

Antidiabetic Studies of *Cnestis ferruginea* Ethanolic Root Extract in Wistar Rats

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ABSTRACT

The need to develop new and affordable antidiabetic drugs with minimal side effects is a growing area of research interest. The medicinal plant *Cnestis ferruginea* DC has diverse pharmacological applications. Its leaves and roots are used for the treatment of diabetes by traditional healers. As a result, the antidiabetic activity of ethanolic extract of *C. ferruginea* (EECF) roots was evaluated in streptozotocin (STZ)-induced diabetic rats. The influence of EECF (200 and 400 mg/kg bw) was studied for three weeks, with glibenclamide as a positive control. The phytochemical screening of EECF was also performed. Like glibenclamide, treatment with EECF caused a significant ($p < 0.05$) reduction in fasting blood glucose (FBG) in diabetic rats. A significant ($p < 0.05$) alteration in serum lipid profile parameters, kidney and liver function parameters, as well as oxidative stress markers in kidney and liver tissues, was observed in diabetic control rats relative to the normal control ones. But the treatment of diabetic rats with EECF resulted in significant ($p < 0.05$) restoration of these parameters to a near-normal level. The phytochemical analysis of EECF showed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatannins, saponins and tannins. Thus, the results offer proof to support the traditional use of *C. ferruginea* roots in the treatment of diabetes. The antidiabetic activity of EECF may be due to its phytoconstituents.

Keywords: *Cnestis ferruginea*, ethanolic root extract, antidiabetic, STZ, glibenclamide, phytochemicals.

Introduction

Diabetes mellitus (DM) is a metabolic disorder that causes hyperglycemia and necessitates ongoing medical attention. DM primarily develops as a result of a partial or total lack of

insulin (Soffe *et al.*, 2019). The most prevalent symptom of diabetes mellitus, hyperglycemia, can be thought of as a cluster of countless disorders. Prolonged hyperglycemia in diabetic patients can cause a wide range of potentially

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fatal health issues, including coronary artery disease, blindness, limb amputation, cardiovascular conditions, renal failure, neurological issues, and early death (Okoro *et al.*, 2022). Type I diabetes mellitus (T1 DM), develops when insulin production is completely absent, while Type II diabetes mellitus (T2 DM), develops when insulin production is partially absent and the body's cells exhibit resistance to the insulin action (Ormazabal *et al.*, 2018). Chronic diseases are becoming a severe danger to people's health, quality of life, and life expectancy due to their rising prevalence. One of the most often identified chronic diseases is diabetes mellitus (DM). According to recent studies, 693 million persons (aged 18 to 99) worldwide are expected to be diabetic in 2045, up from 451 million in 2017 (Cho *et al.*, 2018).

Nowadays, the main techniques for managing hyperglycemia and other diabetes-related problems are dietary changes, insulin injections, and/or the use of oral hypoglycaemic medications (Tang *et al.*, 2017). Unfortunately, almost all of these treatments have some unwanted side effects, making it difficult to maintain diabetes control when using them (Amiel *et al.*, 2008). Examining natural products that are used as medicinal plants and contain secondary metabolites, such as polyphenols and flavonoids, which can exert antioxidant and antidiabetic activities, has led researchers to try to find novel medications with fewer side effects (Tang *et al.*, 2017).

Connaraceae is a family of plants that includes the shrub *Cnestis ferruginea*. West tropical Africa is where it is most frequently found. It is commonly known as “Okpu nkita,” “amunkita” (Igbo); “Fura amarya,” “otito” (Hausa); and “Akara oje”, “Bonyin bonyin” (Yoruba) (Burkill, 1995). According to Ahmed (2017), *Cnestis ferruginea* typically extends from Senegal to West Cameroons. It is a perennial shrub with sarmentose leaves that is 3.0-3.6 m high and occasionally has woody tendrils. In folkloric medicine, the entire plant, including the leaves, roots, and stem bark, is used to treat ailments like fever, gonorrhoea, wounds, gum pain, diabetes, tuberculosis, headaches, dysmenorrhoea, whooping cough, and arthritis (Ahmed, 2017).

Alkaloids, saponins, tannins, reducing sugars, flavonoids, cardiac glycosides, terpenes, phytosterol, oxalate, steroid, cyanogenic glycoside, and anthocyanin are among the phytochemical components that have been identified from the plant (Ahmed, 2017; Ebaná *et al.*, 2019). The plant has been studied for its pharmacological properties, which include anti-inflammatory, antibacterial, aphrodisiac, hepatoprotective, antioxidant, antidiabetic, and analgesic effects (Akharaiyi *et al.*, 2012; Ahmed, 2017).

Yakubu *et al.* (2011) have reported that fruits exhibit antimicrobial characteristics, namely against gram-positive bacteria. Additionally, the aqueous root extract of the plant has been found to possess laxative and antistress qualities, as documented by Ishola and Ashorobi (2007) and Yakubu *et al.* (2011). Furthermore, there have been assertions on the analgesic and anti-inflammatory activities of the methanolic root extract. Additionally, Ishola *et al.* (2011) have reported on the acute toxicity of the root extract.

