5 illustrates the effect of an *E. camaldulensis* leaf-based diet on serum liver function tests. Rats in the diabetic control group had significantly elevated aspartate transaminase (AST), alanine transaminase (ALT) and total protein levels compared to normal control group. However, after a fourteen-day treatment period, diabetic-treated group exhibited statistical insignificance ($p > 0.05$) compared to normal control group and a significant reduction compared to the diabetic control group ($p < 0.05$). The findings emphasize the possible liver-protective effects of the diet including *E. camaldulensis* leaves in rats with diabetes induced by streptozotocin.

Effect of *Eucalyptus camaldulensis* leaf-based diet on serum kidney function

Figure 6 illustrates the impact of a diet enriched with *E. camaldulensis* leaves on kidney function. Our study revealed a substantial increase in creatinine levels and urea concentration in the diabetic control group. Following a treatment period of fourteen days, the diabetic-treated group had a significant reduction ($p < 0.05$) in these levels. The results highlight the potential of the *E. camaldulensis* leaf-supplemented diet to protect the kidneys in diabetic rats induced with streptozotocin.

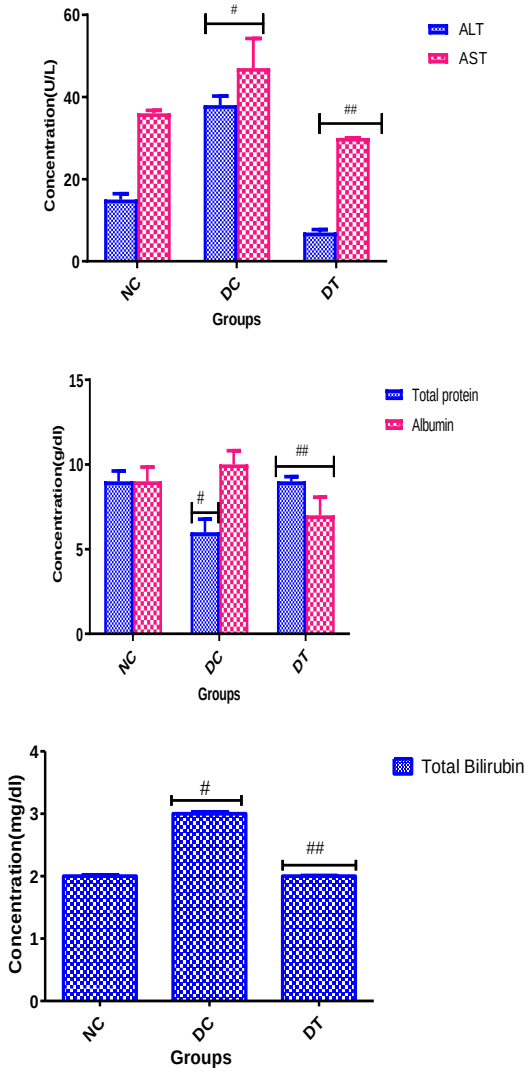

Figure 5: Effect of *Eucalyptus camaldulensis* Leaf-Based Diet on Serum Liver Function Test of Streptozotocin-Induced Diabetic Rats. ***Key:*** n=6. $^{\#}P < 0.05$ vs. normal control (NC); $^{\#\#}P < 0.05$ vs. diabetic control (DC). AST-Aspartate aminotransferase; ALT-Alanine aminotransferase; DT- Diabetic treated

Effect of *Eucalyptus camaldulensis* leaf-based diet on serum insulin and α-amylase

Figure 7 illustrates the effect of an *E. camaldulensis* leaf-based diet on serum insulin and alpha-amylase activity. The findings revealed a significant increase ($p < 0.05$) in amylase levels in the diabetic control group. After Fourteen days post-treatment, diabetic-treated group exhibited a markedly lowered amylase level. Furthermore, the insulin level in the diabetic control group was significantly lower compared to normal control group ($p < 0.05$). However, after a fourteen-day treatment, diabetic-treated group demonstrated a significantly higher insulin level ($p < 0.05$) than the diabetic control group. These outcomes

suggest the potential of the *E. camaldulensis* leaf-based diet in modulating alpha-amylase activity and insulin levels.

