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Evaluation of the anti-diabetic effects of *Tapinanthus preussii* (African mistletoe) aqueous leaf extract in streptozotocin induced diabetic male wistar albino rats

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Abstract
Diabetes mellitus, is a chronic metabolic disorder which has persisted for several decades as a major global public health concern. It is characterized by chronic hyperglycaemia associated with damage, dysfunction and failure of various organs leading to nephropathy, retinopathy, and peripheral neuropathy. This condition is indigenously managed with numerous readily available and affordable medicinal plants but with limited scientific evidence amongst which is *Tapinanthus preussii*. Therefore, this study investigated the anti-diabetic effect of graded dosages of *Tapinanthus preussii* crude aqueous leaf extract, on streptozotocin-induced diabetic male Wistar albino rats. A total of five normal and 25 streptozotocin induced diabetic rats were randomly assigned into six groups of five rats each: Groups 1, 2 and 3 served as normal, negative and positive controls that received water and 250 mg/kg body weight of chlorpropamide (A standard anti-diabetic drug) respectively while groups 4, 5 and 6 received 200, 350 and 500mg/kg body weight of the crude aqueous extract for 28 days. Blood samples were collected at 2,4,6 and 8 hours post administration for the short term study while for the long term study, at 7, 14, 21 and 28 days for the determination of blood glucose concentration. The maximum blood glucose reduction was 17.5% within 2 hours post-administration of 500mg/kg of the aqueous extract, compared to 12.78% for chlorpropamide and 0.38% for the positive negative diabetic control respectively. The 500mg/kg body weight aqueous extract again showed the highest reduction of 59.26% over a 28-day period, outperforming the 17.54% for chlorpropamide. The findings revealed that the extract displayed a stronger ability to lower blood sugar levels than the positive control. Hence, highlighting the dose and time-dependent nature of the anti-diabetic effects, with faster onset of activity observed at high dose of the extract. These findings suggest the potential of *Tapinanthus preussii* as a natural anti-diabetic agent, with promising results in lowering blood glucose levels in diabetic rats. The antidiabetic effects could be attributed to the phytochemicals like flavonoids, carotenoids, anthraquinones alkaloids, tannins, cyanogenic glycosides and saponins present in *Tapinanthus preussii* leaf extract.

Keywords: Diabetes mellitus, African mistletoe, *Tapinanthus preussii*, streptozotocin, chlorpropamide, phytochemicals

1. Introduction
Diabetes mellitus, as defined by the World Health Organization Expert Committee [1], encompasses a range of metabolic hereditary disorders characterized by persistent high blood sugar levels (Hyperglycemia) that exceed the renal threshold, leading to the presence of sugar in urine (Glycosuria). The fasting plasma glucose cut-off level for diagnosis is set at 7.0 mmol/L (126 mg/dl). The hallmark symptoms of chronic diabetes include frequent urination (Polyuria), increased thirst (Polydipsia), heightened hunger (Polyphagia), weight loss, and blurred vision. Severe complications of diabetes include hyperglycemia with ketoacidosis or hyperosmolar syndrome. Long-term effects involve organ damage such as nephropathy, retinopathy, neuropathy, and cardiovascular issues. This condition results from defects in insulin secretion or action within the pancreatic β-cells, influenced by various environmental and genetic factors like age, autoimmune disorders, and obesity. The majority of diabetes cases fall into two main categories: type 1 diabetes, characterized by an absolute insulin deficiency often linked to autoimmune processes and genetic markers; and type 2 diabetes, more common and marked by insulin resistance and inadequate compensatory insulin secretion. Type 2 diabetes can remain asymptomatic for a significant period before

detection through abnormal glucose metabolism tests. The global prevalence of diabetes was 2.8% in 2000 and is estimated to reach 4.4% by 2030, with the number of individuals affected projected to increase from 171 million in 2000 to 366 million in 2030 [2-4]. Various regions report differing prevalence rates of diabetes, with numbers steadily increasing in Africa due to lifestyle changes and urbanization.

Traditional remedies involving over eight hundred medicinal plants and plant-derived products are gaining attention as potential alternatives to conventionally manage diabetes particularly in developing nations [4]. In Nigeria, the utilization of plants and their derivatives, whether in the form of extracts or infusions, is widespread in the treatment and management of various illnesses requiring scientific evaluation to essentially validate their efficacy [5, 6]. Medicinal plants harbor accumulated natural compounds, biologically active substances, and constituents with diverse effects. These active constituents, tend to be synthesized biologically within the plant in minute concentrations relative to the dry material content and accumulate in specific plant parts such as roots, stems, leaves, bark, fruits, and flowers which represent the therapeutic value [7]. Only those plant parts containing active constituents are employed for therapeutic purposes, often administered in the form of extracts, infusions, or decoctions [8].

