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IN VITRO AND IN VIVO EVALUATION OF ANTIDIABETIC AND ORGAN PROTECTIVE EFFECTS OF MAMMEA SURIGA LEAF EXTRACT AND ITS BUTANOL FRACTION

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ABSTRACT
Diabetes mellitus (DM) continues to drive the search for safer, plant-derived alternatives to synthetic hypoglycemic agents. This study evaluated the in vitro and in vivo antidiabetic potential of Mammea suriga (Buch.-Ham. ex Roxb.) Kosterm. leaf ethyl acetate extract (MSEAE) and its butanol fraction (MSBF), together with their organ-protective effects in streptozotocin (STZ) induced diabetic Wistar rats. In vitro, both MSEAE and MSBF inhibited α-amylase (IC_{50} 200.56 and 239.47 µg/mL) and α-glucosidase (IC_{50} 221.57 and 247.45 µg/mL) in a concentration dependent manner, less potently than acarbose, while cytotoxicity screening on HaCaT keratinocytes indicated favourable safety margin and acute oral toxicity testing (OECD 425) showed an LD_{50} exceeding 2000 mg/kg for both preparations. In STZ-induced diabetic rats, 21-day oral administration of MSEAE (100, 200 and 400 mg/kg) and MSBF (400 mg/kg) produced dose dependent reductions in fasting blood glucose, with the 400 mg/kg MSEAE dose lowering glucose from 326.16 ± 26.27 to 165.66 ± 23.46 mg/dL ($p < 0.01$), an effect comparable to glibenclamide. Treatment also restored body weight, normalised the serum lipid profile (total cholesterol, triglycerides, HDL-C and VLDL-C), improved hepatic function markers (AST, ALT, ALP) and renal function markers (urea, creatinine), and ameliorated diabetes associated histopathological damage in the pancreas, liver and kidney, evidenced by partial restoration of islet architecture, hepatocellular arrangement and nephron integrity. These effects, most pronounced at the 400 mg/kg dose and broadly comparable to the standard drug glibenclamide, indicate that M. suriga leaf extract and its butanol fraction possess significant antihyperglycemic, hypolipidemic and organ protective activity, supporting its traditional medicinal use and its potential as candidate for further mechanistic investigation in diabetes management.

KEYWORDS: Antidiabetic activity; histopathology; Mammea suriga; streptozotocin; α-amylase; α-glucosidase

INTRODUCTION

Diabetes mellitus (DM) is chronic metabolic disorder characterized by impaired regulation of blood glucose resulting from defects in insulin secretion, insulin action, or both. Persistent hyperglycemia is associated with progressive development of severe complications, including diabetic nephropathy, neuropathy, retinopathy, and cardiovascular diseases, making diabetes one of the most significant global public health challenges [1,2]. The worldwide prevalence of diabetes continues to rise at an alarming rate, contributing substantially to morbidity, mortality, and healthcare expenditure. In addition to its adverse effects on quality of life, diabetes imposes a considerable socioeconomic burden owing to long-term disability, loss of productivity, and increased treatment costs [3,4,5]. Despite availability of several synthetic antidiabetic drugs, their prolonged use is frequently accompanied by adverse effects, limited therapeutic efficacy in certain patients, and high treatment costs. Consequently, there has been growing interest in identifying safer, cost-effective, and naturally derived therapeutic alternatives. Herbal medicines have gained considerable attention because of their broad pharmacological activities, lower toxicity, improved patient tolerance, and potential to complement conventional antidiabetic therapies [6]. Nevertheless, many medicinal plants used in traditional healthcare systems remain scientifically underexplored, emphasizing the need for systematic pharmacological validation to establish their safety, efficacy, and mechanisms of action [7].

Medicinal plants constitute an abundant source of structurally diverse secondary metabolites, including flavonoids, phenolic compounds, alkaloids, terpenoids, and glycosides, many of which exhibit antioxidant, anti-inflammatory, antimicrobial, antiviral, and antidiabetic properties. These naturally occurring phytochemicals have attracted considerable attention in modern drug discovery owing to their ability to modulate multiple molecular targets involved in chronic diseases while exhibiting comparatively fewer adverse effects than synthetic drugs [8,9]. Among the medicinal plant species distributed in the Western Ghats of India, Mammea suriga (Buch.-Ham. ex Roxb.) Kosterm. is an underutilized species with considerable ethnomedicinal importance. Traditionally, different parts of this plant have been used to manage fever, herpes, dyspepsia, hemorrhoids, and several inflammatory disorders [10]. The literature on preliminary analysis, quantification of phytoconstituents and antioxidant potential of ethyl acetate extract and fraction is previously reported [11].

Histopathological assessment provides direct evidence of tissue injury and regeneration and is considered a critical endpoint for validating the therapeutic efficacy of antidiabetic agents. Persistent hyperglycemia caused by streptozotocin-induced diabetes generates oxidative stress and inflammatory responses that disrupt the normal architecture of pancreatic islets and contribute to hepatic and renal damage, which are among the major complications of diabetes mellitus [12]. Accordingly, the present study includes histological examination of the pancreas, liver, and kidneys to determine whether treatment with Mammea suriga leaf ethyl acetate extract (MSEAE) and its butanol fraction (MSBF) can mitigate diabetes-associated tissue injury. To the best of our knowledge, despite the presence of bioactive constituents in M. suriga, comprehensive studies evaluating its antidiabetic potential and underlying mechanisms of action remain limited. Therefore, the present study designed to evaluate the in vitro α-amylase and α-glucosidase inhibitory activities, cytotoxicity and acute oral toxicity profile, and the in vivo antihyperglycemic, hypolipidemic, hepatoprotective, and nephroprotective effects of MSEAE and MSBF in streptozotocin induced diabetic Wistar rats, supported by histopathological evaluation of pancreas, liver, and kidneys.

MATERIALS AND METHODS

Chemicals and Reagents

All reagents and solvents employed throughout the investigation were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was procured from Sigma-Aldrich (USA), while streptozotocin (STZ) was obtained from HiMedia Laboratories through Sri Durga Laboratory Equipment Supplies, Mangalore, India. Glibenclamide tablets were purchased from a local pharmacy (Vasundara Pharmacy, Mangalore, India). Standard laboratory animal feed was supplied by Champaka Feeds and Foods, Bengaluru, India.

Collection and Authentication of Plant Material

Fresh, fully matured leaves of Mammea suriga (Buch.-Ham. ex Roxb.) Kosterm. were collected from Perdoor village, Udupi District, Karnataka, India. Botanical identification and authentication were carried out at the Department of Applied Botany, Mangalore University, and a voucher specimen (ABMU0124) was deposited in the departmental herbarium for future reference. The collected leaves were thoroughly shade-dried for approximately three weeks, pulverised into a fine powder, and stored in airtight containers until extraction.