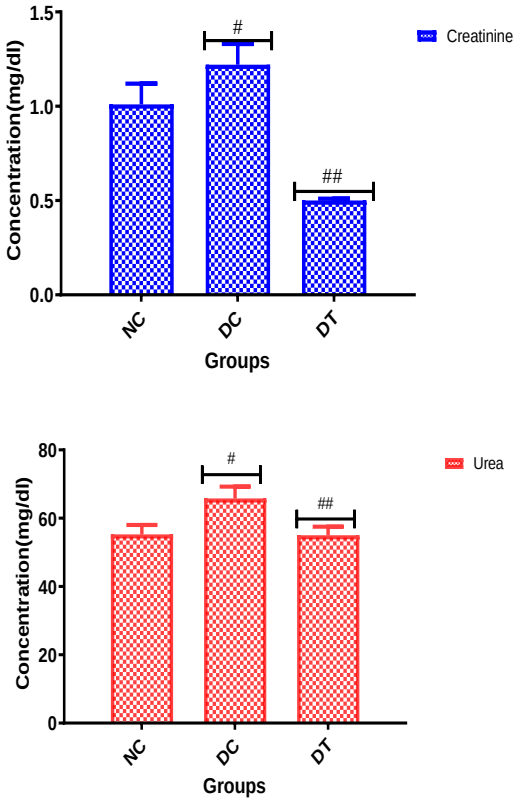

Figure 6: Effect of *Eucalyptus camaldulensis* leaf-based diet on serum kidney function of streptozotocin-induced diabetic rats. ***Key:*** Data are expressed as mean ± SEM, n=6. $^{\#}P < 0.05$ vs. normal control (NC); $^{\#\#}P < 0.05$ vs. diabetic control (DC); DT- Diabetic treated

Histopathology

The photomicrographs below elucidate the comparison between streptozotocin-induced diabetic rats and diabetic rats fed a formulated *E. camaldulensis* leaf-based diet. The liver in the diabetic control group exhibited severe vascular ulceration, congestion, and periportal inflammation of the hepatocytes. Conversely, diabetic-treated group displayed normal hepatocytes and maintained the liver's typical ductal and vascular architecture compared to normal control. This reveals that the *E. camaldulensis-supplemented* diet possesses anti-inflammatory properties and has a commendable reparative effect on the organ, as depicted in Figure 8. The histopathological investigation of the kidney in the diabetic control rats revealed severe vascular distortion and congestion of the glomerulus following streptozotocin-induced diabetes.

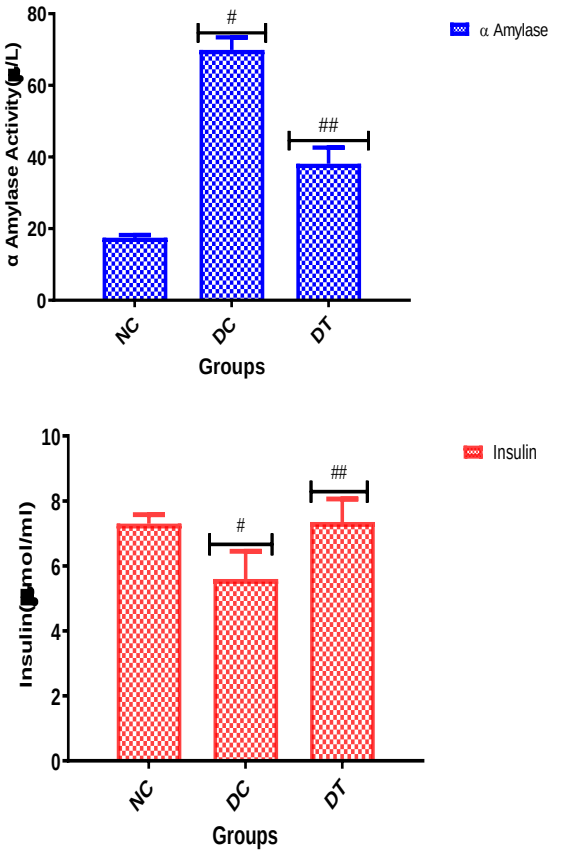

Figure 7: Effect of *Eucalyptus camaldulensis* leaf-Based Diet on Serum Insulin and Alpha-Amylase Activity of Streptozotocin-Induced Diabetic Rats. ***Key:*** Data are expressed as mean ± SEM, n=6. $^{\#}P < 0.05$ vs. normal control (NC); $^{\#\#}P < 0.05$ vs. diabetic control (DC); DT- Diabetic treated