The African mistletoe encompasses various genera that offer a wide range of medicinal benefits, found in different regions, some of which includes amongst others: Helixanthera, Berhautia, Englerina, Globimetula, Agelanthus, Tapinanthus, and Phragmanthera. They are recognized in West Africa, where mistletoes commonly parasitize economically important crop trees like the shea butter, neem, citrus species, cocoa, rubber, hog-plum, African Rauwolfia, kola-nut, sandpaper, teak, breadfruit as well as various forest trees like Terminalia glaucescens and Ficus mucuso [9]. These partially parasitic plants, have abundant antioxidants, flavonoids, saponins, alkaloids, tannins and glycosides, responsible for their diverse therapeutic qualities. Consequently, the African mistletoe has been the subject of research due to its anti-inflammatory, antidiabetic, and anticancer properties, sparking interest in scientific exploration and potential pharmaceutical advancements [10]. Rynchosphylline, a beneficial alkaloid reported by [11], aids in improving cardiac conditions by lowering blood pressure, enhancing circulation, and inhibiting the accumulation of arteriosclerosis plaque and blood clots. Obatomi *et al*. [12] demonstrated the antidiabetic effects of the Northern Nigerian species of African mistletoe (Loranthus bengwensis). Infusions of mistletoe parasitic on *Citrus limon* and guava trees notably lowered serum glucose levels. However, extracts of Loranthus bengwensis parasitic on Jatropha curcas did not exhibit significant reductions in serum glucose. Variations in host trees have also been linked to the antidiabetic activities of Loranthus micranthus [13]. In Nigeria, numerous herbal preparations derived from mistletoe leaves and twigs, such as *Tapinanthus bangwensis* (Engl. and K. Krause) Danser, have gained popularity for treating various ailments, including diabetes and hypertension, which have been on the rise in the country [14]. Obatomi *et al*. [15] demonstrated the significant reduction of mean arterial pressure by the Northern Nigerian species of African mistletoe in both normotensive and spontaneously hypertensive rats. Terpenoids exhibit antimicrobial properties and antiamoebic activity, with antibacterial and antifungal properties attributed to them as well [16]. Agelanthus dodoneifolius, is another African mistletoe, which is ethnomedicinally utilized by the Hausa and Fulani tribes of Northern Nigeria to remedy various human and animal ailments, including stomach ache, diarrhea, dysentery, wound healing, and cancer [17]. Previous studies indicate that Tapinanthus is the most abundant genus in south western Nigeria, known for its potency in treating circulatory problems and acting as an anticancer agent [18]. Preliminary investigations on the Eastern Nigerian species Loranthus micranthus suggest that non-polar constituents possess anti-inflammatory properties. Osadebe and Ukwueze [19] reported on the antimicrobial activities of Loranthus micranthus parasitic on six host trees, possibly mediated by the lipophilic properties of terpenoids disrupting microbial membranes. The presence of anthraquinone in these plants suggests potential use as a mild laxative, since they are known to increase peristaltic action in the gastrointestinal tract [20]. The identification of these chemical compounds underscores the therapeutic potential of mistletoes in treating various diseases. Investigating the hypoglycemic effects and toxicity levels of plants like *Tapinanthus preussii* in animal models can shed light on their potential role in alleviating diabetes and guide future research into their safety and effectiveness.

Understanding the complex interplay of genetic, environmental, and lifestyle factors in diabetes pathogenesis is crucial for developing effective interventions. By exploring the role of traditional medicinal plants alongside conventional treatments, this can broaden the understanding of diabetes management and potentially uncover novel therapeutic avenues. Investigating the efficacy and safety of plants like *Tapinanthus preussii* in animal models can provide insights into their potential role in treating diabetes. This study was therefore aimed to shed light on the global impact of diabetes, the challenges it poses in diverse populations, the potential of natural remedies and its care.

2. Materials and Methods

2.1 Equipment/Apparatus/chemicals

Accu Chek glucometer and test strips, dissecting kit, syringes, measuring cylinder, beakers, micropipettes, spatula, Gallenkamp water bath, Gallenkamp Muffle furnace, Gallenkamp drying oven, desiccator, Mettler H-80 weighing balance, laboratory mill (Corona, Landers Y CIA, SA), refrigerator (Jouan VX 380E), plastic animal cages, Streptozotocin (sigma chemical Co. USA), distilled water, methylated spirit (Sigma Aldrich Laboratory, Germany)

2.2 Collection, identification and authentication of *Tapinanthus preussii* leaves

A substantial amount of *Tapinanthus preussii* leaves, a type of African mistletoe that parasitizes cocoa (Theobroma cacao), was gathered from a cocoa plantation located in Kumba, within the South West region of Cameroon. These leaves underwent identification and authentication by a botanist at the Limbe Botanic Gardens in the same region, with a voucher specimen being placed in the herbarium for future documentation and reference purposes.

2.3 Preliminary Processing and Extraction of *Tapinanthus preussii* Leaves

The freshly harvested leaves underwent a thorough rinsing with clean water to eliminate any impurities. Subsequently,

they were air-dried in the shade for four days at room temperature, followed by further drying in an oven at 40 °C until a consistent weight was achieved. The dried leaves were then finely ground into powder using an electric grinder. Approximately 250 g of this powdered material was subjected to cold maceration in around 1300 ml of distilled water, with intermittent shaking over a 72-hour period. The resulting mixture was filtered through a sieve and cotton wool as per established protocols [21-23]. The crude aqueous extract obtained was freeze-dried until a constant weight was reached. This extract was subsequently retrieved, reconstituted to appropriate concentrations, and prepared for administration to the experimental animals.

2.4 Experimental Animals

For this study, 30 healthy adult male Wistar albino rats weighing between 200-250g were sourced from the animal facility within the Department of Pharmacology, Faculty of Pharmacy, University of Benin. These rats underwent a two-week acclimatization period in the animal facility, maintained at room temperature (25-28 °C) with a natural light-dark cycle of 12 hours each. They were provided with unrestricted access to water and standard rat feed from Bendel Feed Flour Mill, Ewu, Benin City, Edo State. Prior to the commencement of any experiments, all rats were subjected to a 12-hour fasting period.