Preparation of Extract and Solvent Fractionation

20g of powdered Mammea suriga leaves were extracted with ethyl acetate using a Soxhlet extraction system. The obtained extract was concentrated under reduced pressure using a vacuum concentrator and stored at 4 °C until further use [13]. The dried crude extract was subsequently subjected to sequential solvent partitioning with solvents arranged in increasing order of polarity, namely n-hexane, chloroform, acetone, and n-butanol. For the fractionation process, 200 mg of the crude extract was dissolved in 2mL of dimethyl sulfoxide (DMSO), diluted with distilled water, and transferred to a separating funnel. Each solvent extraction was repeated until no additional compounds were recovered in the organic phase. The remaining aqueous layer after successive solvent partitioning was collected as the residual aqueous fraction [14].

Cytotoxicity Assay

The cytotoxic potential of Mammea suriga ethyl acetate extract (MSEAE) and butanol fraction (MSBF) was assessed in HaCaT (human keratinocyte) cell lines. Cells obtained from the Manipal School of Life Sciences were seeded in 96-well plates at a density of 1×10^4 cells/well and cultured for 24 h to permit cell attachment. Attached cells were exposed to 100 µL of F12 medium containing MSEAE or MSBF at concentrations ranging from 25 to 500 µg/mL for an additional 24 h. Cell viability was subsequently determined by MTT assay, in which 5 mg/mL MTT reagent added and incubated for 4 h at 37°C to facilitate conversion of MTT into insoluble formazan crystals by metabolically active cells. The crystals were solubilized in DMSO, and absorbance was recorded at 570nm using a microplate reader [13].

α-Amylase Inhibitory Assay

The α-amylase inhibitory activity of the plant extract and its fractions was evaluated following the method described previously [15]. Briefly, 0.5mL of α-amylase solution (2.0 U/mL) was mixed with 1.0mL of the plant extract or fraction at different concentrations, along with 6 mM NaCl prepared in 0.02 M phosphate buffer (pH 6.8). The reaction mixture was pre-incubated at room temperature for 10 min to facilitate enzyme–inhibitor interaction. Subsequently, 0.5mL of 1% (w/v) soluble starch was added as the substrate, and the enzymatic reaction was allowed to proceed for an additional 10 min. A control mixture without the enzyme was prepared under identical experimental conditions. The reaction was terminated by adding 0.5mL of 1% dinitrosalicylic acid (DNS) reagent, followed by heating the tubes in a boiling water bath for 10 min to develop the characteristic colour. After cooling to room temperature, the reaction mixtures were diluted with 5mL of distilled water, and the absorbance was measured at 540nm using a UV–Visible spectrophotometer.

α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitory activity of the plant extract and its fractions was determined according to the previously reported method [15]. Briefly, 50µL of the extract or fraction at different concentrations was mixed with 100µL of α-glucosidase solution (1.0 U/mL) and pre-incubated for 10 min to allow interaction between the enzyme and the test samples. Following incubation, 50 µL of 3 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) was added as the substrate, and the reaction mixture was further incubated at 37°C for 20 min. The enzymatic reaction was terminated by adding 2mL of 0.1 M sodium carbonate solution. The release of p-nitrophenol, resulting from enzymatic hydrolysis of the substrate, was quantified by measuring the absorbance at 410nm using a UV–Visible spectrophotometer. All reagents, including the

enzyme, substrate, extract, fractions, and sodium carbonate solution, were prepared in 0.2 M sodium phosphate buffer (pH 6.8).

In Vivo Evaluation of Antidiabetic Activity

Experimental Animals and Husbandry

The in vivo antidiabetic study was performed in accordance with the guidelines approved by the Institutional Animal Ethics Committee (IAEC), Mangalore University (Approval No. MU/AZ/IAECMU-RP-25-5/705/2025-26). Healthy adult Albino Wistar rats, aged 8–12 weeks and weighing 180–280g, were procured from Si Sai Biosciences, Bengaluru, Karnataka, India. The animals were maintained in the institutional animal facility under standard laboratory conditions, with an ambient temperature of 23 ± 2°C, relative humidity of 46 –70%, and a 12 h light/12 h dark photoperiod. Before the initiation of the experimental protocol, all animals were acclimatised to the laboratory environment for 7 days and provided with standard pellet feed and water ad libitum.

Acute oral toxicity

MSEAE and MSBF considered for their acute toxicity in accordance with guidelines of Organization for Economic Co-operation and Development (OECD) 425 [16].

Induction of Experimental Diabetes

Experimental diabetes was induced in healthy adult male Wistar albino rats after an overnight fasting of 14 h. Baseline body weight (b.w.) and fasting blood glucose (FBG) levels were recorded prior to induction. Diabetes was induced by single intraperitoneal (i.p) injection of streptozotocin (STZ) at 32mg/kg b.w. [17,18]. This dose was selected within the 30–40 mg/kg range reported to reliably induce a diabetic state while substantially reducing the acute mortality associated with higher doses such as 50 mg/kg. Freshly prepared STZ was dissolved in 0.01 M citrate buffer (pH 4.5) [19]. The injection volume was fixed at 1.0mL/kg b.w.

To avoid acute hypoglycemia, animals were supplied with 5% glucose solution as drinking water for 48h post-injection. Seventy-two hours after STZ administration, fasting blood samples were collected from the tail vein of overnight-fasted rats, and glucose levels were measured using an Accu-Chek Active glucometer (Roche Diabetes Care GmbH, Mannheim, Germany). Rats with FBG levels above 230mg/dL were considered diabetic.

Experimental design

Six rats each in eight groups were randomly divided as presented below:

Group 1: Normal control (NC) rats
Group 2: Vehicle control (VC) rats (1% CMC)
Group 3: Diabetic control (DC) rats
Group 4: DC rats treated with MSEAE suspended in 1% CMC (100 mg/kg b.w.)
Group 5: DC rats treated with MSEAE suspended in 1% CMC (200 mg/kg b.w.)
Group 6: DC rats treated with MSEAE suspended in 1% CMC (400 mg/kg b.w.)
Group 7: DC rats treated with MSBF suspended in 1% CMC (400 mg/kg b.w.)
Group 8: DC rats treated with glibenclamide (10mg/kg b.w.)

Glibenclamide, extract and fraction given orally once daily for 21 days. On days 0, 7, 14 and 21, blood samples drawn from the tail vein, of each group and glucose levels measured (Accu-Check Active glucometer).