However, after a fourteen-day treatment with the *E. camaldulensis* leaf-based diet, the kidney tissue exhibited normal tubular, vascular, and glomerular architecture This underscores the potential of the *E. camaldulensis* leaf-based diet in reversing kidney damage, as illustrated in Figure 9. The pancreas in the diabetic control group exhibited ductal proliferation, severe inflammatory infiltrates, vascular distortion, congestion of acini, and a diminished presence of Langerhans islets. In contrast, diabetic-treated group, compared to the diabetic control and normal control groups, demonstrated normal acini and a resurgence of the Langerhans islets. Additionally, there was activation of pancreaticoduodenal lymphoid, as depicted in Figure 10. These suggest the possible therapeutic impact of the *E. camaldulensis* leaf-based diet on pancreatic tissue in streptozotocin-induced diabetic rats.

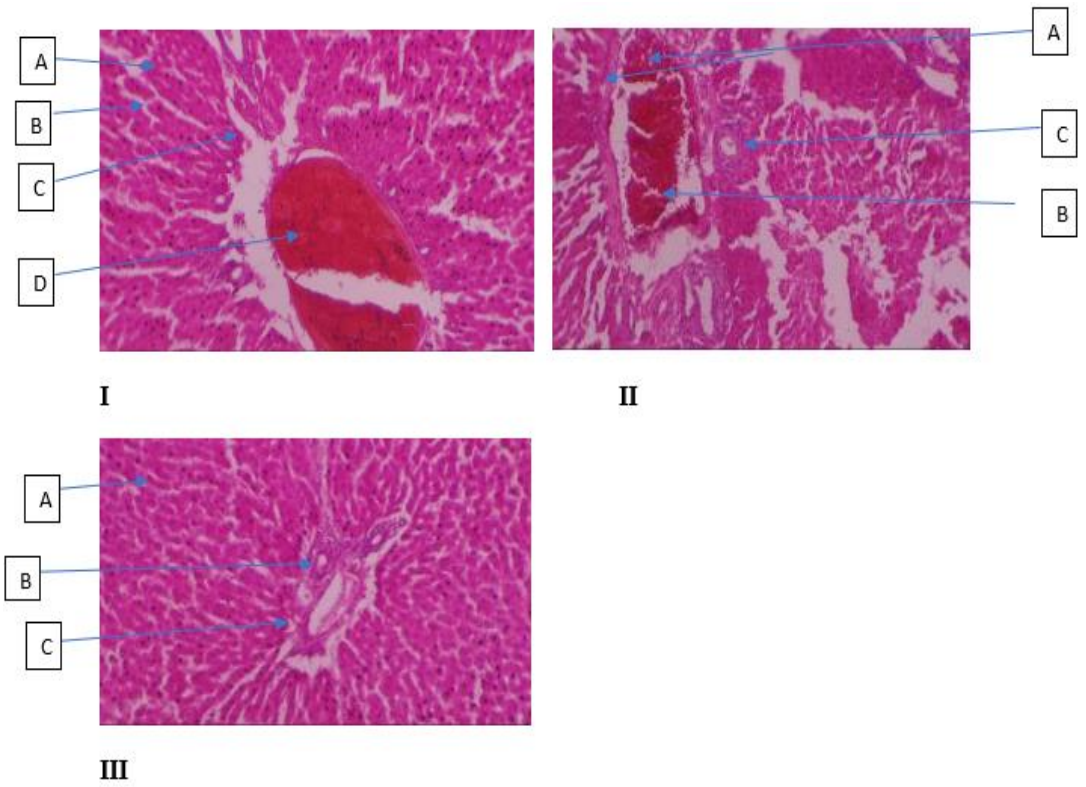

Figure 8: I; Liver of normal control, showing. A: hepatocytes, B: sinusoids, C: hepatic artery and D: portal vein. **II**; Liver of Diabetic control, showing A: severe vascular ulceration, B: congestion and C: Periportal inflammatory infiltrates. **III**; Liver of the Diabetic treated, showing A: normal hepatocytes, B: normal ductal and C: vascular architecture (H&E x100).