2.5 Induction of Diabetes

To induce diabetes, a single intramuscular injection of 60mg of freshly prepared streptozotocin (Sigma, St. Louis, Mo, USA) per kg body weight of the rats was administered, dissolved in 0.1M cold citrate buffer at pH 4.5 as outlined by Zeggwagh *et al*. [24]. Following the injection, the rats were provided with a 5% glucose solution in their cages for the next 24 hours to prevent hypoglycemia. Subsequently, the animals were allowed unrestricted access to food and water for a period of 7 days. After this period, on the 8th day when hyperglycemia had stabilized, rats exhibiting fasting blood glucose levels exceeding 250mg/dl were identified as diabetic and selected for inclusion in the study according to Stanley *et al*. [25].

2.6. Evaluation of *Tapinanthus preussii* Crude Leaf Extracts on Streptozotocin-Induced Diabetic Albino Rats

In this study, twenty-five diabetic rats and five non-diabetic rats were randomly allocated into six groups, each consisting of 5 rats. Group 1 comprised normal rats receiving distilled water, serving as the non-diabetic (normal) control. Group 2 consisted of diabetic rats receiving 1 ml of distilled water, acting as the diabetic control. Group 3, serving as the positive control, included diabetic rats administered 250 mg of chlorpropamide (a standard antidiabetic drug) per kg body weight. Diabetic rats in groups 4, 5, and 6 were given 200, 350, and 500 mg of aqueous leaf extract per kg body weight, respectively. Blood glucose levels were monitored at 0, 2, 4, 6, and 8 hours post-treatment for all groups using the ACCU-CHEK glucometer and test strips. Extract administration continued for 28 days with body weight and fasting blood glucose concentrations recorded on days 7, 14, 21, and 28. After 28 days, the rats were fasted overnight, anesthetized with chloroform vapor, and blood samples were collected from the heart, allowed to clot, centrifuged, and stored at -20 °C for subsequent biochemical analyses. The liver, kidney, spleen, heart, lungs, testis, stomach and pancreas, were excised, weighed, preserved in 10% formal saline.

2.7 Statistical Analysis

The data obtained underwent statistical analysis utilizing SPSS version 23. Results were presented as mean ± SEM. One-way Analysis of Variance (ANOVA) was employed to compare parameter means, with significance set at a 95% confidence level ($p<0.05$) for all treatments in comparison to the control. Duncan's New Multiple Range test was utilized to differentiate between means where significant differences were observed [26].

3. Results

3.1 Induction of Diabetes

Diabetes was confirmed on the seventh day post-administration, with rats exhibiting fasting blood glucose levels exceeding 250 mg/dl being identified as diabetic and selected for the experiment.

3.2 The impact of *Tapinanthus preussii* crude aqueous leaf extract on fasting blood glucose concentration (mg/dl) in Streptozotocin-induced diabetic Wistar albino rats: A Two hourly study

This study investigated the effects of increasing doses (200, 350, and 500mg per kg body weight) of *Tapinanthus preussii* crude aqueous leaf extract, compared with 250mg of chlorpropamide (a standard antidiabetic drug that served as positive control), on blood glucose concentration in Streptozotocin-induced diabetic albino rats over an eight-hour period. The results, illustrated in Figure 3.1, demonstrated varying degrees of antihyperglycemic effects or blood glucose-lowering potentials (6.3%, 8.64%, and 17.5%, respectively) for various doses (200, 350 and 500 mg/kg body weight respectively) of the plant extract compared to both diabetic, positive and normal controls. The percentage maximum reduction in blood glucose levels occurred in the group administered 500mg per kg body weight of *Tapinanthus preussii* aqueous extract, resulting in 17.5% reduction in plasma glucose concentration which was significantly higher compared to the 200 and 350mg/kg extract doses (6.3% and 8.64% respectively), positive control (12.78%), diabetic control (0.38%) and non-diabetic control (4.03%). These findings suggest that, reductions in fasting blood glucose concentrations were found to be both dose and time dependent.

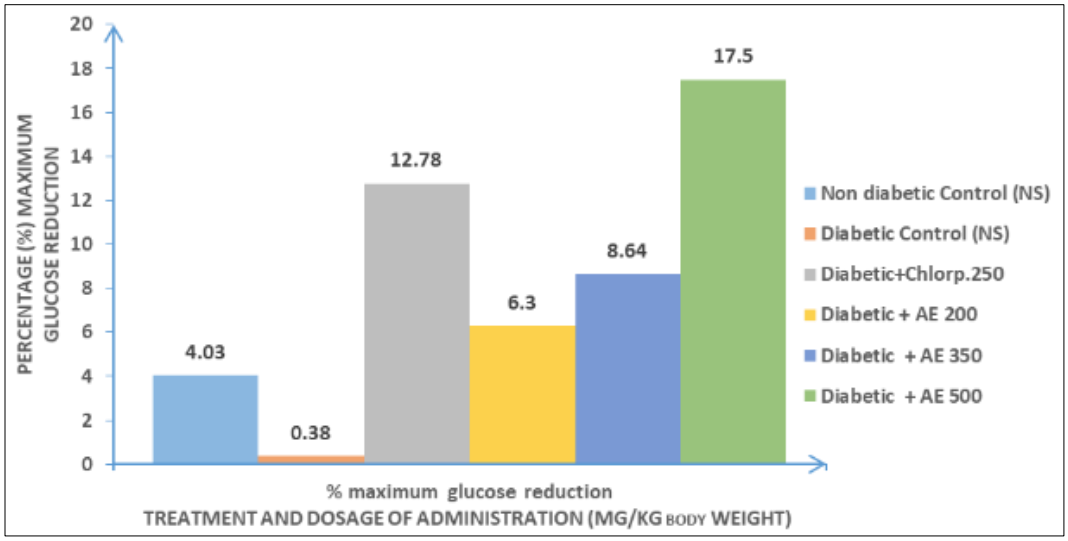

Fig 1: Percentage maximum reduction of fasting blood glucose concentration in streptozotocin induced diabetic Witar albino rats administered *Tapinanthus preussii* crude leaf extract (2 hourly study). NS = normal saline, chlorp. = chlorpropamide, AE = aqueous extract

