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PHYTOCHEMICAL STUDIES, TOXICITY STUDIES, ANTI-DIABETIC AND HAEMOSTATIC EFFECT OF AQUEOUS EXTRACT OF MUSA PARADISIACA STEM ON ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Musa paradisiaca is used in folklore medicine as antiulcer, antidiabetic, antimicrobial, anti-helminthic, anti-dysentery, antimalaria and anti-snake venom. The index study evaluated the antidiabetic, haemostatic properties and subacute toxicity profile, of aqueous stem extract of *Musa paradisiaca* (ASEMP) in rats. In acute toxicity test, animals were grouped (n=5/group) and were treated with varying doses of the stem extract: 500mg/kg, 1000mg/kg and 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg using the method described by OECD. Following induction of diabetes with intraperitoneal alloxan injection, rats were grouped (n=6/group) for subacute toxicity and haemostatic studies. Group 1 served as the normal control while groups 2, 3 were negative and positive controls respectively while groups 4, 5 and 6 received 150mg/kg, 300mg/kg and 600mg/kg of ASEMP via daily gavage for 14 days respectively. All analysis were done using standard laboratory methods. In acute toxicity test, no signs of toxicity and no death was recorded. Animals evaluated for biomarkers: liver, kidney, lipid profile, haematological and antioxidant parameters did not show any sign of toxicity. Animals treated with ASEMP showed lesser clotting and bleeding time when compared to the normal control group. This study shows that ASEMP is safe with potent antibleeding properties.

KEYWORDS: *Musa paradisiaca*, lipid profile, alloxan, diabetes, haemostatic.

INTRODUCTION

Before the advent of conventional medicine, traditional medicine, especially herbal medicine, was dominant globally (Li and Weng 2017). Once dismissed as pseudoscience and met with derision, traditional medicine is experiencing a revival (Amaeze et al. 2018), likely due to factors such as cost, limited access to conventional treatments, antibiotics resistance, and the perceived safety of herbal remedies stemming from their natural origins (Ajijolakewu et al., 2021). Approximately 80% of the global population utilizes herbal medicines (Latif and Nawaz, 2025), and the medicinal plant market was valued at $201 billion in 2023 and is projected to grow from $215.4 billion in 2024 to $375.6 billion by 2032, (Sahar et al, 2025). This illustrates the renewed acceptance that herbal medicine is receiving. While traditional medicine often emphasizes the use of herbs, roots, stems, rhizomes, and barks, fruits and plants also have a significant history of application (Nirumand et al. 2018). Today, these plant parts are considered an inexhaustible and endless source of novel pharmacological agents for the development of new medicines and bioactive foods. For example, many conventional drugs such as morphine and codeine (opioid pain killers), artemisinin and quinine (anti-malaria), vinblastine (anti-cancer), and aspirin (non-steroidal anti-inflammatory drug) (Ukwubile et al., 2023; Ugbogu et al., 2024), paclitaxel (anti-neoplastic) and ephedrine (treatment of asthma) (Renuka et al, 2024), among others, are produced from various medicinal plants.

Diabetes mellitus which is an array of signs and symptoms resulting from defective metabolism of carbohydrates, lipids and proteins is a global health challenge with the number of individuals living with the disease rising from 200 million in 1990 to 830 million in 2022 (WHO, 2024). The prevalence of diabetes has risen more rapidly in the second and third world countries and resulted in the death of 2 million people in 2021 (GBD,

2024). In diabetes, the β-cells of the islets of Langerhans either fails to produce insulin, a fifty-one amino acid peptide hormone produced by the pancreas that regulates glucose level in the body or the cells are insensitive to insulin resulting in a series of metabolic disorders (ADA, 2021; Mukasa, 2024). The defective metabolism of carbohydrate, lipid and protein seen in diabetes mellitus results in hyperglycaemia which remains the cardinal feature of diabetes. Complications of diabetes include nephropathy, retinopathy, vasculopathy, diabetic foot ulcer and gangrene, diabetic ketoacidosis, hyperosmolar non-ketotic coma and obstetric complications while polydipsia, polyuria, nocturia, weight loss and recurrent immunosuppression predisposing to opportunistic infections such as recalcitrant candidiasis are some of the clinical findings seen in diabetic patients. (Ojo et al., 2022; Pickering et al., 2018). This array of clinical features and complications of diabetes mellitus according to Ojo et al, 2022 causes over 1 million global deaths yearly. It is generally classified into type 1 diabetes mellitus (juvenile onset diabetes mellitus of insulin dependent diabetes mellitus) and type 2 diabetes mellitus (adult-onset diabetes mellitus of non-insulin dependent diabetes) based on the pathogenesis and age group affected. The prevalence of type 2 diabetes mellitus is on a steady rise, with an approximately 462 million individuals corresponding to 6.28% of the world's population affected globally. This is equivalent to a prevalence rate of 6,059 cases per 100,00 and is expected to rise to 7,079 individuals per 100,000 by 2030 (Xin et al, 2025.