Measurement of Body Weight and Blood Glucose Levels

Body weight and fasting blood glucose (FBG) levels recorded at the start of the experiment and monitored at 7-day intervals throughout the 21-day treatment period [20]. Baseline FBG values measured before streptozotocin (STZ) administration and continuously monitored during the initial 24 h following diabetes induction. To minimise the risk of acute hypoglycemia induced by STZ, diabetic rats provided with 5% glucose solution for 48 h after injection. The animals were subsequently maintained under diabetic conditions for additional 48 h to ensure stabilisation of diabetic state.

Before initiating treatment, fasting blood glucose levels were measured repeatedly in all experimental groups using glucose oxidase–peroxidase reactive strips in conjunction with an Accu-Chek Active glucometer. Following confirmation of diabetes, the respective treatments were administered orally by gavage once daily for 21 days. Animals received either glibenclamide (10 mg/kg body weight) as the standard drug, the plant extract at doses of 100, 200, or 400 mg/kg body weight/day, or the solvent fraction at a dose of 400 mg/kg body weight/day, according to their assigned experimental groups.

Biochemical Estimation

Blood Collection and Serum Preparation

At the end of the 21-day oral treatment with MSEAE and MSBF, the animals were fasted overnight and euthanised under deep anaesthesia induced by an intramuscular injection of ketamine (55 mg/kg b.w.) and xylazine (5 mg/kg b.w.), followed by cervical dislocation. Prior to euthanasia, approximately 4–5mL of blood was collected from each rat by cardiac puncture using a sterile 23-gauge syringe. The collected blood samples were transferred into non-EDTA tubes and allowed to clot. Serum was separated by centrifugation at 3000 rpm for 10 min using a REMI R-8C centrifuge. The recovered serum was subsequently used for biochemical analyses, while aliquots intended for later analysis were stored at −20°C in a biomedical freezer (Sanyo Biomedical Freezer, Model MDF-U333) until further use.

Assessment of Renal Function, Hepatic Function, and Lipid Profile

Serum biochemical parameters associated with renal function, hepatic function, and lipid metabolism were determined using commercially available ready-to-use diagnostic kits and analysed with an AGD2020 Clinical Chemistry Analyser (AGD Biomedicals, India). All analyses were performed in accordance with the manufacturer's recommended protocols. Renal function was evaluated by measuring serum creatinine and urea concentrations. Hepatic function was assessed by estimating the activities of aspartate aminotransferase (AST; serum glutamic-oxaloacetic transaminase, SGOT), alanine aminotransferase (ALT; serum glutamic-pyruvate transaminase, SGPT), and alkaline phosphatase (ALP). Lipid profile parameters, including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), were also quantified using standard enzymatic colorimetric methods. Friedewald's algorithm was used to determine very low-density lipoprotein cholesterol (VLDL-C) values.

VLDL = Triglycerides/5

Histopathological Examination of the Liver, Kidney, and Pancreas

At the end of the 21-day treatment period, the liver, kidneys, and pancreas were carefully excised from all experimental animals and subjected to gross examination for the detection of any visible pathological alterations or signs of toxicity. The harvested tissues were immediately fixed in 10% neutral buffered formalin to preserve their structural integrity. Following fixation, the tissues were processed using standard histological procedures, embedded in molten paraffin wax, and sectioned into 5-µm-thick slices using a microtome. The tissue sections were subsequently stained with hematoxylin and eosin (H and E) to evaluate histopathological changes. The stained sections were examined and photographed using a Nikon phase-contrast microscope (Y-TV55 ECLIPSE) for microscopic assessment of tissue architecture and pathological alterations.

Statistical Analysis

Data are expressed as mean ± SEM. The IC_{50} values were calculated using GraphPad Prism version 8.4.0 (GraphPad Software, San Diego, CA, USA). Statistical analyses were conducted using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA). Differences between experimental groups were analysed by one-way ANOVA, followed by Dunnett's T3 post hoc multiple comparisons. A probability value of $p < 0.05$ was considered statistically significant, whereas $p < 0.01$ was considered highly significant.

RESULTS

Cytotoxicity Assay

Fig. 1 illustrates cytotoxic effects of MSEAE and MSBF on HaCaT cell lines. As concentration increased cell viability declined. Compared to MSEAE, MSBF showed lower toxicity on HaCaT, with IC_{50} values of 92.46µg/mL and 114.25µg/mL respectively.

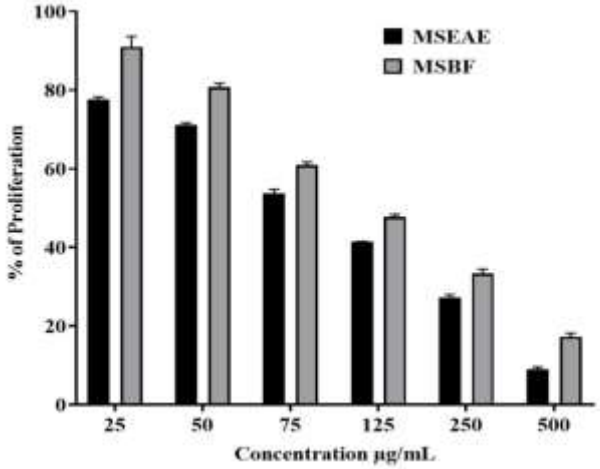

Fig 1. Cytotoxic potential of MSEAE and MSBF on HaCaT cell lines

α-amylase inhibition assay

The in vitro α-amylase inhibition assay demonstrated that both MSEAE and MSBF possess considerable antidiabetic activity through the inhibition of carbohydrate-hydrolyzing enzymes. Both samples exhibited a concentration-dependent increase in α-amylase inhibitory activity across the tested concentrations. The highest inhibitory effect was observed at 250 µg/mL for both MSEAE and MSBF. The IC_{50} values were calculated to be 200.56 µg/mL and 239.47 µg/mL for MSEAE and MSBF, respectively, whereas the standard α-amylase inhibitor, acarbose, exhibited an IC_{50} value of 156.33 µg/mL. Although the inhibitory activity of the plant extract and fraction was lower than that of acarbose, both demonstrated appreciable enzyme inhibition, indicating their potential to regulate postprandial glucose metabolism. The comparative α-amylase inhibitory activities of acarbose, MSEAE and MSBF are presented in Fig 2.