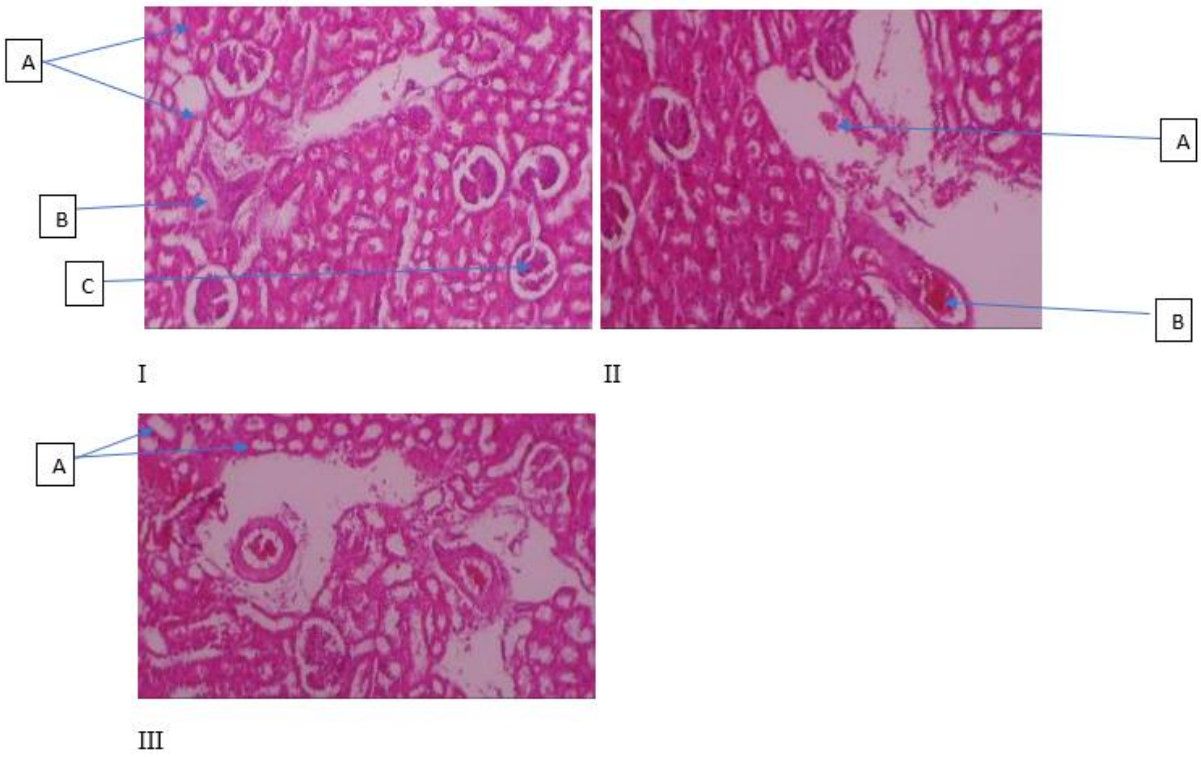

Figure 9: I; Kidney of normal Control, showing A: tubules, B: interstitial space and C: glomeruli, **II**; Kidney of the Diabetic control, showing A: severe vascular distortion and B: congestion, **III**; Kidney of the Diabetic treated, showing A: normal tubular, B: vascular and C: glomerular Architecture (H&E x100)