Musa paradisiaca which is commonly known as plantain is an herbaceous perennial monocotyledonous plant which belongs to the family of Musacaceae (Ekweogu et al, 2024; Ugbogu et al, 2018). Plantain serves both nutritional and therapeutic roles which include wound healing, antiulcer, antidiabetic, antimicrobial, anti-helminthic, anti-dysentery, antimalaria and anti-snake venom properties. They are used to treat warts, reduce inflammations and to make poultice for wounds which reduces pain and swelling (Abdel-Aziz et al, 2020; Cheng et al, 2020.). Pseudo stem of Musa acuminata which is a hybrid of Musa paradisiaca is used in folklore medicine to treat diahorrhea and abdominal discomfort (Uwakwe et al, 2023). These ethnopharmacological properties of *Musa paradisiaca* have been shown by various scientists to be due to the presence of various bioactive compounds in the various parts of the plant (Behiry et al, 2019). Plantain is rich in polyphenols, alkaloids, saponins, flavonoids and tannins (Nurlila et al, 2024; Amutha and Selvakumari, 2016). Previous works of (Abdel-Aziz, 2020 and Behiry et al, 2019) demonstrated the presence of octadecadienoic acid, hexadecenoic acid, - sitosterol, vitamin E, octadecanamide, gallic acid and myricelin. Despite the plurality of studies on the ethnopharmacological potentials of *Musa paradisiaca*, there is paucity of scientific studies on its toxicity, antidiabetic and haemostatic effects. This therefore formed the basis of this study which is to evaluate the toxicity profile, anti-inflammatory and wound healing properties of *Musa paradisiaca* stem on wistar rats.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The stem of Musa paradisiaca was harvested from Okohia farmland in Ideato South Local Government of Imo State and identified in the Department of Plant Science and Biotechnology, Imo State University, Owerri. Ethical clearance was obtained from the Ethical Committee of Animal Care Use of the Faculty of Basic Medical Sciences, Imo State University, Owerri for the use of these animals.

Sample Preparation

Freshly harvested M. paradisiaca stem was cut into tiny pieces, sorted, washed, and air-dried for 24 hours. In line with the local herbal medicine preparation, 1kg of the cut stem was boiled in a flask containing 2 litres of water for 1 hour. After cooling, the boiled extract was filtered using a Whatmann No1. The filtrate was freeze dried at -8^0 C and then stored at 8^0 C until when the need for use arises.

Phytochemical Screening of the Extract and Fractions

Qualitative and quantitative phytochemical screening of ASEPA and fractions were carried out according to the procedures outlined by Harborne (1998) to determine the presence and concentrations of the secondary metabolites.

Animal Experiment

Thirty healthy albino rats with weight between 140-200g were bought from the laboratory animal production unit of the Department of Medical Biochemistry, Imo State University, Owerri. Animals were kept in metal cages in a photoperiod cycle of 12h:12h (light and dark), at a room temperature of 28^0C and fed with standard rat chow and clean drinking water for a period of seven days during which they were allowed to acclimatize. Internationally accepted standards for the use of animals for research were observed throughout the duration of the experiment. Ethical approval for this research was granted by Faculty of Basic Medical Sciences, Imo State University ethics committee.

Acute Toxicity Test

Acute toxicity studies were carried out using the methodology described by OECD guideline 243 (OECD 2001; OECD 2008). Research animals (n=3/group) received a singular oral dosage of ESEPA (0.5g/kg, 1g/kg, 2g/kg, 3g/kg, 4g/kg and 5g/kg) after experimental animals were fasted for 4 hours following which the animals were observed for toxicity signs or death over a 24 hour period and then an additional 14-day observation period.

Induction of diabetes/Determination of Blood Sugar Levels

Animals were fasted overnight and their blood glucose level determined to make sure that their blood glucose level falls within normal range. Except the normal control group animals, others received via intraperitoneal injection, a single dose of 170mg/kg body weight of alloxan monohydrate. Daily fasting glucose level of animals were checked for 7 consecutive days using an Accu-check glucometer manufactured by Roche Diabetes Care Limited, United Kingdom and animals with a stable fasting blood glucose level of 120mg/dl and above were used for the research. Blood sugar levels were checked post-induction on day 0 day 7 and days 14.

Animal grouping

The experimental animals were divided into six (6) groups of six (6) rats per group.

Group I: normal control group (no treatment)

Group II: negative control rats (diabetes induced rats, no treatment)

Group III: diabetes + 3mg/kg glibenclamide daily

Group IV: diabetes + 150mg/kg body weight of *Musa paradisiaca* stem extract 2 times daily

Group V: diabetes + 300mg/kg body weight of *Musa paradisiaca* stem extract 2 times daily

Group IV: diabetes + 600mg/kg body weight of *Musa paradisiaca* stemextract 2 times daily

Subacute Toxicity Study

The animals used for this research were split into six groups of four rats per group. Except group 1 animals that served as normal control and received water and rat chow, groups 2-6 were induced with diabetes. Group 2 alloxan induced diabetic rats received water and rat chow and served as negative control. Group 3 diabetic animals received 3mg/kg of Glibenclamide while groups four, five and six diabetic rats received 150mg/kg, 300mg/kg and 600mg/kg of AEMPS respectively for 14 days via oral gavage. After the 14-day AEMPS treatment, animals were fasted overnight and were sacrificed by dislocation of the cervical bones. Blood samples obtained by puncturing the cardiac muscles were introduced into EDTA containers and plain bottles for haematology and clinical chemistry analysis respectively. Body weight of rats were determined on days 0, 7 and 14 of the experiment.