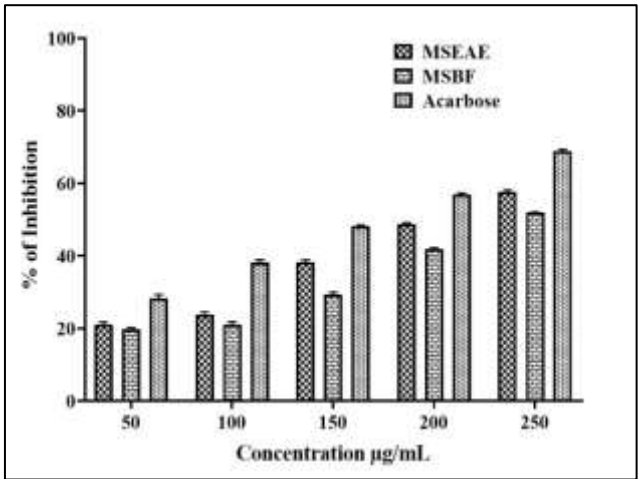

Fig 2. Inhibitory action of MSEAE and MSBF on α-amylase

α-Glucosidase Inhibitory Activity

As shown in Fig. 3, both MSEAE and MSBF inhibited α-glucosidase activity in a concentration-dependent manner. The maximum inhibitory effect was recorded at the highest concentration evaluated. The calculated IC_{50} values were 221.57µg/mL for MSEAE and 247.45 µg/mL of MSBF, while acarbose, used as the positive control, exhibited a lower IC_{50} value of 163.04µg/mL. Although both plant samples displayed lower inhibitory potency than the standard drug, their moderate inhibition of α-glucosidase indicates a potential role in delaying carbohydrate hydrolysis and glucose absorption, thereby supporting their antidiabetic potential.

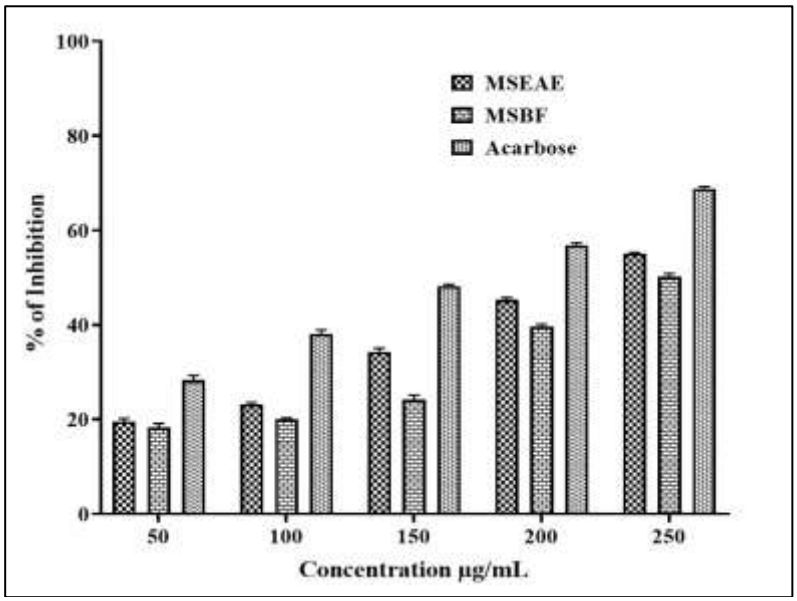

Fig 3. Inhibitory action of MSEAE and MSBF on α-glucosidase

Acute oral toxicity

Animals treated with MSEAE and MSBF did not exhibit any behavioural changes. The b.w., water and food consumption did not change significantly compared to control group. Therefore, acute oral toxicity experiment was terminated. In MSEAE and MSBF, LD_{50} greater than 2000 mg/kg b.w.

Effect of Mammea suriga Ethyl Acetate Extract and Butanol Fraction on Body Weight

Changes in body weight of the experimental animals following treatment with MSEAE and MSBF are summarised in Table 5. The initial body weights did not differ significantly among experimental groups ($p > 0.05$). However, three days after STZ-induced diabetes, the diabetic control group showed a significant decline in b.w. ($p < 0.01$) compared with the normal control group. Treatment with MSEAE, MSBF, and glibenclamide for 21 days significantly ($p < 0.01$) restored b.w. relative to untreated diabetic rats. While normal control group exhibited a steady increase in b.w. throughout the study, animals receiving MSEAE, MSBF or glibenclamide demonstrated a marked improvement by the end of the treatment period, suggesting a protective effect against diabetes-induced weight loss.

Table 5: Effects of ethyl acetate extract and butanol fraction of M. suriga leaves on b.w. in diabetic rats

Groups	0^{th} Day	7^{th} Day	14^{th} Day	21^{th} Day
NC	221.66 ± 9.24	234.33 ± 5.00^{a}	$244.16 \pm 4.66^{a,@}$	$254.83 \pm 3.76^{a,@}$
VC	221.33 ± 6.91	233.50 ± 4.08^{a}	$243.50 \pm 3.83^{a,@}$	$254.00 \pm 5.40^{a,@}$
DC	219.66 ± 4.45	$206.16 \pm 3.65^{@}$	$194.00 \pm 6.54^{\#}$	$180.50 \pm 2.42^{\#}$

DC + E-100	221.83 ± 4.79	208.33 ± 5.88	211.16 ± 7.13	212.66 ± 6.05[a]
DC + E-200	219.16 ± 5.03	219.66 ± 5.99	224.00 ± 5.79[a]	228.16 ± 4.79[a]
DC + E-400	219.83 ± 7.65	225.00 ± 7.74[b]	232.83 ± 8.61[a]	238.16 ± 9.28[a]
DC + F-400	212.16 ± 6.73	221.83 ± 7.57	225.66 ± 4.84[a]	233.16 ± 5.49[a]
DC + Glibenclamide (10 mg/kg)	219.66 ± 10.89	222.66 ± 7.36	224.83 ± 8.54[a]	232.00 ± 10.29[a]

Changes in Body weight (gm) of E (Extract) and F (Fraction) for 21days (3 weeks) NC (Normal control), VC (Vehicle control), DC (Diabetic control), DC + E (100, 200, and 400 mg/kg) and DC + F (400 mg/kg) and DC + Glibenclamide group of rats. Data are expressed as means ± SEM, n = 6 rats per group. $p < 0.05$; $p < 0.01$. Statistical significance was considered at $p < 0.05$ in all cases.

[a]denotes $p < 0.01$ and [b]denotes $p < 0.05$ compared to the DC group.

[#]denotes $p < 0.01$and [@]denotes $p < 0.05$ compared to the DC + Glibenclamide group.

Effect of Mammea suriga Ethyl Acetate Extract and Butanol Fraction on Serum Lipid Profile

As shown in Table 6, diabetes induction significantly disrupted serum lipid homeostasis, as evidenced by increased levels of total cholesterol (TC) and triglycerides (TG), along with decreased HDL-C levels in the DC group. Treatment with MSEAE, MSBF, and glibenclamide significantly ($p < 0.01$) ameliorated these diabetes-associated lipid abnormalities. Rats receiving MSEAE and MSBF exhibited significantly lower TC and TG concentrations and significantly higher HDL-C levels than untreated diabetic animals. The 400 mg/kg dose of both ethyl acetate extract and butanol fraction demonstrated the most pronounced hypolipidemic effect after 21-day treatment period, indicating improved regulation of lipid metabolism in diabetic rats (Table 6).