In a previous experimental investigation, ethyl-acetate and methanol extracts of *C. ferruginea* leaves were found to have antidiabetic properties (Adisa *et al.*, 2010). Although leaves and roots of *Cnestis ferruginea* DC are used for the treatment of diabetes by traditional healers (Konkon *et al.*, 2017), however, no scientific research to the best of our knowledge has yet proven that *C. ferruginea* roots may control blood sugar levels and treat diabetic subjects. Thus, this study aimed to evaluate the phytochemical composition and antidiabetic activity of *C. ferruginea* root extract in STZ-induced diabetic rats.

Materials and Methods

Collection and extraction of plant material

Cnestis ferruginea (roots) were collected from farmland at Abraka, Delta State, Nigeria, and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, with voucher number, UBH-C369. The roots were washed, air dried and powdered by mortar and pestle. The powdered sample (50 g) was dissolved in 200 ml of ethanol for 72 h with

intermittent shaking, and then filtered by Whatman filter paper (No.1). Thereafter, the filtrate was concentrated by a rotary evaporator and freeze-dried.

Preliminary Phytochemical Screening

The extracts were phytochemically tested by standard protocols to determine the presence of phytoconstituents (Harbone, 1998; Evans and Trease, 2002).

Animals for In Vivo Study

The study was done with male Wistar rats (weighing between 150 - 200 g), obtained from the Animal House at the Department of Anatomy, Delta State University, Abraka. They were allowed to acclimatize for two weeks before the start of the experiment with unrestricted access to water and rat chow *ad libitum* during the study. The guidelines for the care and use of laboratory animals were followed when caring for the animals (Olfert *et al.*, 1993).

Experimental design

Diabetes was induced by Streptozotocin (STZ) (50 mg/ kg body weight). Only animals that had been hyperglycemic due to streptozotocin were employed in the study. The rats were separated into five groups, each with five animals, and fasted for 12 hours before the experiment's start. Group I functioned as a control group consisting of healthy animals. Group II served as a control group for diabetic animals induced by STZ. Group III consisted of diabetic rats induced by STZ and treated with glibenclamide at a dosage of 5 mg/kg body weight. Group IV consisted of diabetic rats induced by STZ and treated with *Cnestis ferruginea* ethanolic extract at a dosage of 200 mg/kg body weight. Lastly, group V consisted of diabetic rats induced by STZ and treated with *Cnestis ferruginea* extract at a dosage of 400 mg/kg body weight. The experimental protocol involved a 21-day treatment regimen for rats with diabetes, during

which weekly measurements of fasting blood glucose (FBG) levels and changes in body weight were recorded for the animals. The animals underwent a 12-hour fasting period and were then euthanized on the 22nd day. The blood sample was collected and the organs (liver and kidney) were excised, blotted and homogenized and processed for the various biochemical assays.

Assay of biochemical parameters

Serum biochemical parameters were measured, including kidney function indicators like creatinine (Brod and Sirota, 1948) and urea (Natelson *et al.*, 1951), liver function indicators such as aspartate aminotransferase (AST) (Sood, 1999), alanine aminotransferase (ALT) (Reitman and Frankel, 1957) and alkaline phosphatase (ALP) (Dumas *et al.*, 1971), lipid profiles, including cholesterol (Richmond, 1973), triglycerides (TG) (Trinder, 1969), high-density lipoprotein - cholesterol (HDL - c) (Richmond, 1973), low-density lipoprotein cholesterol (LDL-c) (Friedewald *et al.*, 1972). Tissue homogenate was utilised to assay for the enzymatic antioxidant and lipid peroxidation, the parameters evaluated were catalase (Sinha, 1972), superoxide dismutase (Misra and Fridovich, 1972), and lipid peroxidation (Ohkawa *et al.* (1979).

Statistical analysis

The values are represented as mean \pm SD. Data were subjected to analysis of variance (ANOVA) and Turkey's Multiple Range Test. $p < 0.05$ was deemed statistically significant.

Results and Discussion

Phytochemical screening of *Cnestis ferruginea* root extract.

The ethanolic extract of *Cnestis ferruginea* roots was subjected to phytochemical screening, which revealed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatannins, saponin, and tannins (Table 1.).

Table 1: Phytochemical screening of ethanolic extract of *Cnestis ferruginea* roots

Phytochemicals	Ethanol extract
Alkaloids	+++
Anthraquinones	++
Cardiac Glycoside	++
Flavonoids	++
Phenol	-
Phlobatannins	+
Saponins	++
Steroids	-
Tannins	+

Key: -: Absence +: Presence (+ mild, ++ moderate, +++ high),

The impact of *Cnestis ferruginea* extract on the level of fasting blood glucose

Figure 1 displays the outcomes pertaining to the impact of *C. ferruginea* extract on fasting blood glucose (FBG) levels in rats generated with STZ-induced diabetes. The administration of streptozotocin (STZ) resulted in a gradual elevation of fasting blood glucose (FBG) levels in rats, as observed in the diabetic control group (group B), in comparison to the normal control group (group A) and the remaining groups (C, D, and E). The administration of the plant extract to diabetic rats over a period of 21 days led to a notable enhancement ($p < 0.05$) in their fasting blood glucose levels as compared to the rats in group B. In a similar vein, a statistically significant ($p < 0.05$) reduction in fasting blood glucose (FBG) levels was observed in the rats administered glibenclamide, as compared to the rats in group B.

