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Attenuation of Haematological and Biochemical Alterations in Alloxan-Induced Diabetic Rats by Ethanol Extract of *Annona senegalensis* Persoon (*Annonaceae*) Leaves

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Abstract: Chronic hyperglycemia brought on by abnormalities in insulin production, insulin action, or both is a typical symptom of diabetes mellitus. The study evaluated the ethanol leaves extract of *A. senegalensis* for its potential hypoglycaemic, anti-oxidative, and haematological activities in alloxan-induced diabetic rats. Also, the effect of the extract on the lipid profile, liver, and kidney functions of the rats. Following diabetic induction and treatment of the rats, standard procedures were used in determining the red blood cell/erythrocytes count, white blood cell/leukocyte count, platelets, neutrophils, monocytes, and eosinophils, electrolytes (sodium, potassium, chloride, and bicarbonate), lipid profile parameters (total cholesterol, triacylglycerides, high-density lipoprotein, low-density lipoprotein and very-low-density lipoprotein, kidney, and liver function parameters (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase activities, total protein, total bilirubin serum levels, creatinine, and urea serum levels). There was a reduction in the hyperglycaemic index and significant ($p < 0.05$) elevations in the antioxidant activity and haematological parameters. Na^+ and high-density lipoprotein were respectively significantly ($p < 0.05$) reduced and elevated. There was a significant ($p < 0.05$) decrease in the kidney and liver function parameters. The study allows for more studies, including elucidating the bioactive compounds responsible for the observed pharmacological effects.

Keywords: Diabetes mellitus; Electrolytes; Lipid profile; Hypoglycaemia; Antioxidants

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1. Introduction

Chronic hyperglycemia brought on by abnormalities in insulin production, insulin action, or both is a typical symptom of diabetes mellitus (DM) [1]. According to Mahsud et al. [2], it is a common condition that affects people in both developed and developing nations. By 2030, there are expected to be 366 million diabetics worldwide [3]. Despite developing numerous drugs that can improve the course of the disease, a therapeutic cure for diabetes mellitus has remained elusive [4]. Therefore, it is essential to keep looking into alternative treatment options, particularly those that are more secure, affordable, and accessible. According to recent studies, over 80 % of Africans rely on medicinal plant species for their primary treatment [5]. Also, herbal remedies hold a unique place in

healthcare provision, especially among the rural population, frequently encouraged by the ease of access and affordability of care [6]. Traditionally in China, medicinal plants have also formed basis of treatment of diverse ailments, in ailments, including diabetics, with most of them attributed to their high levels of phenolic compounds, alkaloids, glycosides, flavonoids, and terpenoids [7].

Although this has not been scientifically confirmed, *Annona senegalensis*, also known as "Ubuluocha" in Igbo (Nigeria), is historically used in the control of Diabetic Mellitus (DM). Smallpox, tuberculosis, and yellow fever are all treated using leaves [8]. Hernias and snakebites are treated using stem bark [9]. The root is an antidote for fatal toxins and various infectious ailments and treats conditions like difficulties swallowing, gastritis, snake bites, male sexual impotence, erectile dysfunction, and tuberculosis [10]. According to Faleyimu and Akinyemi [11] and Madièye et al. [12], the juice from the tree is used to cure chickenpox and has anti-inflammatory and antipyretic qualities.