Blood Analysis

Packed cell volume (PCV), haemoglobin (Hb) level, red blood cell (RBC) indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemogloblin concentration (MCHC), platelet level and white blood cell (WBC) counts were determined using an automated hematology analyzer (BC-2300 model, Mindal Medical Co. China) using guidelines provided by the manufacturers.

Biochemical Analysis

Liver function tests (ALT, AST, ALP, albumin, total bilirubin, globulin and total protein), kidney profile (serum urea, creatinine and electrolytes: Na^+, K^+, HCO^{3-} and Cl^-) and lipid profile (triacylglycerol, low density lipoprotein cholesterol, very low-density lipoprotein cholesterol, high density lipoprotein cholesterol and total cholesterol) were determined using standard laboratory tools made available by Randox laboratory ltd co, Antrim, United Kingdom. The method described by Kanu et al. (2016), used to assess the various antioxidant parameters: malondialdehyde, catalase, glutathione peroxidase and superoxide dismutase. Blood sugar levels was determined using ACCU-CHECK glucometer (Roche Diabetes Care Limited, UK).

Method for Haemostasis

Thirty-two (32) mice were purchased and fed with standard pellets and water ad libitum. The animals were divided into 6 groups of 4 animals each as follows: groups 1, 2 and 3 served as normal, negative and positive controls and the animals received normal saline, alloxan and glibenclamide (3 mg/kg) respectively. Groups 4, 5 and 6 received 150,300 and 600 mg/kg AEMPS respectively. Each tail of the animals was cut at 2 cm from the tip and tail lesion blotted with filter paper and weighed to determine the volume of bleeding. The interval from the time of the tail incision to the time that blood no longer dropped on the filter paper was recorded as the bleeding time (seconds) with the aid of stopwatch (Akanji, 2023).

Statistical analysis

A one-way analysis of variance (ANOVA) and a Duncan multiple range comparison tests were carried out on the data generated from the animal study, with a statistical significance difference attained at a 95 % confidence level ($P < 0.05$) while the data for the body weight changes and blood sugar level were analysed using two-way ANOVA. Statistical Products and Service Solutions (SPSS) version 22 was used for the data analysis.

RESULTS

Table 1: Phytochemical screening of the aqueous extract of *Solanum macrocarpon* fruit.

Phytochemicals	Qualitative test results	Amounts
Alkaloids	++	7.16±0.04
Tannins	+	2.05±0.05
Terpenoids	+	1.42±0.03
Saponins	+	3.01±0.05
Flavonoids	++	5.09±0.09

Phenols	++	5.66±0.06
Steroids	+	0.38±0.03

Key
+++ = present in high amount; ++ = present in moderate amount; + = present in little amount
- = not present

Table 2: Acute (oral) toxicity study of rats after 24 h administration of *M. paradisiaca* stem extract.

Dose (mg/kg)	Death
0	0/5
500	0/5
1,000	0/5
2,000	0/5
3,000	0/5
4,000	0/5
5,000	0/5

No mortality was observed across the groups administered graded single oral doses of the extract, even at 5000 mg/kg body weight treatment level, suggesting that the lethal dose value of the extract may be above 5000 mg/kg body weight. However, behavioural and tactic changes observed during the test are stated in the tables below:

Table 3: Body weight changes in rats treated with ASEMP.

Treatment groups	Pre-induction body weight (g)	Post-induction body weight (g)	Post-treatment/final body weight (g)
Normal control	128.10±5.24^{a}	133.20±6.56^{b}	142.30±7.40^{b}
Diabetic control	128.93±7.64^{a}	127.47±7.25^{a,b}	130.10±6.93^{a}
Diabetic + Glibenclamide (3 mg/kg body weight)	126.47±5.91^{a}	125.20±5.94^{a,b}	133.27±5.87^{a,b}
Diabetic + MP extract (150 mg/kg body weight)	129.20±1.41^{a}	127.83±0.68^{a,b}	131.23±1.82^{a}
Diabetic + MP extract (300 mg/kg body weight)	123.13±3.45^{a}	121.93±3.55^{a}	126.00±3.48^{a}
Diabetic + MP extract (600 mg/kg body weight)	126.90±3.16^{a}	125.63±2.97^{a,b}	130.73±4.05^{a}

Values are presented as mean ± standard deviation (n = 6), and values with different letter superscripts are significantly (P < 0.05) different within the column.