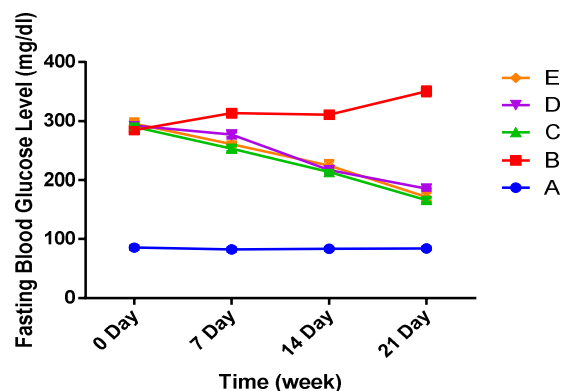


Figure 1: Effects of *Cnestis ferruginea* extract on fasting blood glucose level of STZ-induced diabetic rats

Where: A = normal control (NC), B = diabetic control, C = STZ-induced diabetic rats + glibenclamide (5 mg/kg body weight), D = STZ-induced diabetic rats + 200 mg/kg body weight of *Cnestis ferruginea* ethanolic extract, E = STZ-induced diabetic rats + 400 mg/kg body weight of extract of *Cnestis ferruginea*.

Effects of *Cnestis ferruginea* extract on body weight

Figure 2 illustrates the impact of *Cnestis ferruginea* extract on the body weight of rats with streptozotocin-induced diabetes. A decrease in body weight was seen in rats belonging to group B following the administration of STZ. However, the administration of either the plant extract or the conventional medicine resulted in a statistically significant ($p < 0.05$) enhancement in the body weight of the rats in groups C, D, and E.

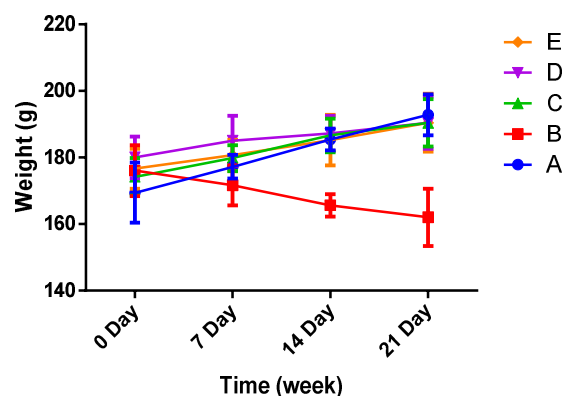


Figure 2: Effects of *Cnestis ferruginea* extract on body weight of STZ-induced diabetic rats. Data are presented as Mean \pm SD ($n=5$). Where A = normal control, B = diabetic control, C = diabetic

rats + glibenclamide (5 mg/kg body weight), D= diabetic rats + 200 mg/kg body weight of *C. ferruginea* extract, E = diabetic rats + 400 mg/kg body weight of *C. ferruginea* extract.

Effects of *Cnestis ferruginea* extract on the total protein of STZ-induced diabetic rats

Presented in Figure 3 are the effects of *C. ferruginea* extract on the total protein of STZ-induced diabetic rats. Induction of diabetes in rats led to a significant ($p < 0.05$) decrease in the total protein of rats when group B rats are compared with the group A rats. However, there was no difference ($p < 0.05$) between the normal control rats and the groups C, D and E rats.

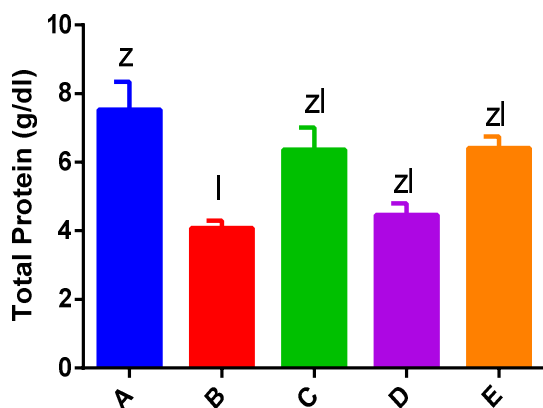


Figure 3: Effects of *Cnestis ferruginea* extract on the total protein of STZ-induced diabetic rats

Data are presented as Mean \pm SD ($n=5$). Different letters on the bar graph(s) show a significant difference (at $p < 0.05$). Where A = normal control, B = diabetic control, C = diabetic rats + glibenclamide (5 mg/kg body weight), D = diabetic rats + 200 mg/kg body weight of *C. ferruginea* extract, E = diabetic rats + 400 mg/kg body weight of *C. ferruginea* extract.

Effects of *Cnestis ferruginea* extract on serum urea and creatinine of STZ-induced diabetic rats

Figure 4 illustrates the impact of *C. ferruginea* extract on the levels of blood urea and creatinine in rats with streptozotocin-induced diabetes. The administration of streptozotocin (STZ) resulted in a statistically significant ($p < 0.05$) elevation in serum urea levels in the diabetic control group compared to the normal control group.

Nevertheless, there was no statistically significant difference ($p < 0.05$) observed in the serum urea levels between the normal control rats and the rats in groups C and D. Likewise, a statistically insignificant difference ($p < 0.05$) was seen in the creatinine level across the experimental groups (A – E).