3.3 The impact of *Tapinanthus preussii* crude aqueous leaf extracts on fasting blood glucose concentration (mg/dl) in streptozotocin-induced diabetic albino rats: A Twenty-eight day study

Figure 3.2 depicts the outcomes of administering increasing doses-200, 350, and 500mg-of *Tapinanthus preussii* crude aqueous leaf extracts, along with 250mg of chlorpropamide (A standard antidiabetic drug that served as positive control) per kg body weight, on plasma glucose concentration in streptozotocin-induced diabetic albino rats over a twenty-eight-day period. The results demonstrated a significant reduction in plasma glucose concentration compared to both diabetic and non-diabetic controls for chlorpropamide and all administered extract doses. The highest percentage reductions were observed with 500 mg/kg of aqueous extract (59.26%), followed by 200 mg/kg of aqueous extract (48.29%), and 350 mg/kg of aqueous extract (19.6%), with chlorpropamide at 250 mg/kg showing a reduction of 17.54%. These findings suggest that 500mg/kg of aqueous extract, 200 mg/kg and 350 mg/kg of aqueous extract demonstrated superior antihyperglycaemic effects compared to the standard antidiabetic drug, chlorpropamide

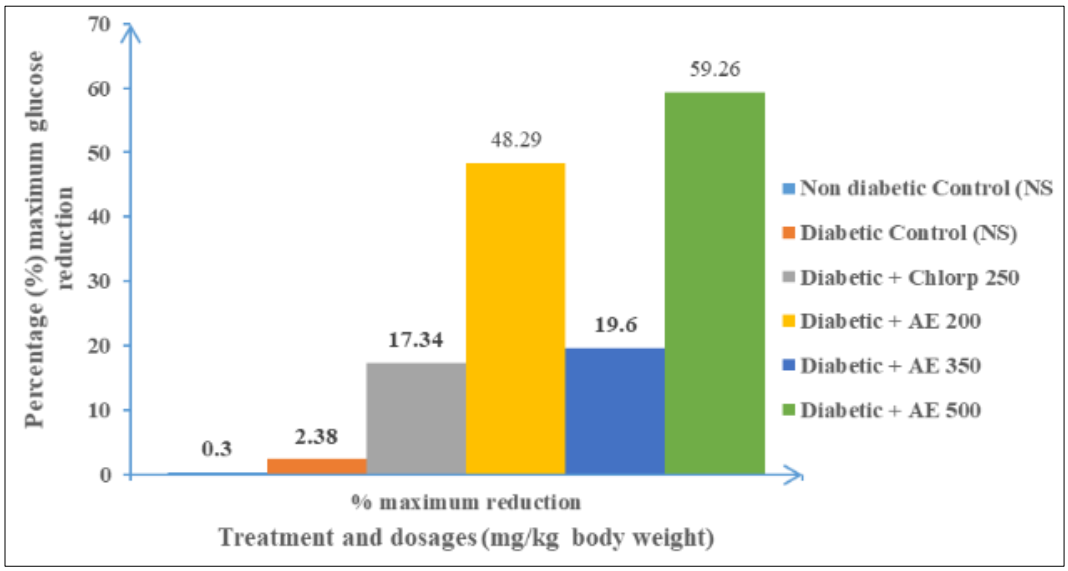

Fig 2: Percentage maximum reduction of fasting blood glucose concentration in streptozotocin induced diabetic Wistar albino rats administered *Tapinanthus preussii* crude aqueous leaf extract (28 day study). NS= normal saline, chlorp = chlorpropamide, AE= Aqueous sxtract

3.4 The impact of *Tapinanthus preussii* crude leaf extracts on the percentage change in body weight (g) of streptozotocin-induced diabetic albino rats

Figure 3.3 illustrates the effects of *Tapinanthus preussii* crude aqueous leaf extracts at various doses-200, 350, and 500 mg-per kg body weight on the percentage change in body weight of streptozotocin-induced diabetic albino rats compared with the positive control administered 250mg/kg body weight of chlorpropamide, normal control and diabetic control over a 28-day period. Generally, untreated diabetic rats induced with streptozotocin (diabetic control) exhibited significantly reduced body weight (-5%) compared to the non-diabetic control group (36.5%) that rather increased significantly. Administration of the extracts led to a slight improvement in weight gain compared to the diabetic control group but remained lower than that of the non-diabetic control group. The most notable increase in the percentage change in body weight was observed in the

group treated with 200 mg of aqueous extract per kg body weight (11.8%) compared to 5.5% in the group administered 250 mg/kg body weight of chlorpropamide (the standard drug and positive diabetic control) showed a lower increase. However it was that the increases (3.3% and 3%) observed in the groups that received 350 and 500 mg/kg body weight of extract respectively.