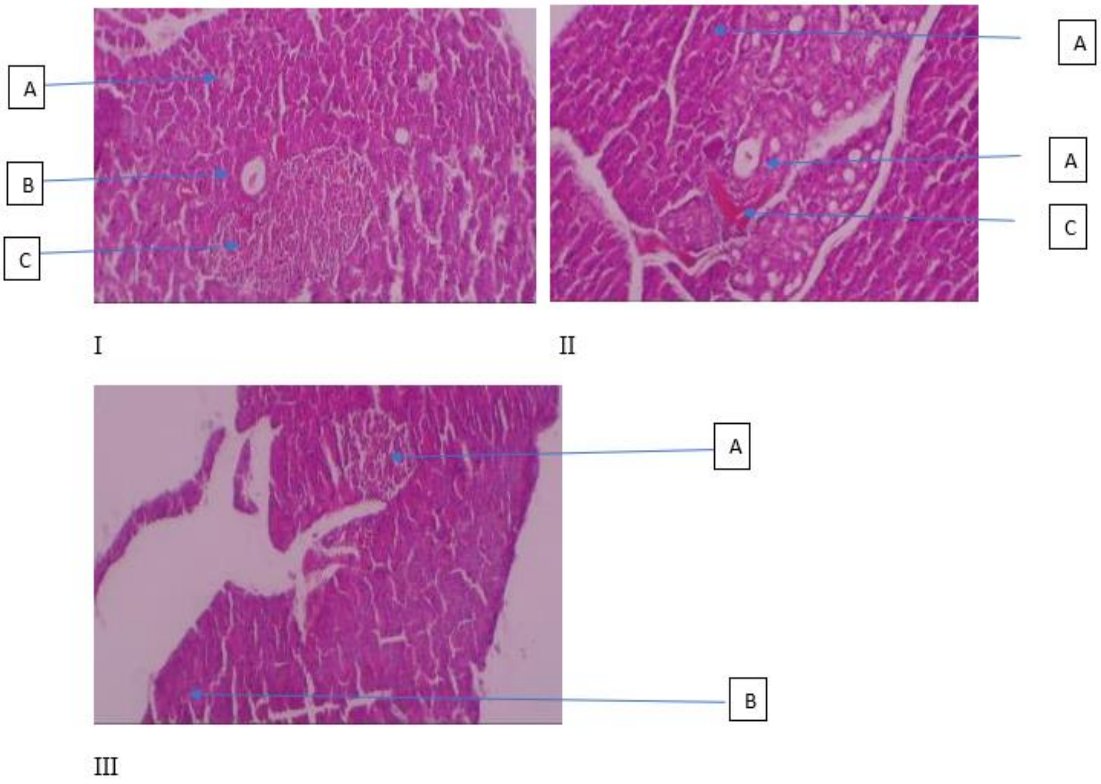

Figure 10: I; Pancreas of normal Control, showing A: acini, B: interlobar duct and C: islets of Langerhans; **II**; Pancreas of the Diabetic control, showing A: ductal proliferation, B: heavy inflammatory infiltrates C: vascular distortion and congestion, **III**; Diabetic treated, showing A: resurgent islets of Langerhans and B: normal Acini (H&E x100)

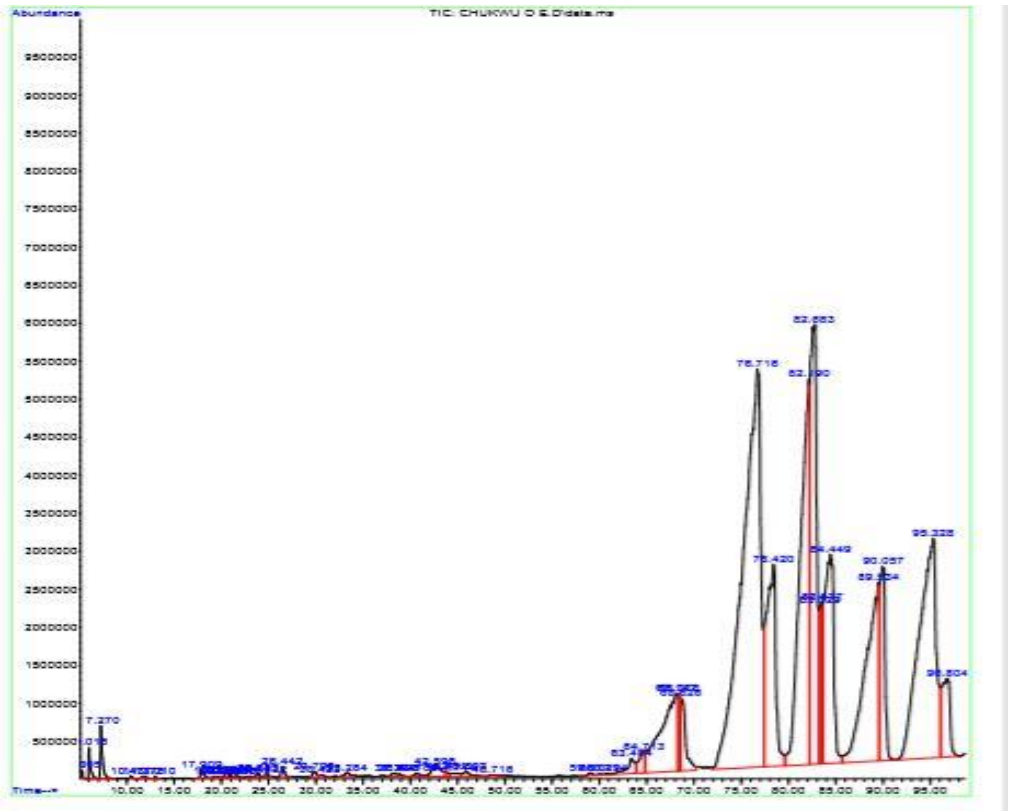

Figure 11: TIC of Methanol Extract of *Eucalyptus camadulensis* Leaf

Table 2: Compounds present in the methanolic extract of *Eucalyptus camaldulensis* leaf