Table 4 shows the effects of ASEMP on the body weight of rats. No significant change (P > 0.05) in weight was seen on both day 7 and day 14 when animals treated with 150mg/kg, 300mg/kg and 600mg/kg were compared with the positive control.

Table 4: Effect of ASEMP on blood sugar levels in rats.

Treatment groups	Pre-induction blood sugar level (mg/dl)	Post-induction blood sugar level (mg/dl)	Day 7 Post-treatment blood sugar level (mg/dl)	Day 14 Post-treatment blood sugar level (mg/dl)	Percentage fall in blood sugar level
Normal control	77.33±2.52^{a}	78.33±3.51^{a}	77.67±2.08^{a}	78.00±2.00^{a}	0.00±0.00^{a}
Diabetic control	78.00±4.58^{a}	331.67±48.76^{c}	333.67±47.96^{c}	337.33±49.10^{c}	-1.72±1.31^{a}
Diabetic + Glibenclamide (3 mg/kg body weight)	79.00±1.00^{a}	312.00±74.91^{b,c}	138.00±8.54^{b}	127.67±5.69^{b}	57.42±10.53^{d}
Diabetic + MP extract (150 mg/kg body weight)	83.67±5.03^{a}	230.33±34.99^{b}	184.33±29.50^{b}	148.33±4.04^{b}	34.45±11.36^{b}
Diabetic + MP extract (300 mg/kg body weight)	81.67±4.93^{a}	246.00±17.52^{b}	171.33±2.08^{b}	143.67±6.03^{b}	41.28±6.62^{b,c}

Diabetic + MP extract (600 mg/kg body weight)	80.33±3.51[a]	281.00±41.94[b,c]	176.67±16.65[b]	139.67±10.02[b]	49.83±4.99[c,d]

Values are presented as mean ± standard deviation (n = 6), and values with different letter superscripts are significantly (P < 0.05) different within the column.

Table 4 shows the effects of ASEMP on the blood sugar levels of rats. Percentage fall in blood sugar seen in animals treated with the maximum dose of 600mg/kg is favourably comparable with that seen in group III animals treated with the orthodox glibenclamide. Also, percentage fall in blood sugar is significantly higher (P < 0.05) in rats treated with 150mg/kg and 300mg/kg when compared with the diabetic control group.

Table 5: Effect of ASEMP on Haematological parameters in rats.

Treatment groups	Normal control	Diabetic control	Diabetic + Glibenclamide (3 mg/kg body weight)	Diabetic + MP extract (150 mg/kg body weight)	Diabetic + MP extract (300 mg/kg body weight)	Diabetic + MP extract (600 mg/kg body weight)
RBC (x106/mm3)	7.02±0.09[d]	4.99±0.12[a]	6.12±0.14[c]	5.51±0.11[b]	5.38±0.12[b]	5.63±0.27[b]
PCV (%)	44.67±0.58[d]	36.33±0.58[a]	41.00±1.00[c]	38.67±0.58[b]	38.33±0.58[b]	39.00±1.00[b]
Hb (g/dl)	15.33±0.47[d]	11.73±0.25[a]	13.50±0.30[c]	12.70±0.26[b]	12.67±0.31[b]	13.00±0.50[b,c]
WBC ($x10^3/mm^3$)	9.05±0.09[d]	7.73±0.15[a]	8.10±0.19[b]	8.28±0.14[b,c]	8.46±0.16[c]	8.52±0.20[c]
PLT ($x10^3/mm^3$)	360.33±6.11[a]	374.33±5.03[a,b]	361.67±4.04[a,b]	380.33±3.32[b]	403.33±10.60[c]	415.33±20.11[c]
MCV (fl)	63.66±0.23[a]	72.87±0.69[e]	66.99±0.21[b]	70.18±0.57[c,d]	71.26±0.57[d]	69.28±1.77[c]
MCH (pg)	21.89±0.41[a]	23.53±0.10[b]	22.06±0.06[a]	23.05±0.23[b]	23.54±0.10[b]	23.09±0.41[b]
MCHC (g/dl)	34.32±0.64[c]	32.29±0.30[a]	32.93±0.07[a,b]	32.85±0.51[a,b]	33.04±0.37[a,b]	33.33±0.43[b]

Values are presented as mean ± standard deviation (n = 6), and values with different letter superscripts are significantly (P < 0.05) different across the row.

RBC, Red Blood Cells; PCV, Packed Cell Volume; Hb, Haemoglobin; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; WBC, White Blood Cell; PLT, Platelet.

Effects of ASEMP in rats is shown in table 5. There was a significant increase (P < 0.05) in the levels of RBC, PCV, Hb and WBC when ASEMP treated groups are compared with negative control groups. Platelet levels in 300mg/kg and 600mg/kg treated groups are also significantly higher than both the positive and negative control groups.

Table 6: Effect of ASEMP on Liver Function parameters in rats.