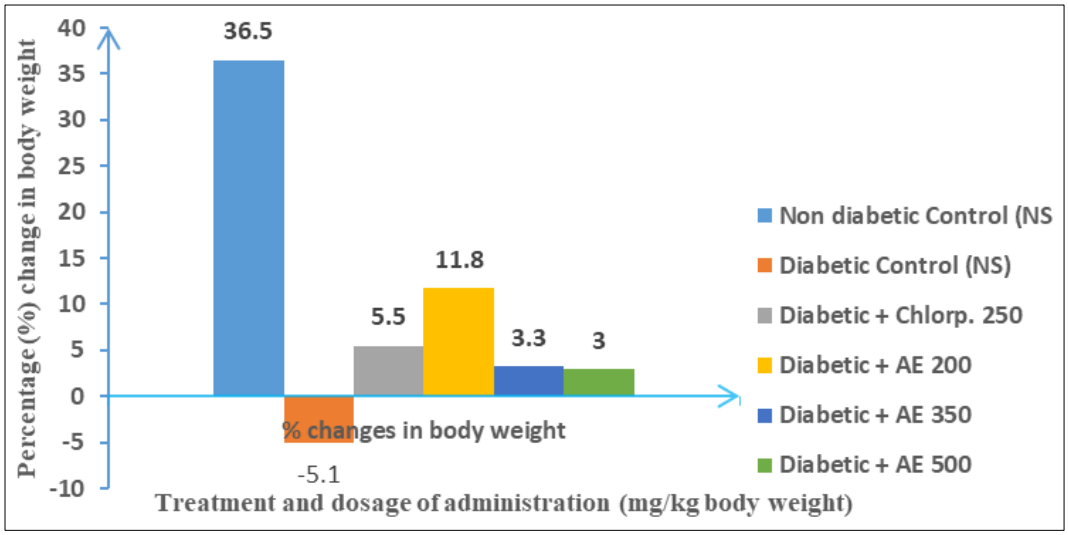

Fig 3: Percentage (%) change in body weight of streptozotocin induced diabetic Wistar albino ras administered *Tapinanthus preussii* crude aqueous leaf extract. NS = Normal saline, Chlorp. = Chlorpropamide

3.5 The impact of *Tapinanthus preussii crude aqueous* leaf extracts on the percentage organ: body weight (g) ratio of streptozotocin induced diabetic albino rats

The impact of *Tapinanthus preussii* crude leaf extracts on the organ-to-body weight ratio of streptozotocin-induced diabetic albino rats was investigated and represented in figure 3.4. Treatment with 200, 350, and 500 mg aqueous crude leaf extract of *Tapinanthus preussii* per kg body weight, along with chlorpropamide, led to a notable reduction in the relative liver, kidney, spleen, heart and pancreas to body weight ratio compared to the negative diabetic control group. However the result of the extract treated groups were very similar to the positive control (chlorpropamide treated) group. It was obvious that the induction of diabetes lead to a decrease in most of the organ to body weight ratio except for the stomach where there was rather an increase in the 500 mg/kg body weight of the extract.

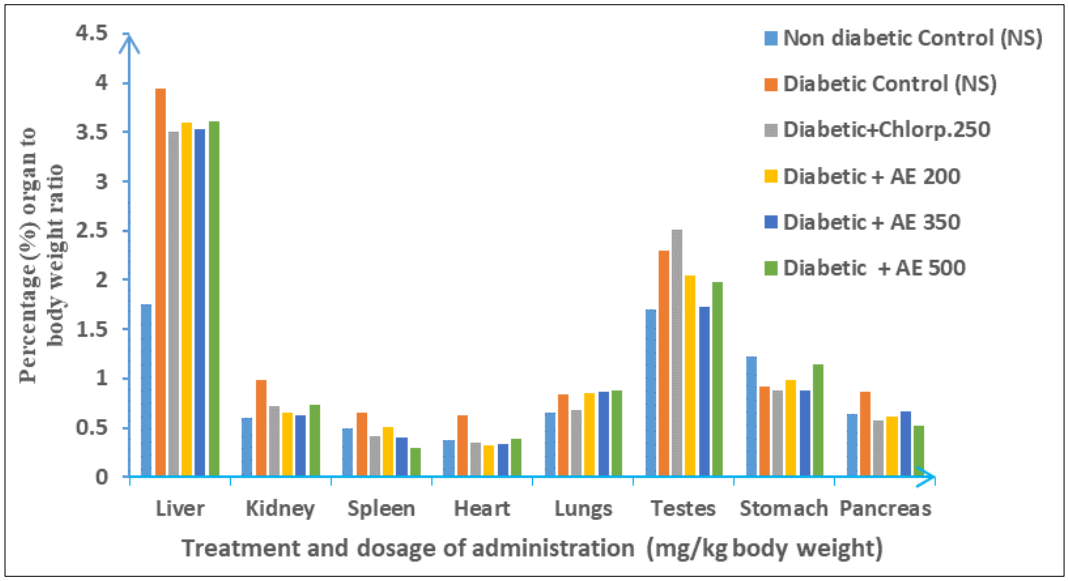

Fig 4: Percentage organ to body weight ratio of streptozotocin induced diabetic Wistar albino rats administered *Tapinanthus preussii* crude aqueousleaf extract. NS = Normal saline, Chlorp. = Chlorpropamide, AE = Aqueous extract