Babalola et al. [13] reviewed various *in vitro* and *in vivo* biological activities of the plant's different parts. These include anti-inflammatory, anti-oxidative, cytotoxic, antiplasmodial, antimicrobial, antitrypanosomal, analgesic, anthelmintic, and hypnotic effects. Specifically, Chedi et al. [14], via *in vivo* studies, reported the acute toxicity (lethal dose at 1131 mg/kg by intraperitoneal route) and antihyperglycaemic effect (insignificant reduction in the fasting serum glucose level and significant reduction in the blood glucose levels) of the ethanol stem bark. Adzu et al. [15] and Chinyere et al. [16] reported *in vivo* anti-snake venom activities of the plant root's methanol and aqueous extracts, respectively. The methanol extracts reduced hyperthermia following exposure to the toxin and had 16 – 33 % detoxification effects. However, it failed to restore some biochemical functions of the liver. The aqueous extract showed inhibitory activity against *Bitisarientans* venom protease and phospholipase A2. Considerable *in vitro* anti-inflammatory and *in vivo* antiulcer prophets were reported using 200 mg/kg of the ethyl-acetate extract of the root bark [17]. Mlozan et al. [18] demonstrated *in vitro* ability of the aqueous root bark extract to inhibit oxidase activity by 83 %, thus, can help to modulate plasma urates, hydrogen peroxide, and superoxide radicals. The antibacterial activities of the lipophilic fraction and kaurenoic acid obtained from the root bark extract were reported against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [19]. Ngbolua et al. [20] reported moderate *in vitro* and weak *in vivo* antiplasmodial activities of the ethanol crude extract against two malaria parasites strains (*Plasmodium falciparum* FcM29 and *Plasmodium yoelii* subsp nigeriensis) in the Democratic Republic of Congo. They also reported toxic effects on P-388 cells. Antitrypanosomal effects of the hexane and aqueous extract at 400 and 300 mg/kg body weights, respectively, were stated by Kabiru et al. [21]. Biseko et al. [22] reported the anti-proliferation potential of petroleum ether extract against cancer cells. The current study aimed to assay the antioxidant, electrolyte, and haematological effects of ethanol leaves extract of *A. senegalensis* on alloxan-induced diabetic rats and additional data on the lipid profile, liver, and kidney functions to determine the safety of its use.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade chemicals and reagents used were from May and Baker, England; Merck, Germany; Sigma-Aldrich, U.K.; and Kieselgel GmbH, Germany. Commercial test kits and glibenclamide products from Randox, UK, Biovendor, Czech Republic, TECO Diagnostics, USA, and Centronic GmbH, Germany, were used as reagents for the assays.

2.2. Ethical clearance

The Faculty and Biosafety Committee, Faculty of Biological Sciences, University of Nigeria Nsukka, granted clearance (number: UNN/FBS/EC/1086) for the experimental methods and the ethical use and care of laboratory animals.

2.3. Animals

Both sexes of 18 adult Swiss albino mice (20–30 g) and 42 adult albino rats (80– 150 g) were procured from the Department of Zoology and Environment Biology animal holding facility, University of Nigeria, Nsukka. Standard housing conditions were used for the animals (25 ± 2 °C, a 12-hour light/dark cycle). The animals were acclimatized for two (2) weeks before being fed standard pellets (Grand Cereals Ltd., Enugu, Nigeria) twice daily. They also had unrestricted access to clean drinking water. The National Institute of Health Guide for Care and Use of Laboratory Animals was followed in all animal research [23].

2.4. Plant material and Extraction

The sample was *A. senegalensis* leaves duly identified (voucher number: BDCP202106) and verified by Mr A. Ozioko, a certified botanist affiliated with the Bioresource Development and Conservation Program (BDCP), Research Center Nsukka, Enugu State. The fresh leaves were collected, cleaned, and dried for three (3) weeks at room temperature (29 to 35 °C) to a constant weight. The dried leaves were ground into a coarse powder using a crestor high-speed milling machine.

For extraction, 400 g of the powdered leaves were soaked and macerated in 95 % ethanol for 72 hours, after which it was filtered with a mesh, followed by a Whatman filter paper. The filtrate was concentrated into a semi-solid residue in an oven at 60 °C to separate the solvent from the extract. The samples were stored in the refrigerator for subsequent studies.

2.5. Phytochemical Screening

The screening for phytochemicals in the plant leaves was carried out using standard procedures [24].

2.6. Determination of Acute Toxicity and Lethality (LD_{50})

The toxicity profile was determined in mice using a standard procedure [25]. Briefly, 10, 100, and 1000 mg/kg of Z.M. slurry were given orally to nine (9) mice randomly divided into three groups (n = 3), and the animals were in the next 24 hours monitored for fatalities. Since there was no mortality yet, 1600, 2900, and 5000 mg/kg of each drug were supplied to a new group of animals (n = 1). The number of fatalities in 24 hours was, however, noted. The geometric mean of the highest non-fatal dose and the lowest deadly dose were used to determine the LD_{50}.

2.7. Induction of diabetes, blood glucose determination, and treatment of diabetes

The method employed by Okwesili et al. [26] with slight modification was used. Following acclimatization, the rats were randomly allotted into seven (7) groups. The animals in groups 2 – 6 were induced with diabetes using 150 mg/kg body weight alloxan, after which group 3 were treated with 2.5 mg/kg body weight of glibenclamide (positive control), and group 4, 5, 6, and 7 treated with 200, 400, 600, and 800 mg/kg body weight of ethanol leaves extract, respectively. Animals in group 2 were not treated, while those in group 1 were not induced with diabetics, both serving as negative controls. The treatments were administered orally once daily for 14 consecutive days. Following treatments, blood was collected by an ocular puncture for biological analysis, after which the animals were sacrificed.