Treatment groups	Normal control	Diabetic control	Diabetic + Glibenclamide (3 mg/kg body weight)	Diabetic + MP extract (150 mg/kg body weight)	Diabetic + MP extract (300 mg/kg body weight)	Diabetic + MP extract (600 mg/kg body weight)
Total protein (g/dl)	5.96±0.11[c]	5.44±0.15[a]	5.80±0.07[b,c]	5.64±0.17[a,b]	5.69±0.10[b]	5.83±0.12[b,c]
Albumin (g/dl)	3.21±0.03[c]	3.07±0.04[a]	3.20±0.07[b,c]	3.11±0.08[a,b]	3.10±0.04[a,b]	3.13±0.03[a,b,c]
Globulin (g/dl)	2.74±0.09[b]	2.36±0.15[a]	2.60±0.06[a,b]	2.53±0.20[a,b]	2.59±0.10[a,b]	2.69±0.15[b]
ALT (U/L)	25.33±1.16[a]	88.00±4.00[d]	43.67±4.04[b]	51.67±2.31[c]	48.67±1.53[b,c]	45.00±4.58[b]
AST (U/L)	35.00±3.00[a]	105.33±9.90[d]	52.00±3.00[b]	63.67±5.13[c]	60.33±1.53[b,c]	60.00±6.25[b,c]
ALP (U/L)	79.00±4.58[a]	170.67±2.08[d]	94.67±2.52[b]	108.67±3.51[c]	102.33±2.08[b,c]	101.67±7.37[b,c]
Total bilirubin (mg/dl)	0.60±0.04[a]	1.37±0.10[c]	0.83±0.04[b]	0.90±0.04[b]	0.85±0.02[b]	0.87±0.04[b]

Values are presented as mean ± standard deviation (n = 6), and values with different letter superscripts are significantly (P < 0.05) different across the row.

AST, Aspartate Aminotransferase; ALT, Alanine Transaminase; ALP, Alkaline Phosphatase Changes in liver function parameters seen in ASEMP treated rats are shown in table 6. Animals treated with ASEMP showed a statistically significant lower levels of ALT, AST ALP

and total bilirubin when compared with the diabetic control. However, 150mg/kg and 300mg/kg ASEMP-treated groups showed no significant change (P >0.05) in albumin and globulin when compared to the negative control.

Table 7: Effect of ASEMP on Renal Function parameters in rats.

Treatment groups	Normal control	Diabetic control	Diabetic + Glibenclamide (3 mg/kg body weight)	Diabetic + MP extract (150 mg/kg body weight)	Diabetic + MP extract (300 mg/kg body weight)	Diabetic + MP extract (600 mg/kg body weight)
Urea (mg/dl)	18.16 ± 0.39^{a}	28.99 ± 1.27^{c}	21.21 ± 0.82^{b}	21.95 ± 1.24^{b}	20.30 ± 0.19^{b}	20.39 ± 0.79^{b}
Creatinine (mg/dl)	0.70 ± 0.04^{a}	1.26 ± 0.08^{c}	0.89 ± 0.05^{b}	0.89 ± 0.02^{b}	0.82 ± 0.02^{b}	0.84 ± 0.06^{b}
Na^{+} (mEq/L)	$128.43\pm2.72^{b,c}$	120.73 ± 2.36^{a}	126.13 ± 1.27^{b}	$127.23\pm1.93^{b,c}$	$128.80\pm3.30^{b,c}$	131.20 ± 1.04^{c}
K^{+} (mEq/L)	4.44 ± 0.12^{b}	4.08 ± 0.05^{a}	4.42 ± 0.08^{b}	4.38 ± 0.10^{b}	4.40 ± 0.05^{b}	4.61 ± 0.07^{c}
Cl^{-} (mEq/L)	89.63 ± 1.50^{b}	84.67 ± 1.04^{a}	86.40 ± 1.93^{a}	$86.90\pm2.43^{a,b}$	$87.57\pm0.95^{a,b}$	$87.63\pm0.90^{a,b}$
HCO_3^{-} (mEq/L)	19.83 ± 0.15^{a}	20.27 ± 0.12^{b}	$19.97\pm0.15^{a,b}$	$19.97\pm0.21^{a,b}$	19.83 ± 0.15^{a}	19.90 ± 0.17^{a}

Values are presented as mean ± standard deviation (n = 6), and values with different letter superscripts are significantly (P < 0.05) different across the row.

Table 7 shows the effects of ASEMP on the renal function parameters of rats. The table above shows a significant decrease in the levels of urea and creatinine when extract treated groups are compared with the group II diabetic rats.

Table 8: Effect of ASEMP on Lipid Profile parameters in rats.