4. Discussion

Diabetes mellitus, a metabolic clinical syndrome characterized by inadequate insulin secretion or insensitivity of target tissues to insulin, has been acknowledged since ancient times and remains a significant global health challenge today. Despite significant progress in understanding the pathophysiology and treatment of this disease, it continues to pose a major health burden worldwide. The potential for managing diabetes through oral hypoglycemic agents has generated considerable interest in recent years [27]. While various oral hypoglycemic agents and insulin are available for treating diabetes, there is a growing patient preference for herbal preparations with antidiabetic properties. Attributed to their perceived

efficacy, cost-effectiveness, fewer side effects, and lower toxicity, despite the often unknown active constituents. The use of herbal remedies has more than tripled over the past decade, giving rise to a new industry known as "nutraceuticals" [28]. However, sparse scientific evidence supports the plethora of herbs utilized to ameliorate metabolic disorders associated with diabetes [29]. For decades, individuals with diabetes have been administered orally with various medicinal plants or their extracts rooted in folk medicine [30, 31]. In accordance with the World Health Organization's recommendations on diabetes mellitus [32], exploring hypoglycemic agents sourced from traditional medicinal plants holds significance. The utilization of herbal remedies for medicinal purposes has held significance across diverse cultures globally, and the quest for antidiabetic solutions will persistently center on plants and other natural reservoirs. Researchers consistently observe unique hypoglycemic properties in several plant products within diabetic animal models [33], with West Africa harboring thousands of such plants [34].

Streptozotocin-induced diabetes is recognized as a valuable experimental model for evaluating the efficacy of antidiabetic agents [35, 36, 37]. Streptozotocin, a naturally occurring beta-cytotoxin and alkylating agent belonging to the glucosamine-nitrosourea class, is commonly used to induce insulin-dependent diabetes mellitus in various experimental animal species due to its toxic impact on pancreatic islet β-cells. The diabetic effect of streptozotocin stems from its ability to cause irreversible harm to pancreatic β-cells, leading to rapid degranulation and loss of insulin-secreting capacity (Necrosis) [38, 39, 36]. This impairment in insulin secretion disrupts glucose homeostasis, ultimately resulting in Type 1 diabetes characterized by the progressive destruction of pancreatic β-cells. These cells are particularly vulnerable to cytotoxic substances like streptozotocin. Intraperitoneal or subcutaneous administration of streptozotocin typically requires 2-3 times higher doses compared to oral administration. Fasting animals have been observed to be more susceptible to streptozotocin [40, 41], while glucose is known to offer protection to beta cells [42, 41, 38].

In this investigation, a single intramuscular injection of freshly prepared streptozotocin in citrate buffer at a dosage of 60mg/kg body weight induced a persistent diabetic condition in rats, characterized by severe hyperglycemia and clinical manifestations of diabetes mellitus after seven days (including reduced motility and piloerection). Rats with fasting blood glucose levels equal to or exceeding 250 mg/dl were selected for this study. In contrast to the current research, Sachin *et al*. [43] illustrated that a single intraperitoneal injection of 180 mg streptozotocin per kg in mice induced type 1 diabetes or insulin-dependent diabetes characterized by a significant increase in blood glucose levels (≥250 mg/dl) and a notable decline in serum insulin levels from the first to the third week. However, administering 100 mg of streptozotocin per kg body weight failed to induce diabetes in mice; only hyperglycemia was observed, with values below the diabetic threshold of 250 mg/dl. Ar'Rajab and Ahren [44] also noted spontaneous recovery from streptozotocin-induced diabetes at lower dose levels of 30-40mg per kg body weight in rats. The current findings aligns partially with the study by Gajdosfk *et al*. [45], which demonstrated that rats injected intravenously with 50 or 60 mg/kg of streptozotocin developed stable diabetes with persistent hyperglycemia exceeding 20 mmol/l (360mg/dl) of plasma glucose, while the 70 mg/kg dosage proved lethal for all animals tested. Another study by Sarika *et al*. [46] corroborated the present research, indicating that experimental type 1 diabetes in rats with fasting blood glucose levels surpassing 250 mg/dl was induced by a single intraperitoneal injection of streptozotocin after an overnight fast at a dose of 60 mg per kg body weight. Hassan and Fahaid [47] classified animals with blood glucose levels exceeding 300 mg/dL as diabetic three days post a single intravenous dose of 65mg streptozotocin per kg body weight injected into the tail vein of rats. Levendal and Frost [48] established that a single intravenous injection of 80 mg streptozotocin per kg body weight in male Wistar rats via the tail vein resulted in fasting blood glucose levels of 250 mg/dl two days post-administration, indicative of diabetes. The dosage of streptozotocin needed to induce consistent diabetes varies based on factors such as animal species, administration route, nutritional status, age, and dosing regimen.