2.8. Lipid profile

Random kits were used to determine the serum cholesterol (CHOL), triacylglycerides (TAG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) [27],[28],[29],[30].

2.9. Antioxidants enzyme activity

According to Arthur and Boyne [31], the Randox Kit measured the superoxide dismutase (SOD) activity. The Sinha method [32] was used to measure the activity of catalase. Glutathione (GSH) concentration and glutathione activities (GPx) were measured according to King and Wootton [33]. The degree of lipid peroxidation, malondialdehyde (MDA), the byproduct of lipid peroxidation, was measured spectrophotometrically, as reported by Wallin et al. [34]

2.10. Haematological indices

The white blood cell (WBC) and red blood cell/erythrocytes (RBC) counts, packed cell volume (PCV), and Haemoglobin (HB) concentration (using the cyanomethaglobin technique) were determined as described by Ochei and Kolhatkar [35].

2.11. Kidney function test

The serum urea and creatinine concentrations were determined using the methods described by Tietz [36].

2.12. Liver function test

Using the serum samples obtained after centrifugation at 3500 g for 10 minutes, the liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities were determined according to the methods described by Joshua et al. [37]. The total protein content was determined using the method described by Plummer [38].

2.13. Determination of serum electrolytes

The serum electrolytes, including sodium, potassium, bicarbonate, and chloride ions, were assayed using the methods described by Tietz et al. [39].

2.14. Statistical Analysis

The data obtained were analyzed using one-way ANOVA in Statistical Package for Social Sciences (SPSS) version 23.0, and the results were expressed as mean ± standard error of the mean (SEM). Differences between the mean of treated and control groups were further subjected to Dunnett's Post Hoc test and considered significant at $p < 0.05$.

3. Results

Qualitative and quantitative phytochemical constituents

The extract's phytochemical analysis revealed the presence of high to moderate bioactive substances, including alkaloids (780.00 ± 8.82), terpenoids (844.34 ± 28.86), flavonoids (1907.40 ± 278.60), steroids (1236.60 ± 145.02), and tannins (222.77 ± 15.68). However, glycosides (52.30 ± 0.81), total phenol (45.08 ± 4.27), saponins (31.40 ± 0.88), and carotenoids (83.60 ± 4.10) were mildly detectable in the extract.

3.1. Acute toxicity and lethality (LD_{50}) Asay

Oral administration of the extract concentrations, up to 5000 mg/kg caused no death in mice, and there was no sign of weakness or decreased activity/movement following the high dose.

3.2. Effect of the extract on blood glucose levels

Before the induction of alloxan and treatments, there was no significant difference ($p > 0.05$) in the blood glucose levels among the experimental animal groups. The effect of alloxan on the glucose levels of the animals after 48h of induction showed a rapid significant ($p < 005$) increase in the induced groups (2, 3, 4, 5, 6, and 7) compared to the normal group (group 1). The treatment groups (4, 5, 6, and 7) blood glucose levels after treatment

showed a significant ($p < 005$) decrease compared to the untreated animals (group 2), as shown in Table 1 below.

Table 1. Effect of the extracts on blood sugar levels.

Groups	Before induction (mg/dl)	After induction (mg/dl)	One week treatment (mg/dl)	Two weeks of treatment (mg/dl)
Group 1	99.50 ± 8.52^{aA}	97.00 ± 4.85^{aA}	94.50 ± 18.99^{aA}	87.16 ± 11.42^{aA}
Group 2	103.50 ± 4.94^{Aa}	531.50 ± 50.20^{bC}	529.50 ± 44.54^{bC}	536.00 ± 32.52^{bF}
Group 3	96.83 ± 5.84^{aA}	513.33 ± 88.15^{dC}	388.33 ± 87.19^{cB}	208.66 ± 58.38^{bB}
Group 4	96.40 ± 8.59^{aA}	459.40 ± 94.73^{dBC}	331.80 ± 107.09^{bB}	227.60 ± 33.22^{cBC}
Group 5	94.00 ± 4.32^{aA}	374.75 ± 46.54^{bB}	302.00 ± 28.36^{bB}	242.00 ± 76.58^{cBCD}
Group 6	101.00 ± 8.83^{aA}	519.00 ± 87.52^{dC}	404.00 ± 69.24^{cB}	285.75 ± 23.15^{bCD}
Group 7	99.00 ± 6.48^{aA}	470.25 ± 67.00^{dBC}	384.250 ± 65.850^{cB}	299.00 ± 42.32^{bD}

*n = 6, results are presented as Mean ± SEM. Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$.