Treatment groups	TC (mg/dl)	HDL-C (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Normal control	93.60 ± 3.63^{a}	63.00 ± 1.25^{c}	80.57 ± 1.82^{a}	14.49 ± 2.66^{a}	16.11 ± 0.36^{a}
Diabetic control	113.27 ± 2.35^{c}	58.50 ± 2.54^{a}	121.60 ± 5.63^{c}	30.45 ± 2.75^{b}	24.32 ± 1.12^{c}
Diabetic + Glibenclamide (3 mg/kg body weight)	99.47 ± 2.46^{b}	$60.20\pm1.25^{a,b}$	101.20 ± 8.14^{b}	19.03 ± 1.05^{a}	20.24 ± 1.63^{b}
Diabetic + MP extract (150 mg/kg body weight)	$98.20\pm2.42^{a,b}$	$61.23\pm0.31^{b,c}$	97.17 ± 5.98^{b}	17.53 ± 3.69^{a}	19.43 ± 1.20^{b}
Diabetic + MP extract (300 mg/kg body weight)	$96.70\pm3.10^{a,b}$	$60.90\pm0.96^{a,b,c}$	95.40 ± 2.91^{b}	16.72 ± 3.68^{a}	19.08 ± 0.58^{b}
Diabetic + MP extract (600 mg/kg body weight)	94.33 ± 1.65^{a}	$61.03\pm0.57^{a,b,c}$	94.23 ± 3.42^{b}	14.45 ± 1.88^{a}	18.85 ± 0.68^{b}

Values are presented as mean ± standard deviation (n = 6), and values with different letter superscripts are significantly (P < 0.05) different within the column.

TC Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; TAG, Triacylglycerol; LDL-C, Low-Density Lipoprotein Cholesterol; VLDL-C, Very Low-Density Lipoprotein Cholesterol.

Table 8 shows the effects of ASEMP on lipid profile parameters in rats. Treatment of experimental animals with extract led to a significant decrease (P < 0.05) in the levels of total cholesterol, TAG, LDL-C and VLDL-C when compared with the negative control group. However, treatment with various dosages of ASEMP significantly increased the level of HDL-C when compared with the diabetic group.

Table 9: Effect of ASEMP on serum Antioxidant parameters in rats.

Treatment groups	GPx (u/l)	SOD (u/l)	CAT (mg/dl)	MDA (mMol/L)
Normal control	49.67 ± 2.52^{d}	37.67 ± 1.53^{c}	24.67 ± 2.31^{b}	0.33 ± 0.02^{a}
Diabetic control	39.33 ± 1.16^{a}	30.67 ± 1.16^{a}	19.67 ± 1.52^{a}	0.68 ± 0.11^{b}
Diabetic + Glibenclamide (3 mg/kg body weight)	$42.00\pm2.00^{a,b}$	35.00 ± 1.72^{b}	22.67 ± 0.58^{b}	0.41 ± 0.02^{a}
Diabetic + MP extract (150 mg/kg body weight)	$43.33\pm1.52^{b,c}$	33.67 ± 1.53^{b}	22.33 ± 1.16^{b}	0.42 ± 0.04^{a}
Diabetic + MP extract (300 mg/kg body weight)	45.67 ± 2.08^{c}	$35.33\pm0.58^{b,c}$	23.67 ± 1.52^{b}	0.40 ± 0.05^{a}
Diabetic + MP extract (600 mg/kg body weight)	$46.67\pm1.53^{c,d}$	$35.33\pm1.16^{b,c}$	24.33 ± 0.58^{b}	0.37 ± 0.03^{a}

Values are presented as mean ± standard deviation (n = 6), and values with different letter superscripts are significantly (P < 0.05) different across the row.

Group. GSH, Glutathione; SOD, Superoxide Dismutase; CAT, Catalase; MDA, Malondialdehyde; GPx, Glutathione Peroxidase

Table 9 is the effects of ASEMP on antioxidant parameters in wistar rats. Animals treated with 150mg/kg, 300mg/kg and 600mg/kg of ASEMP showed a statistically significant decrease in the levels of GPx, SOD and CAT when compared to group 2 animals. The levels of MDA decreased in positive control and extract treated groups when compared with the negative control.

Table 10: Effect of ASEMP on bleeding and clotting times in rats (Haemostatic Tests).

Treatment groups	Clotting time (seconds)	Bleeding time (seconds)
Normal control	58.67±3.51^{d}	69.33±3.52^{c}
Diabetic control	45.67±3.51^{b}	64.33±5.03^{c}
Diabetic + Glibenclamide (3 mg/kg body weight)	51.33±1.16^{c}	56.67±4.73^{b}
Diabetic + MP extract (150 mg/kg body weight)	39.33±3.22^{a}	55.67±3.06^{a,b}
Diabetic + MP extract (300 mg/kg body weight)	35.00±2.00^{a}	53.00±3.61^{a,b}
Diabetic + MP extract (600 mg/kg body weight)	39.67±2.52^{a}	49.00±3.00^{a}

Values are presented as mean ± standard deviation (n = 4), and values with different letter superscripts are significantly ($P < 0.05$) different across the row.

Changes in bleeding and clotting time in rats treated with ASEMP is shown in table 10. ASEMP-treated groups showed a statistically significant decrease ($P < 0.05$) in both the clotting and bleeding time when compared with the diabetic and normal control group.