Effects of *Cnestis ferruginea* extract on serum ALT, AST and ALP of STZ-induced diabetic rats

The effects of *C. ferruginea* extract on serum ALT, AST and ALP of STZ-induced diabetic rats are presented in Figure 5. A Significant ($p < 0.05$) increase was observed in the serum ALT, AST and ALP activities of the diabetic control rats (B) relative to the normal control rats (A). However, treatment of the diabetic rats for 21 days with either glibenclamide or the plant extract led to a significant ($p < 0.05$) decrease in the serum activities of the ALT, AST and ALP compared with the diabetic control rats.

Effects of *Cnestis ferruginea* extract on serum lipid profile parameters of STZ-induced diabetic rats

Shown in Figure 6 are the effects of *C. ferruginea* extract on serum lipid profile parameters of STZ-induced diabetic rats. The induction of diabetes in rats by STZ resulted in a significant ($p < 0.05$) increase in the serum cholesterol, triglyceride and low-density lipoprotein levels in the diabetic control rats (B) compared with the normal control rats (A). Treatment of diabetic rats with the plant extract for 21 days led to a significant ($p < 0.05$) reduction in the above parameters compared with the diabetic control group (B). While significant ($p < 0.05$) decrease in HDL was seen in the group B rats relative to the group A rats. Also, treatment with the plant extract, particularly the higher dose (400 mg/kg) resulted in a significant increase in the HDL level relative to the diabetic control.

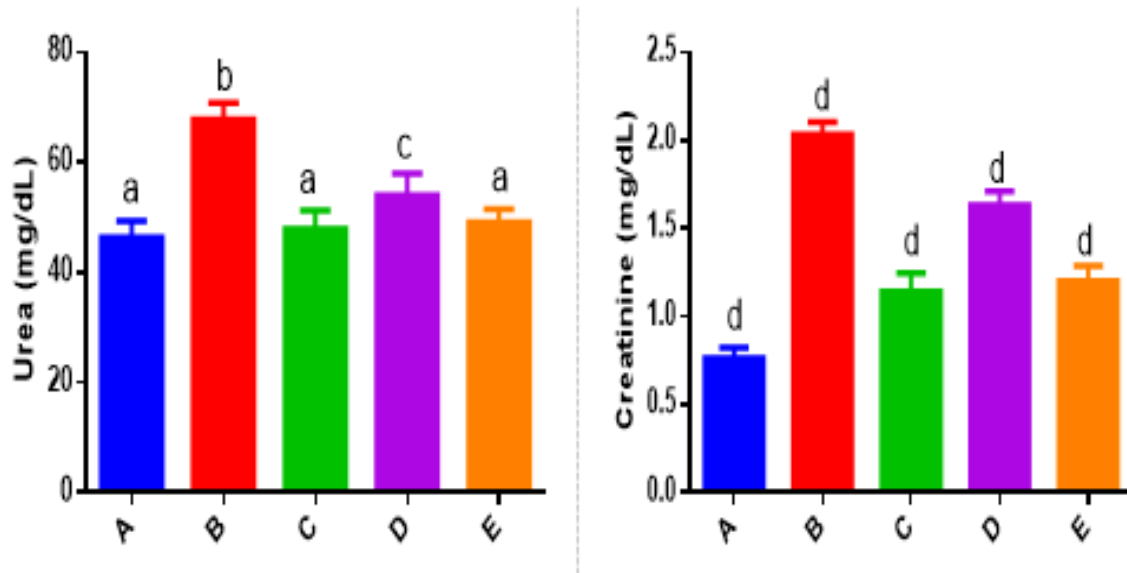


Figure 4: Effects of *Cnestis ferruginea* extract on serum urea and creatinine of STZ-induced diabetic rats.

Data are presented as Mean \pm SD ($n=5$). Different letters on the bar graph(s) show a significant difference (at $p < 0.05$). Where A = normal control, B = diabetic control, C = diabetic rats + glibenclamide (5 mg/kg body weight), D = diabetic rats + 200 mg/kg body weight of *C. ferruginea* extract, E = diabetic rats + 400 mg/kg body weight of *C. ferruginea* extract.

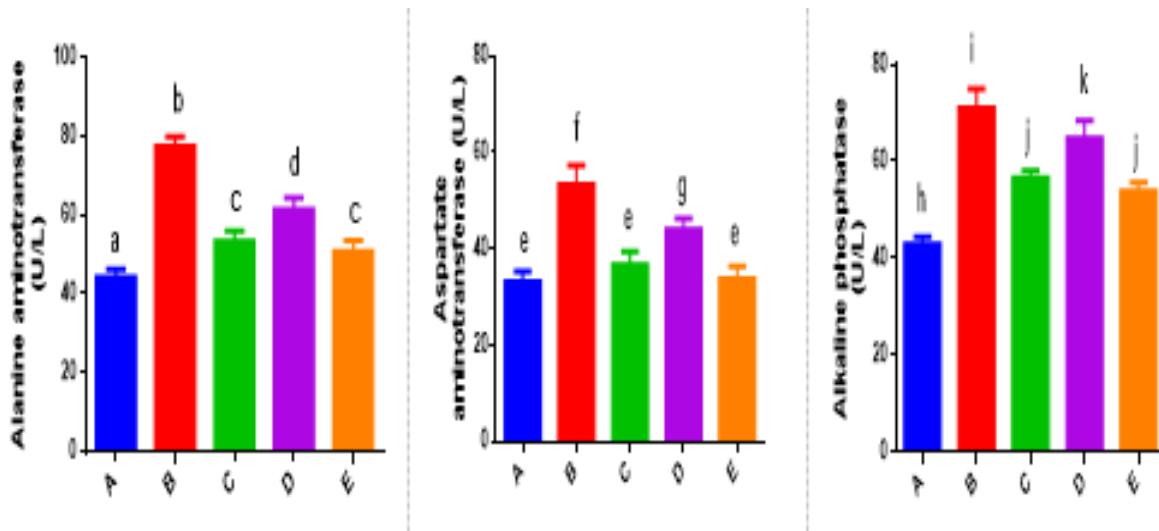


Figure 5: Effects of *Cnestis ferruginea* extract on serum ALT, AST and ALP of STZ-induced diabetic rats.