3.3. Effects of ethanol extract of A. senegalensis leaves on lipid profile.

The serum total cholesterol levels in groups 3, 4, 5, 6, and 7 were significantly ($p < 0.05$) lower compared to group 2. The triacyl glyceride level in group 2 was not significantly different compared to groups 1, 3, 4, 5, 6, and 7. The LDL in groups 1, 3, 4, 5, 6, and 7 were significantly ($p < 0.05$) lower compared to group 2. However, the concentrations of HDL cholesterol in groups 1, 3, 4, 5, 6, and 7 were significantly ($p < 0.05$) higher compared to group 2 (Table 2).

Table 2. Effect of the extract on lipid profile.

Groups	T.CHO. (Mg/dl)	Triglyceride (Mg/l)	LDL (Mg/l)	VLDL (Mg/l)	HDL (Mg/l)
Group 1	117.02 ± 6.76^{a}	142.98 ± 4.23^{a}	33.53 ± 6.19^{a}	28.59 ± 0.85^{a}	55.05 ± 1.68^{c}
Group 2	173.69 ± 10.9^{c}	159.12 ± 6.70^{a}	104.10 ± 12.36^{c}	31.82 ± 1.34^{a}	37.76 ± 0.12^{a}
Group 3	144.61 ± 9.01^{b}	149.94 ± 4.04^{a}	65.67 ± 9.01^{b}	29.99 ± 0.81^{a}	48.95 ± 1.51^{b}
Group 4	144.62 ± 3.67^{b}	153.92 ± 7.11^{a}	66.39 ± 4.97^{b}	30.75 ± 1.43^{a}	47.38 ± 2.92^{b}
Group 5	146.85 ± 3.77^{b}	157.54 ± 4.71^{a}	66.61 ± 2.57^{b}	31.51 ± 0.94^{a}	48.73 ± 0.87^{b}
Group 6	145.47 ± 2.49^{b}	155.31 ± 1.49^{a}	66.76 ± 3.11^{b}	31.06 ± 0.29^{a}	47.65 ± 1.77^{b}
Group 7	148.61 ± 3.36^{b}	155.87 ± 3.69^{a}	67.46 ± 3.72^{b}	31.14 ± 0.72^{a}	$50.01 \pm 0.22b^{c}$

*n = 6, results are presented as Mean ± STD. Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$.

3.4. Effect of the extract on antioxidants enzyme activities

The MDA levels in the treated groups were significantly ($p < 0.05$) lower compared to the group 2 control group and significantly higher compared to the group 1 control group. The GPx activities in groups 1, 3, 4, 5, 6, and 7 were significantly ($p < 0.05$) higher compared to group 2. The SOD activities in groups 1, 3, 4, 5, 6, and 7 were significantly (p

< 0.05) higher compared to group 2. The catalase activities in groups 1, 3, 4, 5, 6, and 7 were significantly ($p < 0.05$) higher compared to group 2. And the concentrations of reduced glutathione (GSH) in groups 1, 3, and 7 were significantly ($p < 0.05$) higher compared to group 2 (Table 3).

Table 3. Effect of ethanol extract of *A. senegalensis* leaves on antioxidants enzyme activities.

Groups	MDA (Mmol/mg)	GPX (u/mg)	SOD (u/mg)	Catalase (u/mg)	GSH (u/mg)
Group 1	3.696 ± 1.052^{a}	33.413 ± 5.358^{b}	70.036 ± 5.925^{c}	5.074 ± 1.024^{c}	25.201 ± 3.395^{b}
Group 2	5.823 ± 0.129^{c}	23.163 ± 2.385^{a}	50.986 ± 6.585^{a}	2.602 ± 0.540^{a}	19.372 ± 0.507^{a}
Group 3	4.055 ± 0.609^{a}	30.781 ± 1.543^{b}	65.643 ± 4.996^{b}	4.266 ± 0.423^{bc}	24.060 ± 2.227^{b}
Group 4	4.732 ± 0.850^{b}	30.018 ± 3.039^{b}	61.478 ± 3.916^{b}	3.703 ± 0.981^{b}	21.919 ± 2.788^{ab}
Group 5	4.625 ± 0.575^{b}	30.391 ± 3.124^{b}	59.559 ± 5.251^{b}	3.850 ± 0.981^{b}	22.690 ± 1.138^{ab}
Group 6	4.317 ± 0.1747^{b}	31.994 ± 1.692^{b}	58.966 ± 4.586^{b}	4.037 ± 0.613^{bc}	22.600 ± 1.382^{ab}
Group 7	4.881 ± 0.643^{b}	29.014 ± 1.944^{b}	59.439 ± 4.520^{b}	3.594 ± 0.228^{ab}	24.007 ± 1.652^{b}

*n = 6, results are presented as Mean ± STD. Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$.