Name	Retention Time (RT)	Area (%)	Molecular weight (g)	Chemical Formula
5-Isothiazolecarboxamide	6.001	0.21	128.2	$C_4H_4N_2OS$
1,4-Naphthalenediol	23.868	0.07	160.2	$C_{10}H_8O_2$
Stilbene	26.456	0.15	180.3	$C_{14}H_{12}$
trimethoxy 3,3'-Diaminodiphenylmethane	30.415	0.07	193.3	$C_{13}H_{14}N_2$
Naphthalene	38.384	0.08	128.2	$C_{10}H_8$
Benzotriazol-4-one	38.841	0.06	133.1	$C_6H_3N_3O$
4H-1-Benzopyran-4-one	42.597	0.42	432.4	$C_{21}H_{20}O_{10}$
4-Isothiazolecarboxamide	44.729	0.08	128.2	$C_4H_4N_2OS$
Coumarin-6-ol	48.739	0.07	237.2	$C_{11}H_{11}NO_5$
5-Formylamino-1H-imidazole-4-carboxylic acid	49.957	0.06	230.2	$C_{11}H_{10}N_4O_2$
6-Methoxy-2-naphthonitrile	63.408	0.41	183.2	$C_{12}H_9NO$
Pyrido[2,3-b] indole	68.128	4.56	168.2	$C_{11}H_8N_2$
Phenyl 4-pyridyl ketone	78.432	6.73	183.2	$C_{12}H_9NO$
Quinoline-3-carbonitrile	82.188	11.90	154.2	$C_{10}H_6N_2$
1-Naphthalenecarbonitrile	89.548	8.12	153.2	$C_{11}H_7N$
2-Amino-1-acenaphthenone	90.056	3.70	183.2	$C_{12}H_9NO$
2-Hydroxycarbazole	95.335	13.14	183.2	$C_{12}H_9NO$