Data are presented as Mean \pm SD ($n=5$). Different letters on the bar graph(s) show a significant difference (at $p < 0.05$). Where A = normal control, B = diabetic control, C = diabetic rats + glibenclamide (5 mg/kg body weight), D = diabetic rats + 200 mg/kg body weight of *C. ferruginea* extract, E = diabetic rats + 400 mg/kg body weight of *C. ferruginea* extract.

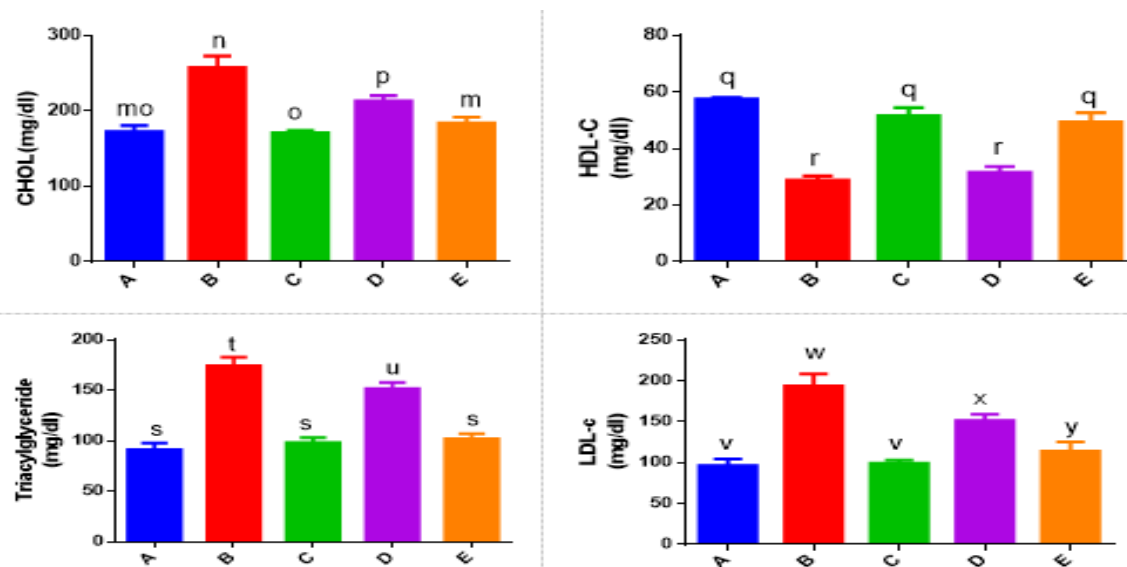


Figure 6: Effects of *Cnestis ferruginea* extract on serum lipid profile parameters of STZ-induced diabetic rats.

Data are presented as Mean \pm SD ($n=5$). Different letters on the bar graph(s) show a significant difference (at $p < 0.05$). Where A = normal control, B = diabetic control, C = diabetic rats + glibenclamide (5 mg/kg body weight), D = diabetic rats + 200 mg/kg body weight of *C. ferruginea* extract, E = diabetic rats + 400 mg/kg body weight of *C. ferruginea* extract.

Effects of Cnestis ferruginea extract on lipid peroxidation level in the liver and kidney of STZ-induced diabetic rats

Figure 7 illustrates the impact of *C. ferruginea* extract on the levels of lipid peroxidation in the liver and kidney of rats with streptozotocin-induced diabetes. A statistically significant ($p < 0.05$) elevation in lipid peroxidation levels was seen in the liver and kidney of diabetic rats when comparing group B rats to group A rats. Nonetheless, the administration of the plant extract to diabetic rats over a period of 21 days led to a noteworthy reduction ($p < 0.05$) in the levels of malondialdehyde (MDA) in both the liver and kidney. This reduction was observed when comparing the groups of rats treated with the extract (D and E) to the group of diabetic control rats.

Effects of Cnestis ferruginea extract on superoxide dismutase (SOD) activities in the liver and kidney of STZ-induced diabetic rats

The effects of *C. ferruginea* extract on superoxide dismutase (SOD) activity in the liver and kidney of streptozotocin (STZ)-induced diabetic rats are illustrated in Figure 8. The administration of streptozotocin (STZ) led to a statistically significant ($p < 0.05$) decrease in superoxide dismutase (SOD) activity in the liver and kidney of diabetic control rats (group B) compared to normal control rats (group A). The administration of a larger dose (400 mg/kg) of plant extract to diabetic rats led to a statistically significant ($p < 0.05$) elevation in superoxide dismutase (SOD) activity in both the liver and kidney.