3.5. Effect of the extract on haematological indices.

Group 7 showed a significant ($p < 0.05$) increase in value compared to group 2. There was a significant ($p < 0.05$) decrease in Hb concentrations in groups 2, 3, and 5 compared to group 1. Also, the Hb level in group 7 showed a significant ($p < 0.05$) increase compared to group 2. The WBC decreased significantly ($p < 0.05$) in groups 2, 3, 4, 5, 6, and 7 compared to group 1, and there was no significant change compared to group 2. There was a significant ($p < 0.05$) change in platelet levels in groups 3 and 5 compared to group 1 (Table 4).

Table 4. Effect of the extract on haematological indices.

Groups	PCV	HB	RBC	WBC	Platelets	Neutrophil (%)	Leucocyte (%)	Monocyte (%)	Eosinophil (%)
Group 1	35.67 ± 1.33^{a}	21.46 ± 1.10^{c}	3.47 ± 0.18^{a}	6283.33 ± 297.12^{b}	98.00 ± 9.52^{b}	60.33 ± 1.20^{a}	35.67 ± 1.05^{a}	2.50 ± 0.34^{a}	1.50 ± 0.22^{a}
Group 2	28.50 ± 4.50^{a}	16.43 ± 1.27^{a}	3.25 ± 0.25^{a}	4700.00 ± 700.00^{a}	103.00 ± 3.00^{b}	56.50 ± 0.50^{a}	39.00 ± 0.00^{ab}	3.00 ± 1.00^{a}	1.50 ± 0.50^{a}
Group 3	33.83 ± 2.167^{a}	18.99 ± 0.98^{abc}	2.98 ± 0.15^{a}	4900.00 ± 276.88^{a}	95.67 ± 2.44^{b}	56.17 ± 0.75^{a}	39.67 ± 0.84^{b}	2.67 ± 0.33^{a}	1.50 ± 0.22^{a}
Group 4	30.60 ± 1.80^{ab}	20.85 ± 0.50^{bc}	2.88 ± 0.33^{a}	4880.00 ± 412.79^{a}	86.40 ± 8.21^{a}	58.00 ± 1.38^{a}	37.80 ± 1.28^{ab}	2.40 ± 0.51^{a}	2.00 ± 0.32^{a}
Group 5	32.00 ± 2.45^{ab}	18.27 ± 0.34^{ab}	3.13 ± 0.16^{a}	4450.00 ± 236.29^{a}	59.25 ± 12.17^{b}	57.50 ± 2.22^{a}	39.00 ± 1.83^{ab}	1.75 ± 0.48^{a}	1.75 ± 0.25^{a}
Group 6	33.75 ± 2.59^{ab}	19.39 ± 0.66^{abc}	3.58 ± 0.33^{a}	4750.00 ± 262.99^{a}	97.75 ± 11.34^{b}	58.25 ± 0.63^{a}	37.50 ± 0.87^{ab}	2.25 ± 0.25^{a}	2.00 ± 0.41^{a}
Group 7	34.00 ± 3.22^{b}	20.54 ± 0.54^{bc}	3.70 ± 0.26^{a}	4600.00 ± 294.39^{a}	110.00 ± 6.06^{b}	57.00 ± 1.00^{a}	38.40 ± 0.68^{ab}	2.80 ± 0.37^{a}	1.80 ± 0.20^{a}

*n = 6, results are presented as Mean ± STD. Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$.

3.6. Effect of the extract on kidney function

There was a significant ($p < 0.05$) increase in urea concentration in group 2 (untreated group) compared to normal control (group 1). There was a significant ($p < 0.05$) increase in the level of creatinine in group 2 when compared to group 1 (Table 5).