DISCUSSION

In the recent past, there has been a drift towards the use of plant based formulations as against orthodox medications in the management of various diseases especially those without officially established curative treatment such as diabetes mellitus and bleeding disorders. (Nwankpa et al, 2025; Weremfo et al, 2011). Present and previous studies have shown that the stem, leaves and fruits of *Musa paradisiaca* have previously been shown by various researchers to contain phytoconstituents such as saponins, alkaloids, tannins phenols which are effective against hyperglycaemia, obesity, pain, arthritis and a form of blood cancer called acute myelogenous leukemia. (Abdel-aziz et al 2020). Different parts of *Musa paradisiaca* and other plants are therefore employed as raw materials for the formulation of different classes of drugs, be it anticancer, antimalaria, antifungal, antiviral and antiulcer medications (Ngbolua et al, 2019; Ekweogu et al 2024). This preference for the current use of herbal formulations is based on the fact that the bioactive compounds present in these plants cause little or no side effects and complications, these plants are relatively available, accessible and affordable to the natives that use them. The present study therefore aimed to assess the toxicity, antidiabetic and antibleeding properties of Musa paradisiaca in rats.

Acute toxicity study involves the treatment of animals with varying doses of a plant extract to establish the safety of such a plant material before human trial (Nakakawaal et al, 2023; Uche et al, 2024). In this study, experimental rats did not show any features of acute toxicity and no deaths were recorded when the animals were exposed to the graded doses of ASEMP (10mg/kg to 5000mg/kg). From our findings, the LD50 of the ASEMP is greater than 5000mg/kg following the method described by OECD, 2001 and is safe, non-toxic and can be used for therapeutic purposes.(OECD, 2008; Ugbogu et al, 2018). Besides mortality and behavioral changes which serve as important parameters for assessment of toxicity, changes in body weight of animals also serve as viable indicator of exposure of animals to toxic substances (Michael et al., 2007). Previous findings have shown that body weight loss may be used to calibrate the level of toxicity of a plant material (Ekweogu et al, 2019). In the index study, animals treated with ASEMP did not lose weight throughout the course of the experiment implying that ASEMP is non-toxic.

The hypoglycaemic effects of ASEMP in alloxan induced diabetic animals were shown by steady increase in the percentage fall in blood glucose level of animals treated with ASEMP. The percentage fall in blood glucose level seen in rats that received 600mg/kg of extract is comparable to the levels seen in animals that received the standard oral antidiabetic drug, glibenclamide. The antidiabetic capability seen in our study is similar to that of Ajay Singh Bisht et al 2017. The phenolic and flavanoidal compounds are present in stem part of the *Musa paradisiaca* can inhibit both alpha amylase and alpha glucosidase enzymes in vitro in a dose dependent manner and bring about reduction in blood sugar level in rats. The antidiabetic effect of *M. paradisiaca* is probably due to the presence of tannins, alkaloids which are known to enhance secretion of insulin, like sulphonyl ureas (glibenclamide) (Suneetha et al, 2010).

Blood parameters such as Hb, RBC, M CV, MCH, MCHC and platelets are viable tools for monitoring the adverse effects of drugs and other xenobiotics as well as diseases such as anemia, thrombocytopenia, leucopenia, leukaemia. Blood parameters such as MCV, HB, PCV, RBC, MCH and MCHC are often used to monitor animal and human progress during treatment for anemia.(Uche et al, 2024. Treatment with ASEMP did not disrupt the haematopoetic machinery of treated rats as evidenced by increase in the levels of RBC, PCV and Hb in animals

that received the extract (Asuquo and Udobi, 2022). Antioxidant capability of ASEMP may be responsible for the haematopoeitic effects since free radical attack has been previously linked to the impairment of blood forming process (Umeh et al, 2024).

The liver is the main site for the metabolism of most biochemical compounds such as carbohydrates, proteins, haem, secretion of bile, storage of iron and xenobiotic detoxification. Diseases such as Liver cirrhosis, fibrosis, hepatitis and jaundice become manifest when the liver is damaged from intake of toxins (Adeyemi et al, 2015; Ugbogu et al, 2024). These results in the elevation of AST, ALT, ALP, bilirubin while levels of globulin, albumin and total protein decrease. In the index study, animals treated with ASEMP showed a statistically significant lower levels of ALT, AST ALP and total bilirubin when compared with the diabetic control while 150mg/kg and 300mg/kg ASEMP-treated groups showed no significant change ($P > 0.05$) in albumin and globulin when compared to the negative control. Therefore, ASEMP is hepatoprotective. The findings in this study are similar to those of Ahmed et al., (2021) where treatment of rats with *Musa paradisiaca* extract led to a decrease in AST level in animals with nicotinamide/streptozocin induced diabetes Also, Olorunfemi et al (2010) reported a decrease in the levels of AST, ALT, ALP and conjugated bilirubin when animals that received 1000mg/kg of *Musa paradisiaca* extract were compared with the control animals. Serum urea, creatine and electrolytes such as potassium, sodium, chloride and bicarbonate are all reliable indices used in assessing kidney function.[54] Increased serum urea and creatine are diagnostic of kidney pathology (Ekweogu et al, 2024; Emmanuel et al, 2021). The result of our study shows a significant decrease in the levels of urea and creatinine when extract treated groups are compared with the group II diabetic rats. Olorunfemi et al., (2010) carried out similar research where the administration of 1000mg/kg aqueous extract of *Musa paradisiaca* did not cause any nephrotoxicity in tested animals as was depicted by a significant reduction ($p<0.05$) in the levels of urea, creatinine and sodium in such animals.