Effects of Cnestis ferruginea extract on catalase (CAT) activities in the liver and kidney of STZ-induced diabetic rats

Figure 9 illustrates the impact of *C. ferruginea* extract on catalase (CAT) activities in the liver and kidney of rats with streptozotocin (STZ)-induced diabetes. A statistically significant decrease ($p < 0.05$) in catalase activity was seen

in the diabetic control rats (group B) when compared to the normal control rats, in both organs. However, administration of the plant extract to rats with diabetes, specifically at a

higher dose of 400 mg/kg, resulted in a noteworthy enhancement in catalase activity compared to the control group of diabetic rats, with statistical significance ($p < 0.05$).

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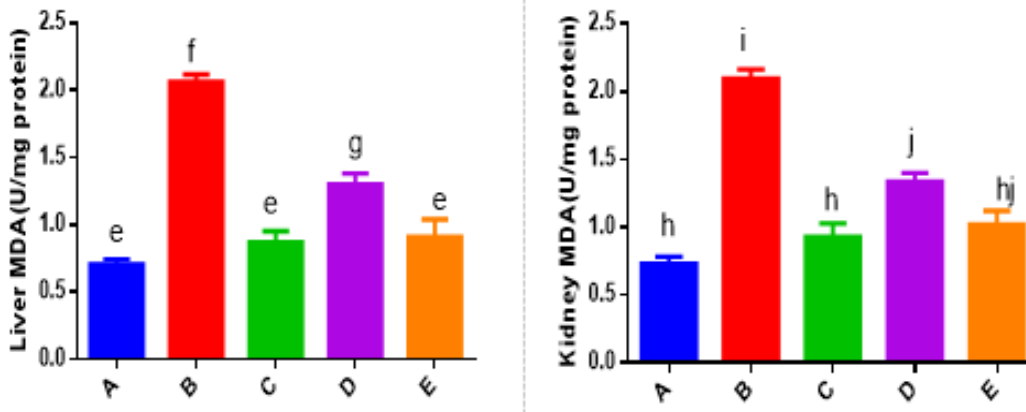


Figure 7: Effects of *Cnestis ferruginea* extract on lipid peroxidation level in the liver and kidney of STZ-induced diabetic rats.

Data are presented as Mean \pm SD (n=5). Different letters on the bar graph(s) show a significant difference (at $p < 0.05$). Where A = normal control, B = diabetic control, C = diabetic rats + glibenclamide (5 mg/kg body weight), D= diabetic rats + 200 mg/kg body weight of *C. ferruginea* extract, E = diabetic rats + 400 mg/kg body weight of *C. ferruginea* extract.

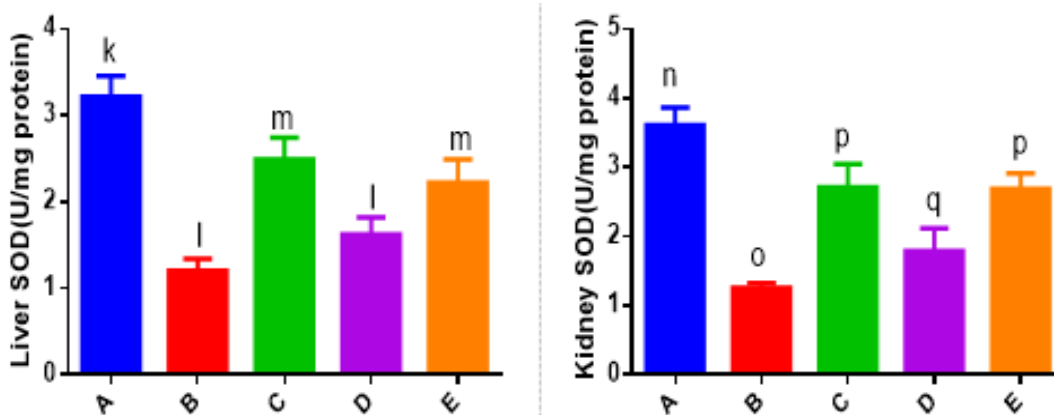


Figure 8: Effects of *Cnestis ferruginea* extract on superoxide dismutase (SOD) activities in the liver and kidney of STZ-induced diabetic rats.

Data are presented as Mean \pm SD (n=5). Different letters on the bar graph(s) show a significant difference (at $p < 0.05$). Where A = normal control, B = diabetic control, C = diabetic rats + glibenclamide (5 mg/kg body weight), D= diabetic rats + 200 mg/kg body weight of *C. ferruginea* extract, E = diabetic rats + 400 mg/kg body weight of *C. ferruginea* extract.

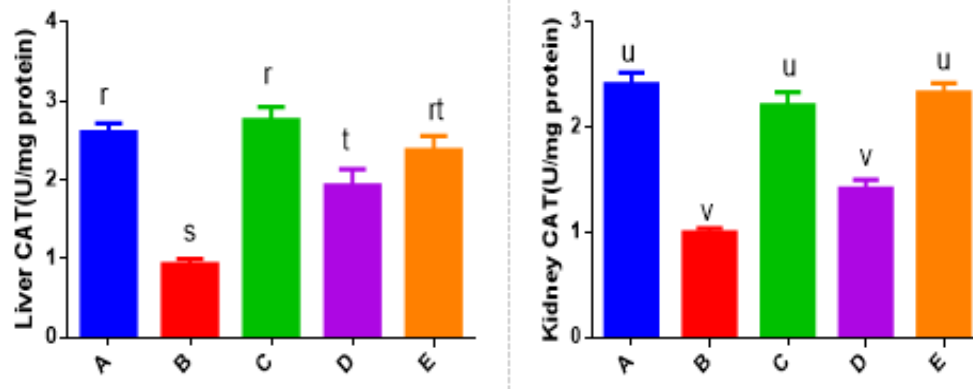


Figure 9: Effects of *Cnestis ferruginea* extract on catalase (CAT) activities in the liver and kidney of STZ-induced diabetic rats.