Table 5. Effect of extract of on kidney function.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Group 1	30.97 ± 7.24^{a}	0.77 ± 0.09^{a}

Group 2	42.54 ± 9.01^{b}	0.90 ± 0.03^{b}
Group 3	35.38 ± 4.78^{ab}	0.77 ± 0.08^{ab}
Group 4	40.12 ± 4.96^{ab}	0.84 ± 0.03^{ab}
Group 5	38.22 ± 5.94^{ab}	0.82 ± 0.03^{ab}
Group 6	36.98 ± 6.97^{ab}	0.86 ± 0.04^{ab}
Group 7	32.51 ± 5.08^{a}	0.79 ± 0.04^{a}

*n = 6, results are presented as Mean ± STD. Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$.

3.7. Effect of the extract on liver function

Compared to group 2, the total protein levels in groups 1, 3, 4, 5, and 6 were considerably higher ($p < 0.05$). Compared to group 1, the AST activities in groups 2 and 7 were found to be considerably ($p < 0.05$) greater. Compared to group 1, the ALT activities in groups 2, 3, 4, 5, 6, and 7 were found to be considerably ($p < 0.05$) higher. Compared to group 1, the ALP activity in groups 2 and 7 was found to be considerably ($p < 0.05$) higher. Compared to group 2, the ALP activities in groups 1, 3, 4, 5, 6, and 7 were considerably ($p < 0.05$) lower. Additionally, compared to all the groups, group 2's ALP activity and bilirubin concentration were considerably ($p < 0.05$) higher (Table 6).

Table 6. Effect of the extract on liver function parameters.

Groups	T. Protein (g/dl)	AST (u/l)	ALT (u/l)	ALP (u/l)	T. Bilirubin (Mg/dl)
Group 1	6.32 ± 0.99^{b}	32.00 ± 5.01^{a}	46.83 ± 3.97^{a}	37.83 ± 7.47^{a}	0.44 ± 0.07^{a}
Group 2	3.55 ± 1.08^{a}	47.00 ± 1.41^{b}	64.00 ± 4.24^{c}	52.98 ± 4.77^{b}	0.83 ± 0.14^{b}
Group 3	5.17 ± 0.76^{b}	36.50 ± 2.50^{ab}	54.16 ± 5.38^{b}	40.71 ± 2.21^{a}	0.52 ± 0.15^{a}
Group 4	4.79 ± 0.52^{b}	35.40 ± 3.20^{a}	55.20 ± 4.60^{b}	40.89 ± 3.32^{a}	0.64 ± 0.13^{a}
Group 5	4.96 ± 0.16^{b}	38.00 ± 6.37^{ab}	55.25 ± 2.98^{b}	43.76 ± 5.97^{a}	0.50 ± 0.05^{a}
Group 6	5.08 ± 0.20^{b}	32.00 ± 2.00^{a}	58.00 ± 4.61^{bc}	40.72 ± 3.50^{a}	0.52 ± 0.09^{a}
Group 7	4.51 ± 0.43^{a}	43.25 ± 8.38^{b}	54.25 ± 5.56^{b}	45.60 ± 1.44^{a}	0.48 ± 0.06^{a}

*n = 6, results are presented as Mean ± STD. Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$.

3.8. Effect of the extract on serum electrolytes

Na^{+} concentrations in group 2 were significantly ($p < 0.05$) lower compared to groups 1, 4, 5, 6, and 7. The k^{+} concentrations in groups 1, 3, 4, 5, 6, and 7 exhibited significant ($p < 0.05$) higher values compared to group 2 (Table 7).

Table 7. Effect of the extract on serum electrolyte concentration.

Groups	Sodium (mEq/l)	Potassium (mEq/l)	Bicarbonate (Mmol/l)	Chloride (mEq/l)
Group 1	243.473 ± 40.219^{b}	0.707 ± 0.213^{b}	28.333 ± 3.929^{a}	144.661 ± 30.627^{b}
Group 2	149.321 ± 21.195a	0.324 ± 0.007^{a}	24.342 ± 5.980^{a}	108.139 ± 9.866^{a}
Group 3	198.437 ± 44.004^{ab}	0.611 ± 0.130^{b}	25.244 ± 3.203^{a}	124.927 ± 24.872^{ab}
Group 4	204.983 ± 31.715^{b}	0.615 ± 0.058^{b}	25.856 ± 4.443^{a}	130.459 ± 12.614^{ab}
Group 5	234.083 ± 17.065^{b}	$0{\cdot}603 \pm 0.018^{b}$	21.964 ± 2.361^{a}	112.500 ± 15.697^{ab}
Group 6	222.105 ± 23.407^{b}	0.540 ± 0.069^{b}	25.535 ± 5.096^{a}	127.284 ± 4.208^{ab}
Group 7	236.700 ± 32.477^{b}	0.539 ± 0.099^{b}	25.387 ± 2.883^{a}	122.543 ± 10.292^{ab}

*n=6, results are presented as Mean ± STD. Mean values with different letters as superscripts across the column are considered significant at $p < 0.05$.