The impact of *Tapinanthus preussii* crude leaf extracts on fasting blood glucose levels in streptozotocin-induced diabetic albino rats was examined. The study assessed the antidiabetic properties of the crude aqueous leaf extract over short-term (8 hours) and long-term (28 days) periods. The results indicated a notable dose-dependent decrease in fasting blood glucose levels in the diabetic rats treated with the aqueous leaf extract, compared to the normal, negative and positive control groups. The extracts exhibited antidiabetic activity, with the 500 mg aqueous extract per kg body weight demonstrating the highest percentage reduction in both short-term (17.54%) and long-term (59.26%) studies. In the sub-chronic study, all dosages of the aqueous extract displayed superior antihyperglycemic potential compared to the positive control. The sustained effectiveness of the crude leaf extracts in reducing blood glucose levels in diabetic rats over the long term may be attributed to a progressive dose-dependent mechanism leading to a drop in blood glucose levels. The presence of phytochemicals like saponins, tannins, and flavonoids in the extract is believed to contribute to its antidiabetic effects through various mechanisms such as slowing down digestion, inhibiting enzymes, and regulating glucose transport [10]. Previous research has suggested that the synergistic effects of heterogeneous phytoconstituents in crude plant preparations and insulin mimicry may play a role in lowering blood glucose levels [49-52]. Research has shown that the aqueous and 50% ethanol extracts of *Triplochiton scleroxylon* may lower plasma glucose levels by stimulating insulin release from the pancreas' β-cells or influencing rapid mobilization of glucose across insulin receptors. This effect could lead to increased hepatic glycogen content through enhanced glycogenesis and reduced glycogenolysis and gluconeogenesis [53]. The significant reduction in blood glucose levels in streptozotocin-induced diabetic rats by *Tapinanthus preussii* leaf extract at various doses suggests it may act in unidentified ways beyond stimulating insulin production from damaged pancreatic islets. The extracts may stimulate sulfonylurea receptors in undamaged pancreatic islets to produce insulin, better than the action of chlorpropamide, a first-generation sulfonylurea [54]. Additionally, the long-term effectiveness of plant extracts in lowering blood glucose levels may be attributed to beta cell regeneration and

restoration due to the presence of various phytoconstituents like flavonoids, carotenoids, and saponins. This regeneration of pancreatic islet cells had been implicated in the studies by some scientists using Carica papaya leaf extracts, indicating a similar potential of *Tapinanthus preussii* aqueous extracts to counteract streptozotocin-induced hyperglycemia [55-57].

Given the induction of type 1 diabetes with a high streptozotocin dose, chlorpropamide likely engaged in extrapancreatic processes to achieve the minimal hypoglycemic effect observed. Sulphonylureas are known to induce hypoglycemia by boosting insulin secretion from the pancreas. Additionally, there is a suggestion that this class of drugs has extra pancreatic mechanisms beyond insulin release, such as increasing cellular insulin receptors, reducing glucagon secretion, enhancing insulin action on target tissues, and decreasing hepatic glucose production. The substantial decrease in blood glucose levels across all aqueous extract dosages implies a mechanism unrelated to insulin secretion from pancreatic β-cells. Therefore, the extract likely lowered fasting blood glucose levels through various extrapancreatic mechanisms, indicating that *Tapinanthus preussii* may stimulate insulin production and glucose utilization akin to chlorpropamide, contributing to its hypoglycemic effect in the experimental model used. It has been documented that, the possible diverse causes and points of intervention in the biochemical processes associated with diabetes, involves somatostatin, gastrointestinal hormones, corticosteroids, prostaglandins, and vascular alterations in the pancreas affecting insulin production [58, 59, 60]. This multifaceted action is mirrored in plant extracts, which contain various biologically active constituents such as flavonoids, saponins, steroids, alkaloids, and tannins. Secondary metabolites of plants with diverse chemical structures demonstrate antidiabetic properties in numerous experimental models [61]. Some studies suggest that certain flavonoids and saponins isolated from medicinal plants notably decrease blood glucose levels [62-65]. It is crucial to consider the crude preparation of plants themselves when evaluating their medicinal worth. Flavonoids also exhibit potent antioxidant properties, scavenging free radicals, which may counteract free radical-induced damage to insulin-producing cells implicated in diabetes in streptozotocin-induced rats [66, 67]. Therefore this study's findings confirm the antidiabetic potential of the crude aqueous leaf extract of *Tapinanthus preussii*.

The impact of crude leaf extracts from *Tapinanthus preussii* on absolute body weight in albino rats induced with streptozotocin was also monitored.Throughout the 28-day oral experimental phase, diabetic control rats experienced a decline in body weight, whereas rats treated with the aqueous leaf exhibited a dose-independent increase in body weight. Decreases in body weight are often associated with Type I diabetes mellitus [68], stemming from disruptions in metabolic processes, degradation of structural proteins, and muscle wasting following the onset of this pathological condition. This decline in body weight among diabetic rats aligns with findings by the works carried out by several researchers, who reported similar effects on streptozotocin-induced diabetic rats [69-72]. However, oral administrations of *Tapinanthus preussii* crude leaf extracts at all doses, as well as chlorpropamide, effectively improved the body weight of the animals, contrasting with the untreated diabetic group. likely due to evident therapeutic benefits, improved glucose management, and protective effects in controlling muscle wasting, such as the reversal of gluconeogenesis and induced adipogenesis [73].

Other studies have indicated that cannabinoids increase adrenocorticotropin (ACTH) secretion and are involved in the stress response [74, 75]. Given that these physiological processes aim to mobilize energy through the activation of lipolysis and protein catabolism, the differences in body composition observed in the experimental animals could be attributed to these physiological responses. Consistent with the present study, several researchers have employed improvements in body weight as an indicator of the ameliorative properties of plant materials in diabetic animal models [76-78]. According to Pierre *et al.* [79], the abilities of aqueous and methanolic extracts of B. engleriana to normalize fasting blood glucose levels in nicotinamide / streptozotocin-induced diabetic rats were fairly correlated with corresponding improvements in body weight in this study. Furthermore, the pattern of improvement in body weight among groups treated with various dosages of the aqueous extract aligned reasonably well with rats treated with the standard antidiabetic drug, chlorpropamide [79].