Data are presented as Mean \pm SD (n=5). Different letters on the bar graph(s) show a significant difference (at $p < 0.05$). Where A = normal control, B = diabetic control, C = diabetic rats + glibenclamide (5 mg/kg body weight), D = diabetic rats + 200 mg/kg body weight of *C. ferruginea* extract, E = diabetic rats + 400 mg/kg body weight of *C. ferruginea* extract.

Discussion

Diabetes is a metabolic condition characterized by either insufficient insulin production or the development of insulin resistance. Despite the existence of numerous anti-diabetic medications on the pharmaceutical market, the efficacy of plant-based therapies in the management of diabetes has repeatedly demonstrated positive outcomes. The therapeutic efficiency of natural medicines, namely plant components, has been demonstrated to be remarkable, exhibiting little side effects and low toxicity (Okoro *et al.*, 2015a; Kadiri *et al.*, 2020). Consequently, the ongoing pursuit of plant-based natural medicine with antidiabetic properties remains imperative (Okoro, 2020). Although *Cnestis ferruginea* has been utilized by traditional healers for managing diabetes by employing its leaves and roots (Konkon *et al.*, 2017), there is currently a lack of scientific evidence supporting the efficacy of *C. ferruginea* roots in regulating blood glucose levels and treating individuals with diabetes. This study aimed to assess the antidiabetic effects of the ethanolic extract derived from the root of *C. ferruginea* in rats with diabetes caused by STZ.

Furthermore, the extract underwent phytochemical screening.

In this research, when compared to diabetic control, the roots' extract of *C. ferruginea* at 200 and 400 mg/kg bw doses showed a substantial hypoglycemic property. In diabetic rats, administration of ethanol root extract of *C. ferruginea* (200 mg and 400 mg/kg body weight) resulted in a dose-dependent and significant decrease in hyperglycemia. Thus, on the seventh day, fourteenth day, and twenty-first day of the study, the extract particularly the higher dose (400 mg/kg bw) demonstrated a significant hypoglycemic effect. At 200 mg/kg b. wt. and 400 mg/kg, the blood glucose level reduction was 47.84% and 54.41%, respectively, with a reduction in fasting blood glucose level of the higher dose being comparable to 61.54 % reduction in fasting blood glucose level seen in the standard group rats on the 21st day of the study. The STZ-induced hyperglycemia seen in this study may be linked to free radical generation through glucose breakdown, oxidative degradation, or non-enzymatic glycation of proteins (Okoro *et al.*, 2015b). The antihyperglycemic effect of the root extract

observed in this investigation agrees with earlier reports on the antidiabetic properties of *C. ferruginea* leaves (Adisa *et al.*, 2010).

Loss in body weight is known as an important feature in STZ-induced diabetes, which results from protein wasting increase and the unavailability of glucose for energy utilisation (Okoro *et al.*, 2015a). In this experiment, STZ-induced reduction in body weight was observed in diabetic control rats. But the treatment of diabetic rats with the plant extract led to significant improvement in the body weight of animals. Thus, there was a substantial increase in body weight of extract-treated diabetic rats, which may be a result of improvement in glycaemic regulation following an increase in structural protein synthesis (Eliza *et al.*, 2009). Therefore, the administration of plant extract to diabetic rats led to an effective decrease in FBG levels and an increase in body weight. This improvement in body weight and reduction in the FBG level of extract-treated diabetic rats signify the hypoglycemic influence of *C. ferruginea* root extract.

According to Upwar *et al.* (2011), hyperlipidemia and diabetes are related. After 21 days of this research, diabetic rats had a lower level of high-density lipoprotein and higher levels of triglycerides, low-density lipoprotein and total cholesterol in comparison to the healthy control group. However, the treatment of diabetic rats with the plant extract for 21 days led to a significant improvement in the lipid profile parameters compared with the diabetic control group. When compared to diabetic controls, the groups treated with 200 mg/kg and 400 mg/kg b.w. doses of *C. ferruginea* extract significantly reduced their levels of triglycerides, low-density lipoprotein and total cholesterol. These findings demonstrated *C. ferruginea*'s capability to significantly reduce diabetes-related hyperlipidemia.