4. Discussion

There is a need for bioprospection and the development of ethnomedicinal plants as an alternative to current hypoglycaemic drugs which are often costly with side effects. A high safety profile with no recorded death was revealed by the extract's acute lethality (LD_{50}). The mice in all groups survived for the test duration after receiving the various doses of the ethanol leaves extract of *A. senegalensis*. After oral administrations, neither

treatment-related adverse symptoms nor mortality was seen, indicating relative safety. Also, none of the mice exhibited aberrant behavioural responses. Compared to the control groups, there was no difference in behaviour, body weight, temperature, food and water intake, skin effects, fur coating, eyes, mucous membranes, and respiratory activities.

In agreement with Theophine et al. [40], the phytochemical screening revealed the varying presence of steroids, saponins, carotenoids, glycosides, terpenoids, phenol, flavonoids, tannins, and alkaloids were found in varying amounts during phytochemical screening. N'Diaye et al. [41]. These secondary metabolites may be essential in managing several conditions, including diabetes and inflammation. In the alloxan-induced diabetic rats, the plant extract dramatically decreased hyperglycemia. This might be explained by flavonoids' antihyperglycemic characteristics, which increase the body's sensitivity to insulin [42]. Additionally, saponin has an antihyperglycemic effect, according to Kambouche et al. [43]. Thus, the antihyperglycaemic action of the extract could be attributed to the metabolites. [41].

The examination of the extract's antidiabetic potential revealed substantial improvements in the antioxidants, haematological indices, serum electrolytes, lipid profile, and kidney and liver function parameters. One of the most prevalent symptoms of diabetes is loss of body weight. Aside from the increased appetite, insulin insufficiency slows down all anabolic processes while speeding up catabolic ones, which furthers the loss of body weight led by polyuria and glycosuria [44]. The weights of the animals did not differ significantly from the normal control weight following treatment, consistent with the findings of Yisa et al. [45]. However, differed with a significant decrease at higher doses, revealing a wasting effect of the extract, probably caused by the presence of saponin in the plant [46].

Compared to the positive control and the elevated HDL concentrations in the treated rats, the total blood lipids (cholesterol and LDL) levels in the treated animals were statistically lower. This could explain the use in treating diabetes and high blood pressure. From studies, a rise in blood TC, LDL, and atherogenic index levels, as well as a fall in HDL concentrations, enhance the risk of cardiovascular disease. Longe et al. [46] and Mattar and Helal [47] also noted a comparable decline in serum total lipids as seen in this study. However, there were no appreciable variations from the control in the serum triacyl glyceride and VLDL levels. The drop in free fatty acids possibly coincided with the reduction in the total lipid [48]. In the case of fat build-up in adipocytes, such a decrease is anticipated in terms of decreased fatty acid liberation and synthesis.

Studies have demonstrated a link between diabetes, increased free radical production, and decreased antioxidant capability. Our study indicated that diabetic rats had lower blood levels of SOD, CAT, and GPx activities and higher tissue levels of MDA. The oxidative response was enhanced by the administration of the plant extract. The rats' SOD, CAT, and GPx activities were revived. However, the extract reduced the MDA levels in the treatment groups. Two pathways could account for the antioxidant effects. First, the extract reduced the harmful effects of free radicals and prevented protein glycosylation and peroxidation by interacting with them. Second, the antioxidant enzymes' protein production was stimulated by the plant extract. According to earlier research, polyphenolic substances boosted the transcriptional expression of the SOD and GPx enzymes [49].

GSH, a significant non-protein in living things, also coordinates the body's antioxidant defence mechanisms and results in severe biological effects when altered [50]. The decrease in GSH concentration in diabetic-induced rats' serum and the subsequent rise to near normal in the rats treated with the extracts demonstrate the antioxidant activity of the plant leaves. Also, the Free radical destruction and prevention of GSH depletion explain the potential mechanism underlying this medication's antioxidant capabilities [51]. These findings are consistent with an earlier report {52].