Table 6: Effects of ethyl acetate extract and butanol fraction of M. suriga leaves on lipid profile in diabetic rats.

Groups	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	VLDL-C (mg/dL)
NC	104.83 ± 10.16[a,@]	92.50 ± 9.60[a,@]	41.16 ± 5.56[b]	18.50 ± 1.92[a,@]
VC	105.83 ± 9.19[a,@]	92.00 ± 7.23[a,@]	41.83 ± 8.13[b]	18.10 ±1.75[a,@]
DC	266.33 ± 28.66[#]	193.33 ± 16.21[#]	23.00 ± 5.65	38.66 ± 3.24[#]
DC + E-100	194.83 ± 10.92[b,#]	155.16 ± 18.06	26.16 ± 4.44	31.03 ± 3.61
DC + E-200	160.66 ± 10.23[b,@]	130.16 ±10.00[a]	32.00 ± 5.44	26.03 ± 2.00[a]
DC + E-400	144.00 ± 12.19[a]	122.33 ± 11.14[a]	36.00 ± 3.74[b]	24.46 ± 2.22[a]
DC + F-400	149.16 ± 5.30[b]	129.83 ± 6.27[b]	29.83 ± 3.31	25.96 ± 1.25[b]
DC+Glibenclamide (10 mg/kg)	134.50 ± 8.24[a]	118.16 ± 7.02[a]	33.33 ± 3.66	23.63 ± 1.40[a]

Changes in lipid profile of E (Extract) and F (Fraction) for 21days (3 weeks) NC (Normal control), VC (Vehicle control), DC (Diabetic control), DC + E (100, 200, and 400 mg/kg) and DC + F (400 mg/kg) and DC + Glibenclamide group of rats. Data are expressed as means ± SEM, n = 6 rats per group. $p < 0.05$; $p < 0.01$. Statistical significance was considered at $p < 0.05$ in all cases.

[a]denotes $p < 0.01$ and [b]denotes $p < 0.05$ compared to the DC group.

[#]denotes $p < 0.01$and [@]denotes $p < 0.05$ compared to the DC + Glibenclamide group.

Effect of Mammea suriga Ethyl Acetate Extract and Butanol Fraction on Hepatic Function

The effects of MSEAE and MSBF on hepatic function are presented in Table 7. STZ-induced diabetic rats exhibited a marked elevation in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities compared with the normal control group, indicating diabetes-associated hepatic injury. Oral administration of MSEAE, MSBF significantly reduced the elevated levels of these liver enzymes relative to the untreated diabetic control group. The most pronounced improvement was observed in animals treated with 400 mg/kg of MSEAE, MSBF which showed a highly significant ($p < 0.01$) reduction in serum transaminase activities (Table 7).

Table 7: Effects of ethyl acetate extract and butanol fraction of M. suriga leaves on liver function in diabetic rats.

Groups	TP (g/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
NC	6.86 ± 1.22[b]	98.18 ± 15.67[b]	31.45 ± 4.81[a,@]	109.28 ± 17.59[a,#]
VC	7.05 ± 0.53[a]	100.38 ± 17.84[a]	34.08 ± 6.44[a,@]	80.52 ±15.09[a,#]
DC	4.18 ± 0.22[#]	242.40 ± 19.13[a,#]	123.13± 11.89[#]	268.81 ± 38.78[@]
DC + E-100	5.29 ± 0.49[b,#]	187.38 ± 22.70[b,@]	95.88 ± 8.96[b,@]	173.95 ± 8.44[b]
DC + E-200	6.48 ± 0.46[a]	153.76±16.52[a]	80.41 ± 7.55[b]	143.03 ± 9.29[b]
DC + E-400	7.10 ± 0.51[a]	133.56 ±14.71[a]	62.36 ± 7.53[a]	125.86 ± 10.94[b,@]
DC + F-400	7.70 ± 0.51[a]	127.75 ± 8.88[a]	68.26 ± 7.61[a]	140.41 ± 9.16[b]
DC + Glibenclamide (10 mg/kg)	6.65 ± 0.43[a]	118.05 ± 9.65[a]	61.98 ± 8.64[a]	160.80 ± 11.73[b]

Changes in liver functions of E (Extract) and F (Fraction) for 21days (3 weeks) NC (Normal control), VC (Vehicle control), DC (Diabetic control), DC + E (100, 200, and 400 mg/kg) and DC + F (400 mg/kg) and DC + Glibenclamide group of rats. Data expressed as means ± SEM, n = 6 rats per group. $p < 0.05$; $p < 0.01$. Statistical significance considered at $p < 0.05$ in all cases.

[a]denotes $p < 0.01$ and [b]denotes $p < 0.05$ compared to the DC group.

[#]denotes $p < 0.01$and [@]denotes $p < 0.05$ compared to the DC + Glibenclamide group.

Effect of Mammea suriga Ethyl Acetate Extract and Butanol Fraction on Renal Function

The effects of the treatments on renal function are summarised in Table 8. Diabetic control rats exhibited significantly increased serum creatinine and urea concentrations compared with normal control animals, indicating impaired renal function. Treatment with MSEAE and MSBF effectively attenuated these alterations, with 400 mg/kg treatment groups showing a significant ($p < 0.01$) decrease in both creatinine and urea levels compared with untreated diabetic rats. These findings suggest that both ethyl acetate extract and butanol fraction of M. suriga exert protective effects against diabetes-induced hepatic and renal dysfunction.

Table 8: Effect of M. suriga ethyl acetate extract and their butanol fraction on kidney function in diabetic rats.

Groups	Kidney Parameters	
	Urea (mg/dL)	**Creatinine (mg/dL)**
NC	21.75 ± 3.10[a,#]	0.35 ± 0.06[a]
VC	23.48 ± 3.49[a]	0.26 ± 0.06[a]
DC	49.11 ± 5.0[@]	1.31 ± 0.22[#]
DC + E-100	39.76 ± 5.52	1.13 ± 0.27[@]
DC + E-200	35.69 ± 3.27[b]	0.89 ± 0.02[@]
DC + E-400	29.58 ± 1.97[a]	0.34 ± 0.09[a]
DC + F-400	35.91 ± 3.95[b]	0.41 ± 0.07[b]
DC+Glibenclamide (10mg/kg)	29.71 ± 2.15[a]	0.40 ± 0.12[a]

Changes in kidney function of E (Extract) and F (Fraction) for 21days (3 weeks) NC (Normal control), VC (Vehicle control), DC (Diabetic control), DC + E (100, 200, and 400 mg/kg) and DC + F (400 mg/kg) and DC + Glibenclamide group of rats. Data expressed as means ± SEM, n = 6 rats per group. $p < 0.05$; $p < 0.01$. Statistical significance considered at $p < 0.05$ in all cases.