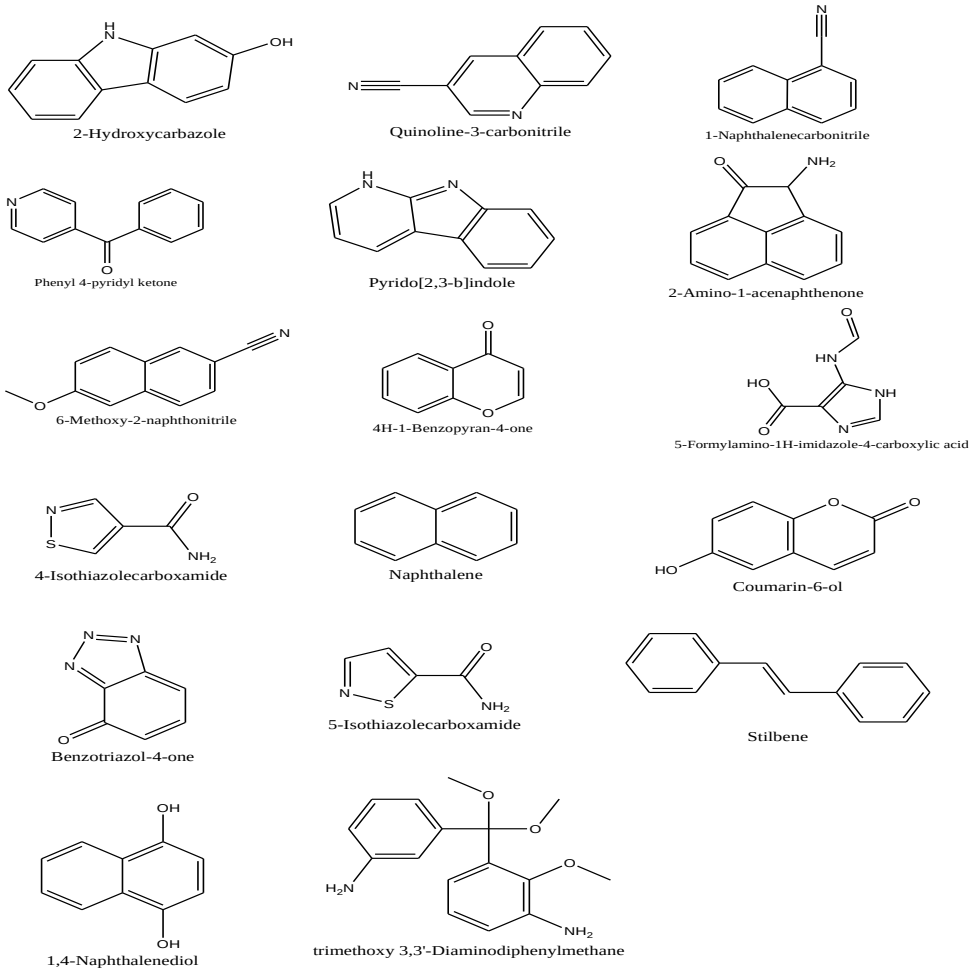

Figure 12: Structure of compounds identified from methanolic extract of *Eucalyptus camadulensis* Leaf. (1) 2-Hydroxycarbazole; (2) Quinoline-3-carbonitrile; (3) 1-Naphthalenecarbonitrile; (4) Phenyl 4-pyridyl ketone; (5) Pyrido(2,3-b) indole; (6) 2-Amino-1-acenaphthenone; (7) 6-Methoxy-2-naphthonitrile; (8) 4H-1-Benzopyran-4-one, (9) 5-Formylamino-1H-imidazole-4-carboxylic acid; (10) 4-Isothiazolecarboxamide; (11) Naphthalene; (12) Coumarin-6-ol; (13) Benzotriazol-4-one; (14) 5-Isothiazolecarboxamide; (15) Stilbene; (16) 1,4-Naphthalenediol; (17) trimethoxy 3,3'-Diaminodiphenylmethane

DISCUSSION

Eucalyptus camaldulensis is relevant in health and ethnomedicine. According to Azza *et al* [21], *E. camaldulensis* leaf demonstrated an antihyperglycemic effect using a diet supplemented with *E. camaldulensis* leaf, although the percentage supplementation was not specified. The administration of streptozotocin to Wistar rats resulted in diabetes with an increase in the fasting blood sugar. However, upon feeding with 10 % supplemented *E. camaldulensis* leaf-based diet for fourteen days, the fasting blood sugar level normalized. The result revealed that the fasting blood glucose level of diabetic-treated animals was the same as that of normal control group. This is probably because 10 % supplemented *E. camaldulensis* leaf-based diet stimulated the regeneration of both alpha and beta cells of the pancreas, which is evident from the elevated serum insulin and decreased amylase levels. Diabetic-treated rats had decreased amylase levels and plasma insulin levels that were comparable to normal control group, showing that the 10 % supplemented *E. camaldulensis* leaf-based diet was able to stimulate the regeneration of the beta cells, which made those regenerated cells start secreting insulin that stabilized the blood glucose level.

Similarly, the histopathology of the pancreas showed the diabetic control rats had ductal proliferation, severe inflammatory infiltrates, vascular distortion and congestion of acini, and little or no islets of Langerhans was seen. In contrast, the group B animals showed normal acini, the resurgence of the islets of Langerhans and a florid activation of pancreaticoduodenal lymphoid. This result is consistent with that reported by Azza *et al* [21], who observed that *E. camaldulensis* leaves exhibited actions similar to glibenclamide, stimulating the surviving beta cells to release insulin. Subsequently, our study showed that 10 % supplemented *E. camaldulensis* leaf-based diet resuscitated damaged beta cells. The serum liver function test indicated liver damage after administration of streptozotocin because plasma levels rapidly decrease within 15 min after administration and concentrate in the liver [22]. This concentration within the liver is capable of causing damage via inflammation, necrosis, and ulceration of the organ's cells. This was seen in the diabetic control group, which showed high serum AST and ALT levels. Also, the total protein concentration decreased below average, while the albumin and bilirubin concentrations increased significantly. However, the 10 % supplemented *E. camaldulensis* leaf-based diet protected the liver by lowering the liver enzymes. The albumin and bilirubin concentrations were also comparable with the normal group. The histopathology of the liver of the diabetic control rats showed severe vascular ulceration, congestion and periportal inflammation of the hepatocytes. In contrast, diabetic-treated group showed normal hepatocytes and normal ductal and vascular architecture of the liver. This indicates that the 10% *E. camaldulensis* supplemented leaf-based diet possesses anti-inflammatory properties, which repair the organ remarkably. Furthermore, the kidney function tests indicated destruction of the kidney after the induction with diabetes, as observed in the diabetic control group. Under normal conditions, creatinine is constantly excreted by the kidneys. Still, because the kidney was damaged by streptozotocin, the serum creatinine level was elevated in the diabetic control group. However, 10% supplemented *E. camaldulensis* leaf-based diet reversed the damage caused by streptozotocin in diabetic-treated group, thereby normalizing the serum creatinine level to the level of the control group.