Dietary modifications and lifestyle changes such as exercise are among the effective ways of managing dyslipidaemia with its attendant complications such as stroke, coronary artery disease and high blood pressure,diabetes mellitus (Enechi and Ozougwu, 2014) Hyperlipidaemia is associated with elevated levels of serum cholesterol, triacylglycerol, phospholipids and alterations of lipoprotein levels.[50] While elevated HDL protects against coronary heart diseases, LDL, VLDL and serum cholesterol when elevated predisposes to cardiovascular diseases. Treatment of experimental animals with extract led to a significant decrease ($P < 0.05$) in the levels of total cholesterol, TAG, LDL-C and VLDL-C when compared with the negative control group, while treatment with various dosages of ASEMP significantly increased the level of HDL-C implying that ASEMP administration caused hypolipidaemic effect. According to Ahmed et al., (2021), treatment of experimental *rats* with Musa paradisiaca leaf and fruit extracts reversed the elevated total cholesterol, LDL cholesterol, triglyceride seen in rats with nicotinamide/streptozocin induced diabetes *corroborating* the antihyperlipidemic effects of the extract. Ugbogu et al., 2018 also *described* the anti-hyperlipidemic effect of Musa paradisiaca extract by reporting a decrease in the levels of LDL, VLDL and TC in rats treated with aqueous fermented extracts of Musa paradisiaca fruit.

Oxidative damage occurs at an early stage in diabetes, resulting to the appearance of complications such as diabetic ketoacidosis, gangrene, infections, fetal macrosomia, neuropathy and diabetic nephropathy. Hyperglycemia aggravates endothelial ROS generation by a variety of mechanisms such as activation of protein kinase-C isoform (Koya and King, 1998), increased formation of advanced glycation end products (Turko et al, 2001) and increased glucose flux through aldose reductase pathways. These are some of the known biochemical mechanisms of hyperglycemia brings about organ damage. Free radicals can be eliminated by a number of enzymatic and non enzymatic antioxidants and thus protecting tissues and organs against damage from oxidative stress. In the present study, we estimated both enzymatic and nonenzymatic antioxidants in pancreas in vivo. Animals treated with 150mg/kg, 300mg/kg and 600mg/kg of ASEMP showed a statistically significant decrease in the levels of GPx, SOD and CAT when compared to group 2 animals. The levels of MDA decreased in positive control and extract treated groups when compared with the negative control. Findings from this study are also similar to the findings of Ahmed et al., (2021) who reported the antioxidative capabilities of *Musa paradisiaca* leaf and fruit peel in rats which streptozocin induced toxicity.

Haemostasis is the spontaneous arrest of bleeding from damaged blood vessels which is important for initiation of tissue repair processes and prevention of tissue death through haemorrhage (Dapper et al, 2007). The stages for haemostasis include: vasoconstriction, platelet response and blood coagulation. A fourth stage occurs when the clot is dissolved following repair of the blood vessel (Weremfo et al, 2011). Changes in bleeding and clotting time in rats treated with ASEMP was assessed in the index study. ASEMP-treated groups showed a statistically significant decrease ($P < 0.05$) in both the clotting and bleeding time when compared with the diabetic and normal control group. Whereas the clotting time measures the intrinsic clotting factors (I, II, V, VIII, IX, X, XI and XII), bleeding time evaluates the vascular and platelet responses associated with haemostasis (Weremfo et al, 2011).

The significant decrease in clotting time in this study may be indicative of the fact that there was an increase in

one or more of the clotting factors involved in the intrinsic pathway. Also, the marked decrease in bleeding time suggests that the stem juice of *Musa paradisiaca* has positive effect on haemostasis possibly by acting on the integrity of the blood vessel or involvement of platelets forming the haemostatic plug or both. Another plausible explanation may be the inhibition the formation of prostaglandin by the vessel walls during injury. Prostaglandins released during injury are responsible for vessel relaxation, which leads to increased blood loss (Dapper et al, 2007).

CONCLUSION

Acute and subacute toxicity studies demonstrated that aqueous fruit extract of ASEMP is not toxic but safe for use in the treatment of various diseases. Treatment of the animals with the extract for 14 days showed that ASEMP has hepatoprotective, nephron-protective, hypolipidaemic, antioxidant and antidiabetic effects. The phytochemical screening revealed that it contains a wide range of physiologically active chemicals with different pharmacological effects. This study also showed that ASEMP has haemostatic effects. The findings from this study support the use of ASEMP in traditional medicine for the treatment of diabetes and bleeding disorders. We recommend that further long-term studies on anti-diabetic effects of ASEMP and the clinical trial of ASEMP and its bioactive compounds should be ccarried out to determine the therapeutic doses effective for treatment of various ailments.

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