[a]denotes $p < 0.01$ and [b]denotes $p < 0.05$ compared to the DC group.

[#]denotes $p < 0.01$and [@]denotes $p < 0.05$ compared to the DC + Glibenclamide group.

Effect of Mammea suriga Ethyl Acetate Extract and Butanol Fraction on Blood Glucose Levels

The antihyperglycemic effects of MSEAE and MSBF in streptozotocin (STZ)-induced diabetic rats are presented in Table 9. Oral administration of MSEAE and MSBF for 21 consecutive days resulted in a significant reduction in fasting blood glucose levels compared with the untreated diabetic control group. Treatment with MSEAE at doses of 100 mg/kg and 200 mg/kg reduced blood glucose concentrations from 346.83 ± 44.19 to 255.16 ± 37.27mg/dL and 360.83 ± 39.58 to 221.00 ± 13.43 mg/dL, respectively, with the reduction at 200 mg/kg being statistically significant ($p < 0.05$). A more pronounced antihyperglycemic effect was observed at the 400 mg/kg dose of MSEAE, where blood glucose levels declined from 326.16 ± 26.27 to 165.66 ± 23.46 mg/dL. Similarly, treatment with MSBF (400 mg/kg) decreased blood glucose levels from 307.16 ± 22.30 to 201.66 ± 29.46 mg/dL, with both treatments demonstrating highly significant improvements ($p < 0.01$) compared with the diabetic control group. By the end of the 21-day treatment period, all treatment groups exhibited marked reductions in fasting blood glucose levels, confirming the antihyperglycemic potential of M. suriga ethyl acetate extract and its butanol fraction in STZ-induced diabetic rats.

Table 9: Effect of M. suriga ethyl acetate extract and their butanol fraction on blood glucose level in diabetic rats.

Groups	Glucose Level (mg/dL)			
	Day-0	**Day-7**	**Day-14**	**Day-21**
NC	96.83 ± 5.08[a,#]	97.66 ± 7.08[a,@]	91.50 ± 7.39[a,@]	97.33 ± 10.48[a,@]
VC	98.66 ± 6.05[a,#]	97.33 ± 3.66[a,@]	96.16 ± 8.37[a,@]	102.50 ± 5.75[a,@]
DC	327.16 ± 63.51	418.83 ± 56.35[#]	503.83 ± 40.67[#]	565.16 ± 25.13[#]
DC + E-100	346.83 ± 44.19	326.33 ± 56.9[@]	270.16 ± 37.53[a]	255.16 ± 37.27[a,@]
DC + E-200	360.83 ± 39.58	308.16 ± 28.35[@]	259.16 ± 23.10[a,@]	221.00 ± 13.43[a,@]
DC + E-400	326.16 ± 26.27	182.33 ± 14.51[b]	201.16 ± 12.87[a]	165.66 ± 23.46[a]
DC + F-400	307.16 ± 22.30	187.83 ± 132.56[a]	204.16 ± 26.94[a]	201.66 ± 29.46[a]
DC + Glibenclamide (10mg/kg)	308.50 ± 18.89	188.00 ± 33.17	196.33 ± 26.21[a]	162.33 ± 16.32[a]

Changes in blood glucose level of E (Extract) and F (Fraction) for 21days (3 weeks) NC (Normal control), VC (Vehicle control), DC (Diabetic control), DC + E (100, 200, and 400 mg/kg) and DC + F (400 mg/kg) and DC + Glibenclamide group of rats. Data expressed as means ± SEM, n = 6 rats per group. $p < 0.05$; $p < 0.01$. Statistical significance considered at $p < 0.05$ in all cases.

[a]denotes $p < 0.01$ and [b]denotes $p < 0.05$ compared to the DC group.

[#]denotes $p < 0.01$and [@]denotes $p < 0.05$ compared to the DC + Glibenclamide group.

Histopathological Examination

Histopathological evaluation of the pancreas (Fig. 4) revealed normal architecture in the normal NC and VC groups, characterised by well-defined pancreatic islets with intact cellular morphology and normal distribution of endocrine cells.

In contrast, DC group exhibited marked degenerative changes, including shrunken pancreatic islets, reduced islet size, and degeneration of β-cells. Treatment with glibenclamide, MSEAE, and MSBF markedly ameliorated these pathological alterations, as evidenced by restoration of islet architecture, increased cellular density, and improved organisation of the endocrine tissue, indicating partial regeneration of pancreatic β-cells.

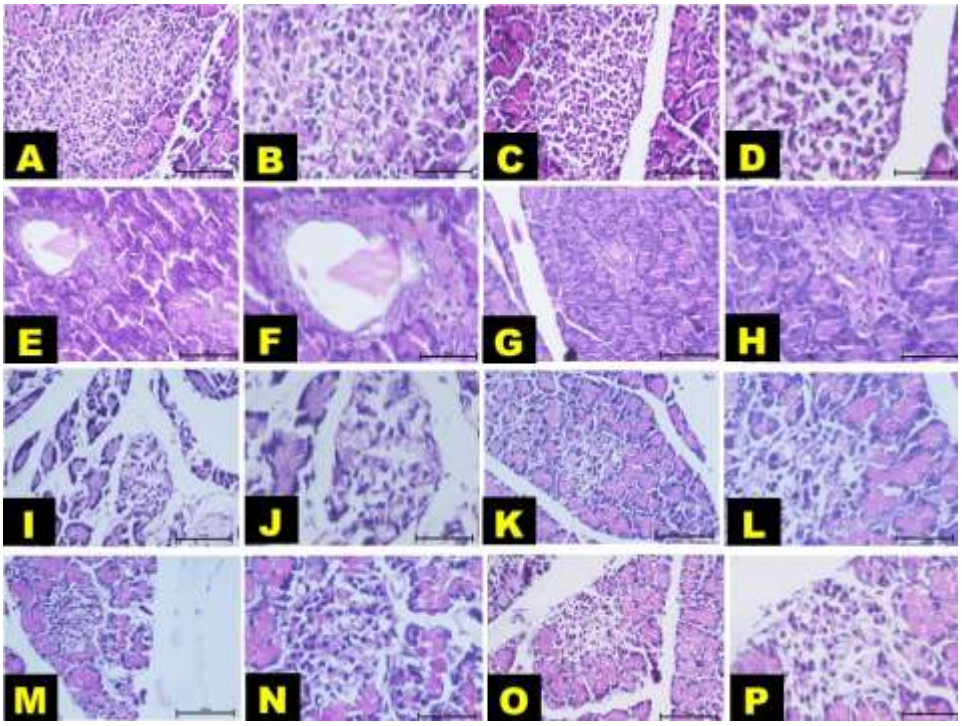

Fig.4. Histopathology of the pancreatic tissue sample of diabetes induced Wistar rats treated with M. suriga extract and fraction. (A) NC- indicates normal islets of Langerhans 20X. (B) 40X. (C) VC- indicates normal islets of Langerhans 20X. (D) 40X. (E) DC- degenerated, shrunken islets of Langerhans 20X. (F) 40X. (G) MSEAE (100mg/kg) treated group- indicates partially recovered islets of Langerhans – 20X. (H) 40X. (I) MSEAE (200mg/kg) treated group- indicates partially recovered islets of Langerhans 20X, (J) 40X, (K) MSEAE (400mg/kg) treated group- indicates recovered islets of Langerhans after being destroyed due to disease progression 20X, (L) 40X, (M) MSBF (400mg/kg) treated group- indicates partially recovered islets of Langerhans 20X, (N) 40X, (O) Glibenclamide treated group- indicates partially recovered islets of Langerhans 20X, (P) 40X.