The present study aimed to assess liver-associated biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), in serum samples. Based on available findings, individuals diagnosed with type 2 diabetes exhibit a higher propensity for encountering deviations in liver function tests compared to individuals without any underlying health conditions. In accordance with the findings of Harris (2005), it has been observed that mild and persistent increases in the levels of transaminases, specifically aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), often indicate the presence of underlying insulin resistance. The utilization of these measures is prevalent in clinical settings for the purpose of diagnosing liver disease. The AST and ALT enzymes are predominantly localized in the cytosol, whereas the ALP enzyme is primarily associated with the cell membrane. The presence of heightened concentrations of these enzymes in the bloodstream is indicative of hepatic impairment (Saleh *et al.*, 2018). According to Saleh *et al.* (2018), an elevation in the blood concentrations of these enzymes indicates hepatocyte damage in diabetic rats induced by STZ. During the course of this study, it was observed that diabetic rats had elevated levels of aminotransferases, specifically aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as alkaline phosphatase (ALP) after a period of 21 days. Nevertheless, the administration of the plant extract to the diabetic rats for a period of 21 days resulted in a noteworthy reduction in the blood activity of ALT, AST, and ALP in comparison to the diabetic control rats. Therefore, in comparison to the diabetic control rats, the experimental groups administered with *C. ferruginea* extract at doses of 200 mg/kg and 400 mg/kg exhibited significant reductions in ALP, ALT, and AST levels. The observed phenomenon could

potentially be attributed to the inherent stability of the cell membrane and the process of cellular restoration, as suggested by Madrigal-Santillán *et al.* (2013). The aforementioned findings indicate the potential hepatoprotective properties of the extract derived from *C. ferruginea*.

A common complication of diabetes is diabetic nephropathy. According to the results of this study, the diabetic control had higher levels of blood urea and serum creatinine after 21 days, which indicated kidney damage. When compared to diabetic control, the groups treated with extract significantly reduced their serum urea levels. The outcomes demonstrated a dose-dependent effect in regulating blood urea and creatinine.

The activity of free radicals has been linked to diabetes-induced tissue damage, which results from an attack on the membrane by lipid peroxidation. This ultimately results in membrane dysfunction and damage (Alfy *et al.*, 2005). One of the mechanisms by which diabetic complications are prevented is improvement in the antioxidant status and reduction of lipid peroxidation. In this experiment, there was a significant increase in the lipid peroxidation level for both the liver and the kidney of diabetic rats when the diabetic control rats were compared with the normal control rats. However, treatment of diabetic rats with *C. ferruginea* root extract caused a significant reduction in lipid peroxidation levels in both the liver and kidney, which can be attributed to the antioxidant actions of its phytochemicals (Okoro and Kadiri, 2019).

The observed elevation of lipid peroxidation level in diabetic rats was accompanied by a reduction in the activity of the antioxidant enzymes (superoxide dismutase and catalases), which are prominent in the scavenging of toxic intermediates generated in the course of incomplete oxidation, resulting in the availability of superoxide anions (O_2^-) in excess and hydrogen peroxide, so producing hydroxyl radicals accountable for lipid peroxidation's initiation and

propagation (Asagba *et al.*, 2010). But there was an improvement in the activities of the antioxidant enzymes following the treatment of diabetic animals with *C. ferruginea* root extract for 21 days, therefore confirming the antioxidant properties of the extract.

To evaluate the phytochemicals present in the plant extract, the preliminary phytochemical screening of the ethanolic extract of *Cnestis ferruginea* roots was done, which showed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatannins, saponin and tannins (Okoro, 2020b). The anti-diabetic properties of various medicinal plants have been attributed to the activities of saponins (Metwally *et al.*, 2012). Saponin stimulates insulin production and has oxidative stress ameliorative effects (Jiang, 1999). Saponins cause serum insulin level elevation and assuage hyperglycemia by inhibiting disaccharidase activity and reduction of lipid levels (Zheng *et al.*, 2012).

The antidiabetic and antioxidant properties of alkaloids have been reported for some medicinal plants (Tiong *et al.*, 2013; Agrawal *et al.*, 2013; Okoro *et al.*, 2015b). Alkaloids have been reported to stimulate the proper functioning of insulin hormone and also have antihyperglycemic and hypolipidemic activities (Singh *et al.*, 2014). Similarly, the antioxidant and antidiabetic properties of some food items have been linked to condensed tannins (Kunyanga *et al.*, 2011). The antidiabetic properties of glycoside (Verma *et al.*, 2008), and cardiac glycoside (Tofigli *et al.*, 2016) have been reported. Research has similarly revealed that glycosides and terpenoids possess anti-diabetic actions through modulation of insulin production and activity. Besides, these phytochemicals are known to inhibit α -glucosidase activity, regulate glycemic index, and aid pancreatic β -cell recovery (Ben-Younes *et al.*, 2018).

The antidiabetic and antioxidant properties of flavonoids had been mentioned. Earlier research

indicates that quercetin (a flavonoid), moderates insulin secretion, stimulates insulin action in tissues, augments glucose uptake, and aids the restoration of pancreatic β -cells (Al-Ishaq *et al.*, 2019). Therefore, the antidiabetic influence of *C. ferruginea* extract may be a result of its phytoconstituents (Ansari, 2022).

Conclusion

The findings of our study demonstrate a noteworthy decrease in fasting blood glucose levels among diabetic rats that were administered *Cnestis ferruginea* ethanolic root extract for a duration of 21 days, as compared to diabetic control rats. The extract-treated rats exhibited a reversal of deviations in serum lipid profile parameters, kidney and liver function parameters, and oxidative stress markers in kidney and liver tissues, which were initially observed in diabetic control rats. Notably, the administration of a higher dose (400 mg/kg bw) of the extract yielded the most favourable outcomes. The analysis revealed that the extract contained a wide range of phytochemicals. The antidiabetic activities of the ethanolic root extract of *Cnestis ferruginea* may be attributed to its phytoconstituents. The results of our study provide evidence that supports the historical utilization of *C. ferruginea* roots for managing diabetes.

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