In this study, the assessment of visceral organ weight was based on organ-to-body weight ratio rather than absolute organ weight. This approach aimed to mitigate variability arising from fluctuations and inconsistencies in the body weight of experimental animals, thereby ensuring more accurate interpretations of observed values. Additionally, it facilitated the evaluation of treatment-specific effects on organs and the exploration of any potential chronic toxicity through microscopic examination. Changes in visceral organ weights are widely recognized indicators of disruptions in physicochemical integrity, potentially signaling underlying pathological conditions resulting from exposure to toxicants [80, 81, 82, 77, 83]. They similarly utilized changes in relative organ-to-body weight ratio for toxicological assessment in different experimental setups. The impacts of streptozotocin on various organs have undergone extensive investigation, revealing its diverse biological actions, which include inducing both acute and chronic cellular injury, carcinogenesis, teratogenesis, and mutagenesis [84]. This was further demonstrated in several other studies that streptozotocin has hepatotoxic and nephrotoxic properties, as well as the ability to cause gastric ulceration [85, 86, 59].

In the present study, untreated diabetic control rats exhibited a paradoxical increase (Hypertrophy) in the liver-to-body weight ratio compared to non-diabetic control, despite the decrease (-5.1%) in the percentage change in body weight. However there was a relative decrease in the extract and chlorpropamide treated groups. These findings align with those reported by Zafar and Naeem [67]. This increase in the untreated diabetic rats may signify liver disarrangement characterized by features such as fatty infiltration and triglyceride accumulation, along with liver enlargement and tissue hyperplasia induced by poor glycemic control (Hypoinsulinemia) and impaired lipoprotein secretion from the liver due to deficient apolipoprotein B synthesis in experimental rats [67]. There was therefore some amelioration in the treated groups due to the phytoconstituents that the extracts possess. Conversely, Amarnath and Saralaya [87] found that streptozotocin treatment significantly reduced the liver, heart, and pancreas-to-body weight ratio, indicating direct effects on these organs. Furthermore, significant kidney hypertrophy relative to body weight ratio was observed in untreated

diabetic control rats compared to normal control, despite a decrease in the percentage change in body weight. This finding is consistent with reports by Zafar and Naeem [67] and Omonkhua *et al*. [88]. However, it contradicts the findings of Chikezie and Iheanacho [75], who found no significant alteration in kidney weight in rats without glycemic control. This discrepancy may be attributed to differences in the duration of the experiments, with shorter-term investigations potentially failing to manifest renal hypertrophy compared to long-term studies. Kidney hypertrophy in streptozotocin-induced diabetic rats represents an early event in glomerular pathology, occurring before mesangial expansion [89]. It was also observed in this study that, there was a significant increase in the spleen-to-body weight ratio in untreated diabetic control rats compared to non-diabetic control. This alteration could be attributed to splenic disarrangement tending toward restoration to normal physiological weight following administration of *Tapinanthus preussii* leaf extracts within the experimental time constraints. This aligns with the findings of Chikezie and Iheanacho [75], who reported similar restoration of normal splenic physiological weight after treatment with Viscum album leaf extracts. Additionally, the pancreas-to-body weight ratio was significantly increased in diabetic control rats compared to non-diabetic controls. This result is consistent with the findings of Omonkhua *et al*. [87] and contradicts those of Zafar and Naeem [67]. Treatment with *Tapinanthus preussii* effectively ameliorated the pancreas-to-body weight ratio, bringing it to values similar to those of normal control rats, as reported by Omonkhua *et al*. [87]. Summarily the results of this study indicate that treatment with varied dosages of *Tapinanthus preussii* crude leaf extracts led to a reduction in the relative liver, kidney, spleen, heart, and pancreas to body weight ratio in streptozotocin-induced diabetic albino rats. This reduction was similar to that observed in the chlorpropamide treated group, suggesting that the extract has a similar effect to the standard anti-diabetic drug in reducing organ weight in diabetic rats. The induction of diabetes led to an increase in most of the organ to body weight ratio, except for the stomach where there was a decrease relative to the normal control. The anti-diabetic effects of *Tapinanthus preussii* extract may also be attributed to its ability to trap the DPPH radical and its curative antidiabetic effects on the type 1 diabetes model [90]. The result from this study was consistent with that of Eleazu *et al*., [91] where the ameliorative potentials of ginger on relative organ weights in streptozotocin-induced diabetic rats were also been reported, with a significant decrease in relative liver weight and a corresponding amelioration of elevated urinary protein, sugars, specific gravity, and renal growth. The study suggests that *Tapinanthus preussii* extract has potential as a natural anti-diabetic agent, with promising results in reducing organ weight and improving insulin resistance in diabetic rats.

5. Conclusion

In conclusion, this study has provided scientific evidence for the anti-diabetic potential of *Tapinanthus preussii* crude aqueous leaf extract in streptozotocin-induced diabetic male Wistar albino rats. The extract exhibited a dose and time-dependent antihyperglycemic effect, outperforming the standard anti-diabetic drug chlorpropamide in reducing blood glucose levels. The findings suggest that the phytochemicals present in *Tapinanthus preussii* leaf extract, such as flavonoids, carotenoids, anthraquinones, alkaloids, tannins, cyanogenic glycosides, and saponins, may contribute to its antidiabetic effects. These results highlight the potential of *Tapinanthus preussii* as a natural anti-diabetic agent for the management of diabetes mellitus which could provide a cost-effective and accessible treatment option for individuals living in low-resource settings.

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