Microscopic examination of the liver (Fig. 5) demonstrated normal hepatic architecture in NC and VC groups, with regularly arranged hepatocytes and the absence of pathological lesions. In contrast, liver sections from DC rats exhibited enlarged hepatocytes, focal areas of necrosis, and lipid accumulation, reflecting diabetes-associated hepatic injury. Treatment with glibenclamide, MSEAE, and MSBF markedly attenuated these histopathological abnormalities, as evidenced by restoration of normal hepatocellular arrangement, reduced lipid deposition, and the absence of necrotic lesions, indicating a protective effect against hepatic damage induced by diabetes.

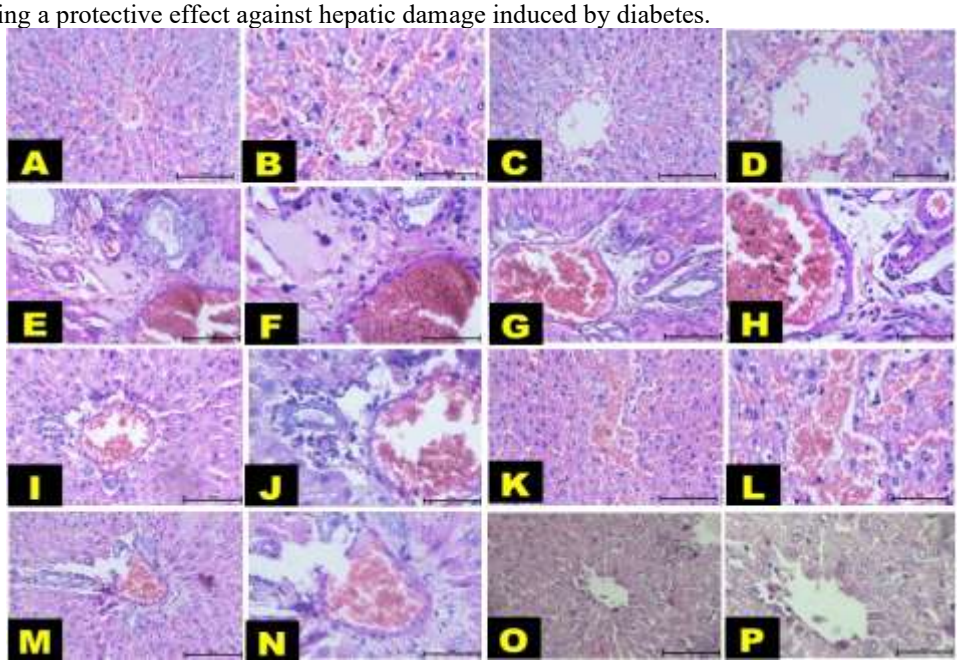

Fig.5. Histopathology of the liver tissue sample of diabetes induced Wistar rats treated with M. suriga extract and fraction. (A) NC- indicates normal arrangement of hepatic cells 20X. (B) 40X. (C) VC- indicates normal arrangement of hepatic

cells 20X. (D) 40X. (E) DC- indicates inappropriate arrangement of hepatic cells 20X. (F) 40X. (G) MSEAE (100mg/kg) treated group- indicates partial recovered hepatic cells 20X. (H) 40X. (I) MSEAE (200mg/kg) treated group- indicates partial recovered hepatic cells 20X, (J) 40X, (K) MSEAE (400mg/kg) treated group- indicates recovered hepatic cells 20X, (L) 40X, (M) MSBF (400mg/kg) treated group- indicates recovered hepatic cells 20X, (N) 40X, (O) Glibenclamide treated group- indicates recovered normal hepatic cell arrangement 20X, (P) 40X.

Histological examination of the kidney (Fig.6) showed normal renal architecture in NC and VC groups, with well-organised nephrons and intact glomeruli. Conversely, kidneys from DC group displayed pronounced pathological changes, including disorganised nephron architecture and numerous endocytic vacuoles, indicative of diabetes-induced renal damage. Administration of glibenclamide, MSEAE, and MSBF significantly improved renal histoarchitecture, resulting in better-organised nephron structures and preservation of glomerular integrity compared with the untreated diabetic group.