During diabetes, the immune system and several haematological parameters change [53]. Additionally, toxicological research has shown that ingesting medications/medicinal plants can change usual haematological values [54]. Therefore, haematological measures

may be crucial in determining a drug's adverse effects and those of medicinal plants and their extracts [55]. According to a report on the minor increase in RBC and its indicators by *N. laevis* leaves extracts [56]; the increase may be attributable to the extract's stimulation of the formation of stromal cells and macrophages in the bone marrow's erythropoietin and colony-stimulating factor. These effects could be brought on by phytoconstituents, such as flavonoids, found in *N. laevis* leaves tissue [56]. White blood cell (WBC), packed cell volume (PCV), and haemoglobin (Hb) concentration values were lowered in diabetic rats in the current investigation. However, treatment with the ethanol leaves extract of *A. senegalensis* increased the WBC, PCV, and Hb concentration values in diabetic rats. This is consistent with earlier studies that have established the incidence of anaemia in people with diabetes [57]. In addition, the count of platelets, neutrophils, lymphocytes, eosinophils, basophils, and monocytes somewhat increased, which conflicts with the findings of Olotu et al. [58].

The primary excretory organ is the kidney, and renal function testing is designed to find any potential renal injury. One of the most sensitive signs of kidney damage is elevated urea and creatinine serum levels. These increases are frequently brought on by hyperglycemia. Alarcon-Aguilar et al. [59] stated that alloxan-induced hyperglycemia caused serum urea and creatinine levels to rise. It has been hypothesized that activating gluconeogenesis as an alternative glucose source due to insulin insufficiency causes elevated serum urea in diabetic control rats [60]. Increased proteolysis releases glucogenic amino acids, which are then deaminated in the liver and result in high quantities of urea, sustaining gluconeogenesis [61]. These indicators were marginally and dose-dependently decreased after receiving the plant extract for 14 days. The stabilization of these variables points to an improvement in renal function, which may be explained by the extract's antihyperglycaemic effects, which lead to an increase in insulin action and a decrease in proteolysis. Similar findings have been reported [61].

To determine the early harmful effects, enzyme activity in the tissues is often utilized as "markers" during the administration of foreign chemicals to experimental animals and results in to increase in ALT, AST, and ALP activities. The elevated levels of the markers in the serum are a sign of cellular leakage and a deterioration of the liver's functional cellular membrane integrity [62]. The administration of the extract mildly decreased the increased serum levels of these parameters towards the corresponding normal control. This suggests that with sustained use, the plant extract may stabilize the plasma membrane and aid in healing the injury to the hepatic tissue, as observed by Effiong and Akpan [63]. A valuable tool for determining the extent of liver damage is total serum protein, a marker of the liver's synthetic function [64]. The possibility of hepatocyte injury brought on by alloxan and prolonged hyperglycemia can explain the decrease in protein levels in diabetic control rats [65]. This might happen due to enhanced protein synthesis brought on by higher insulin levels, which might be related to the extract's ability to protect the liver from oxidative damage [66]. Since total bilirubin is conjugated in the liver for potential excretion via the kidneys or bile, it is also a significant indicator of liver function. Liver function was affected in diabetic control rats because of an elevated total bilirubin level. This finding is in line with a previous report [61]. A decrease in liver absorption, conjugation, or an increase in bilirubin production after liver damage is all possible cause of the rise in plasma bilirubin [67].

An imbalance in the homeostasis of the electrolytes results from the increased volume and metabolite excretions through the kidneys, which are often above normal thresholds [68]. According to Onunogbo et al. [69], the increased electrolyte and water levels commonly seen in diabetes may cause a depletion of extracellular fluid electrolytes and, as a result, cause parietal and non-parietal cells to excrete electrolytes. This may explain the significant drop in serum sodium ions (Na^+) and potassium ions (K^+) in diabetic untreated rats compared to the normal control. However, the decrease in electrolytes after oral administration of the extract shows the successful increase of the treated rats' altered extracellular fluid electrolyte levels [69]. Further evidence that the extract improves

rehydration and prevents metabolic acidosis comes from the study's observation of a slight increase in blood Cl^- and HCO_3^- levels in treated groups compared to diabetes controls.

5. Conclusion

The study demonstrated that *A. senegalensis* leaves extract could control hyperglycemia in alloxan-induced diabetic rats. Also, it increases antioxidant levels and haematological abnormalities and improves kidney, liver, and electrolyte derangements. The study supports the use of *A. senegalensis* leaves extract in treating diabetes and its consequences. It also opens the way for more studies, including elucidating the bioactive compounds responsible for the pharmacological effects.

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