Also, under diabetic conditions, increased breakdown of amino acids for energy generation due to the cell's inability to use glucose effectively lead to a corresponding increase in urea level. The kidney is the organ involved in excretion of urea. If there is kidney damage, the serum urea level may significantly increase, as shown in diabetic control group. Nevertheless, a 10 % supplemented *E. camaldulensis* leaf-based diet ameliorated this condition by reversing the damaged kidney to a significant extent, which caused the serum urea level of diabetic-treated group to normalize to the level of normal control group. This is further corroborated by the histopathology investigation of the kidney, which showed the diabetic control rats to have severe vascular distortion and congestion of the glomerulus after induction of diabetes with streptozotocin. Still, upon treatment with *E. camaldulensis* leaf-based diet for fourteen days, the kidney had normal tubular, vascular and glomerular architecture. This established that *E. camaldulensis* leaf-based diet could reverse kidney damage.

This study also evaluated the antioxidant potential of *E. camaldulensis* leaf-based diet. The result shows that after the induction of diabetes, the rate of oxidative stress increased, which was evident among the diabetic control group. The oxidative stress caused the antioxidant enzyme activities of the diabetic control group to decrease. Oxidative stress can lead to cell

damage because of increased free radicals, and most plants are rich in antioxidants. Malondialdehyde, a serum biomarker for oxidative stress, was very high in the diabetic control group. However, the *E. camaldulensis* leaf-based diet reduced the malondialdehyde level. Also, the leaf-based diet of *E. camaldulensis* significantly increased the superoxide dismutase and glutathione peroxidase activities, decreasing catalase activity. This suggests that *the E. camaldulensis* leaf-based diet reduced oxidative stress by mopping up free radicals, thereby preventing lipid peroxidation.

The lipid profile indicated that triglycerides and total cholesterol levels of the diabetic control group were high, and this is possible because, under diabetic conditions, glucose is in excess and cannot be utilized. This leads to an increase in the breakdown of lipids or fats as an alternative energy source. This, in turn, will lead to a rise in triglycerides. Also, acetyl CoA is increased because of increased fatty acid breakdown, and some of this acetyl CoA is diverted to cholesterol synthesis, leading to hypercholesterolemia [23]. Under diabetic conditions, plasma lipoproteins are compromised; the changes in the lipoproteins cause the high-density lipoprotein cholesterol to be lowered while the LDL-C and VLDL-C are raised. However, *the E. camaldulensis* leaf-based diet significantly decreased the level of LDL-C and VLDL-C, which was even lower than that of normal control group, suggesting that *E. camaldulensis* leaf-based diet is hypolipidemic.
In the bid to evaluate and determine the compound(s) responsible for the medicinal effect of *E. camaldulensis* leaf-based diet, Gas Chromatography-Mass Spectrometry was carried out on the methanolic extract of *E. camaldulensis* leaf. The GC-MS revealed seventeen compounds.

The GC-MS fingerprint showed that the predominant compound identified was 2-hydroxycarbazole, as indicated by the highest peak in the chromatogram. Previous studies, such as Agata [24], have highlighted carbazole's enhanced efficacy against fungi and parasites, alongside their notable anti-inflammatory properties with potential applications in treating neurological disorders. Additionally, Patel *et al* [22] reported the anti-diabetic activity of carbazoles. Further support for the anti-diabetic and hypolipidemic effects of carbazoles is provided by Dineshkumar *et al* [25]. These findings collectively suggest the potential pharmacological significance of 2-hydroxycarbazole, and related compounds derived from *E. camaldulensis* leaves**.**

CONCLUSION

Eucalyptus camaldulensis leaf-based diet display anti-diabetic and hypolipidemic effect in streptozotocin-induced diabetic rats. A leaf-based diet of *E. camaldulensis* may also be a good source of antioxidants. GC-MS analysis of the methanol plant extract has shown that carbazole may be the potential anti-diabetic and hypolipidemic agent in *E. camaldulensis* leaf-based diet.

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Ethical approval

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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