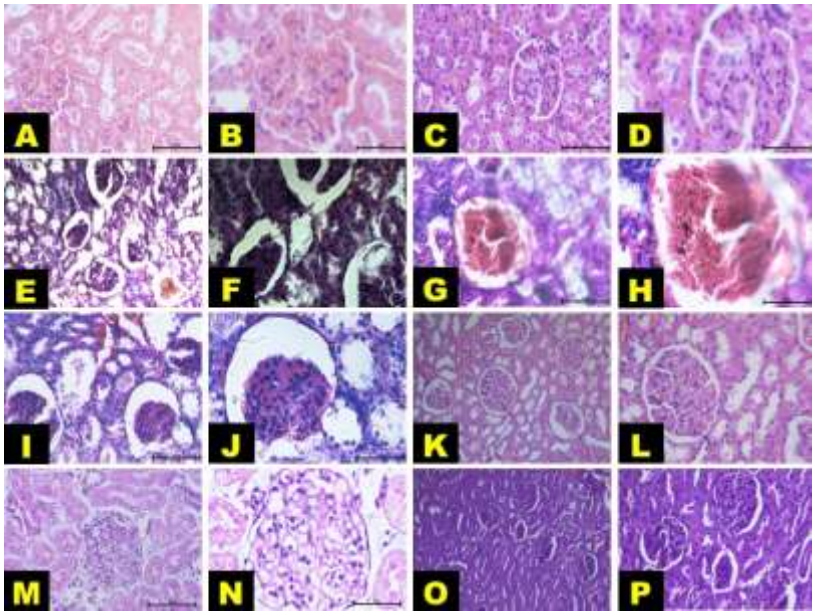

Fig.6. Histopathology of the kidney tissue sample of diabetes induced Wistar rats treated with M. suriga extract and fraction. (A) NC- indicates normal arrangements of glomerulus and tubule 20X. (B) 40X. (C) VC- indicates normal arrangements of glomerulus and tubule 20X. (D) 40X. (E) DC- indicates improper arrangements of glomerulus and tubule 20X. (F) 40X. (G) MSEAE (100mg/kg) treated group- indicates partially recovered arrangements of glomerulus and tubule 20X. (H) 40X. (I) MSEAE (200mg/kg) treated group- indicates partially recovered arrangements of glomerulus and tubule 20X, (J) 40X, (K) MSEAE (400mg/kg) treated group- indicates recovered arrangements of glomerulus and tubule with low endocytic vacuoles 20X, (L) 40X, (M) MSBF (400mg/kg) treated group- indicates recovered arrangements of glomerulus and tubule with low endocytic vacuoles 20X, (N) 40X, (O) Glibenclamide treated group indicates recovered arrangements of glomerulus and tubule with low endocytic vacuoles 20X, (P) 40X.

DISCUSSION

Diabetes mellitus (DM) is complex metabolic disease that causes problems including hyperlipidemia and multiorgan dysfunction under continuous hyperglycemia by interfering with the normal metabolism of proteins, lipids, and carbohydrates because of insufficient insulin production and insulin resistance [21]. This makes it necessary to look for novel antidiabetic medications that affect the metabolism of fats and carbohydrates. In metabolic diseases, recent therapeutic methods have focused on altering lipid control and pertinent cardiovascular events [22, 23].

Inhibition of carbohydrate-hydrolysing enzymes α-amylase and α-glucosidase is a well-recognised therapeutic approach for reducing postprandial hyperglycemia in type 2 diabetes by delaying carbohydrate digestion and intestinal glucose absorption [24, 25]. In the present study, both MSEAE and MSBF exhibited concentration-dependent inhibitory activity against these enzymes. The α-amylase IC_{50} values were 200.56 µg/mL for MSEAE and 239.47 µg/mL for MSBF, while acarbose showed a lower IC_{50} value of 156.33 µg/mL, indicating stronger inhibitory potency. Similarly, the α-glucosidase IC_{50} values of 221.57 µg/mL (MSEAE) and 247.45 µg/mL (MSBF) were higher than that of acarbose (163.04 µg/mL), suggesting moderate but appreciable enzyme inhibition.

The comparatively better inhibitory activity of MSEAE than MSBF indicates that the ethyl acetate extract may contain a higher concentration of bioactive phytochemicals, particularly phenolic compounds and flavonoids, which are known to inhibit digestive enzymes through interactions with their catalytic sites and by modifying enzyme conformation [26]. Although the inhibitory effects were lower than those of acarbose, moderate inhibition of these enzymes is considered advantageous because it may effectively reduce postprandial glucose excursions while minimizing the gastrointestinal adverse effects frequently associated with potent synthetic α-glucosidase inhibitors [27].

Histopathological evaluation provides direct evidence of tissue injury and recovery following therapeutic intervention and complements biochemical findings in experimental diabetes. Streptozotocin (STZ) is known to induce pancreatic β-cell destruction through oxidative stress and DNA alkylation, resulting in insulin deficiency and subsequent pathological alterations in the liver and kidneys [12, 28]. In the present study, histological observations were consistent with biochemical outcomes, confirming the protective effects of MSEAE and MSBF against diabetes induced organ damage.

Pancreatic sections from DC group (Fig. 4) exhibited severe degeneration of islets of Langerhans, characterized by reduced islet size, cellular shrinkage, and β-cell degeneration, indicating STZ-mediated pancreatic injury. In contrast, treatment with MSEAE and MSBF improved pancreatic architecture, with partial restoration of islet morphology and increased cellular organisation. Regenerative effect was more evident in 400 mg/kg MSEAE group, although glibenclamide showed the greatest recovery. Preservation of pancreatic islet integrity suggests that extract may protect β-cells from oxidative damage and improve endogenous insulin secretion, which agrees with previous reports describing the β-cell protective effects of polyphenol-rich medicinal plants [26, 29].

Similarly, liver histology (Fig. 5) demonstrated marked hepatocellular degeneration, focal necrosis, and lipid accumulation in diabetic rats, reflecting metabolic disturbances associated with chronic hyperglycemia. Treatment with MSEAE and MSBF markedly restored normal hepatic architecture by reducing necrotic lesions and lipid deposition, with the highest dose of MSEAE exhibiting greater hepatoprotective activity than MSBF. These improvements are consistent with observed normalisation of serum ALT, AST, and ALP levels, indicating attenuation of diabetes-associated hepatic injury. Such hepatoprotective effects have been attributed to antioxidant and anti-inflammatory properties of plant-derived phenolics and flavonoids [30].

Renal histopathology (Fig. 6) further supported nephroprotective potential of the treatments. DC group showed distorted nephron architecture, glomerular abnormalities, and prominent endocytic vacuoles, all of which are characteristic features of diabetic nephropathy. Administration of MSEAE and MSBF improved renal morphology by restoring glomerular integrity and tubular organisation while reducing vacuolar degeneration. These structural improvements correlated well with significant reductions in serum creatinine and urea levels observed after treatment, suggesting preservation of renal function. Similar renoprotective effects of phytochemical-rich plant extracts have been reported in experimental diabetic models through the suppression of oxidative stress, inflammation, and apoptosis [31, 32].

Collectively, the histopathological findings (Figs. 4–6) corroborate biochemical and antihyperglycemic results, demonstrating that MSEAE and MSBF effectively mitigate STZ-induced tissue damage in pancreas, liver, and kidneys. Although their protective effects were slightly less pronounced than those of glibenclamide, substantial restoration of tissue architecture indicates that M. suriga exerts significant organ-protective activity, likely mediated by its bioactive phytoconstituents with antioxidant and cytoprotective properties.

CONCLUSION

The present study demonstrates that Mammea suriga leaf ethyl acetate extract (MSEAE) and butanol fraction (MSBF) possess promising antidiabetic activity through inhibition of α-amylase and α-glucosidase, leading to improved glycemic control. In STZ induced diabetic rats, both treatments significantly reduced blood glucose levels, improved body weight, normalised lipid profile, and restored liver and kidney function. Histopathological findings further confirmed their protective effects by preserving the structural integrity of pancreas, liver, and kidneys. Among the tested samples, MSEAE exhibited greater therapeutic efficacy than MSBF, although both were less potent than glibenclamide. Overall, these findings suggest that M. suriga is a promising source of natural antidiabetic agents with no side effects and assures further investigation to isolate its bioactive constituents and elucidate their molecular mechanisms before clinical application.

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