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Effect of Different Fractions of *Lawsonia inermis* Linn on Haematobiochemical Changes, Osmotic Fragility and Lipid Profile in Streptozotocin-induced Diabetic Wistar Rats

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ABSTRACT

Diabetes mellitus is a major health challenge that has harmful effects on the quality of life globally as a result of its numerous complications. This study aimed to evaluate the positive modulatory effect of *Lawsonia inermis* (LI) Linn on various haemobiochemical parameters and lipid profiles in streptozotocin-induced diabetic rats. Ten groups of diabetic rats (n = 5) were orally administered 50 and 100 mg/kg of three different fractions of *Lawsonia inermis*: metformin (500 mg/kg) and glibenclamide (5 mg/kg), whereas untreated hyperglycaemic and normoglycaemic rats received distilled water. The results showed increased red blood cells (RBC) in treated rats compared to untreated diabetic rats. Hb, MCV, MCH, and MCHC decreased nonsignificantly, whereas WBC increased nonsignificantly. Neutrophil increased non-significantly in diabetic, untreated rats, while all the treatment groups decreased non-significantly. Lymphocytes and monocytes increased non-significantly ($p > 0.05$). Most treatment groups showed a non-significant ($p > 0.05$) increase in the platelet count. The ALT levels decreased non-significantly ($p > 0.05$) compared to normoglycemic rats. The AST levels decreased significantly ($p<0.01$). ALP decreased non-significantly in both treated and untreated groups, whereas bilirubin did not show any significant changes. Creatinine and urea levels in untreated diabetic rats increased non-significantly, while treatment groups decreased non-significantly. *Lawsonia inermis*-treated rats showed significant improvement in erythrocytic fragility, while glycated haemoglobin in untreated-diabetic and glibenclamide-treated rats increased significantly ($p > 0.001$). Triglycerides and high-density lipoprotein decreased non-significantly, while low-density lipoprotein increased non-significantly ($p > 0.05$) in diabetic untreated rats. All treatment groups showed a non-significant ($P > 0.05$) decrease in low-density lipoprotein. *Lawsonia inermis* showed a significant positive modulatory effect on the haemobiochemical changes, glycated Hb, osmotic fragility, and lipid profile in streptozotocin-induced diabetic rats.

Keywords: **:** Diabetes, Glycated Hb, Haematology, *Lawsonia inermis*, Serum chemistry, Wistar rats.

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INTRODUCTION

Diabetes mellitus has a mortality rate of 6–7%, and World Health Organisation has declared it the seventh leading cause of death worldwide. Between 2000 and 2016, reports have shown that diabetes mellitus is directly linked to 5% of premature deaths that occur in most developing countries **(Xiling *et al.*, 2020).**

Diabetes mellitus has been linked to several haematological abnormalities, though there were no specific pathologic findings in the blood of the patient **(Banerjee *et al.*, 2020).** It was observed that these changes may affect the red blood cells (RBCs), white blood cells (WBCs), platelets, and their coagulatory factors **(Noori *et al.*, 2022).** It's interesting to observe that diabetes patients' differential WBC counts increased overall despite no change in the proportion of monocytes, including basophils, eosinophils, and neutrophils **(Suvarna *et al.*, 2023).** Studies have shown that increased platelet reactivation in diabetic patients

will likely result in less cardiovascular protection **(Mainoli *et al.*, 2021)** because many studies have shown that hyperinsulinemia and insulin resistance are directly linked to erythroid progenitors' stimulation, leading to increased pro-inflammatory markers that predispose patients to diabetic complications **(Ellinger *et al.*, 2018).** It has been reported that the osmotic fragility of RBC is usually higher in patients with diabetes mellitus (especially type 2) when compared to normoglycaemic patients. The fragility of RBC is usually correlated to glycosylated haemoglobin **(Kung *et al.*, 2009).**

Several factors affect lipid levels in diabetic patient and lipid abnormalities play a vital role in ensuring vascular anomalies associated with diabetes mellitus thus making it pertinent to evaluate the lipid profile in most diabetic patients so as to ensure prompt management **(Al Ghadeer *et al.*, 2022).** Reports have shown that persistent uncontrolled dyslipidemia leads to numerous medical complications such as increased in the incidence of coronary heart disease most especially in diabetic patient **(Antwi-Baffour *et al.*, 2018).**

Lawsonia inermis Linn. is a very valuable herb used all around the world. The powdered leaves have been used to dye beards, hands, and nails, among other body parts **(Sen *et al.*, 2023).** Numerous accounts have demonstrated that *L. inermis* leaves are used to treat a variety of illnesses, particularly in the south-western region of Nigeria, including measles, diabetes, and poliomyelitis **(Oladunmoye and Kehinde, 2011).** However, due to its deodorising properties, *L. inermis* seed is reported to have been employed in the treatment of gynaecological conditions such as vaginal discharge, menorrhagia, and leucorrhoea **(Abdulfatai and Ayotunde, 2022).**

Several studies have been conducted to compare the haematological parameters and lipid profiles of diabetic patients, but there is a paucity of information showing the modulatory effect of medicinal plants used in treating diabetes mellitus. This study aimed to evaluate the positive modulatory effect of *Lawsonia inermis* Linn leaves on the haemobiochemical parameters, glycated haemoglobin and lipid profiles of a streptozotocin-induced diabetic rat model.

MATERIALS AND METHODS

Plant Harvesting, Identification and Preparation

Leaves of *Lawsonia inermis* Linn were harvested from farmland in the Ilorin East area council of Kwara State, Nigeria. It was both identified and authenticated at University of Ibadan Herbarium, and a specimen was deposited and assigned a voucher number of **UIH-22460.** The leaves of *Lawsonia inermis* Linn were dried at room temperature (25 ^{o}C) under shade.

Extraction and Separation of *Lawsonia Inermis*

5 kg of powdery leaves of the *Lawsonia inermis* were soaked in 4 litres of *n*-hexane, ethyl acetate, and methanol for 72 hours. The mixture was gently decanted and filtered using filtered paper. The filtrate was immediately evaporated at 40 $^{\circ}$C using a rotary evaporator. The concentrate (wet residue from different solvents) was dried and stored at 4 ^{o}C in the refrigerator.

Fractionation of a crude methanolic extract of *Lawsonia inermis* Linn leaves

The crude methanol extract of *Lawsonia inermis* Linn leaves (200 g) was subsequently extracted with N-hexane, ethyl acetate, and methanol in order to increase polarity.

Phytochemical screenings

Dry solid samples of crude methanol extract were assayed for phytochemical content because of their high solubility compared to n-hexane and ethyl acetate. This was done following the methods described by **Trease and Evans (1989)** as follows:.

Tannins

10 mL of distilled water was diluted with 0.5 g of henna extract and heated for 15 minutes. The mixture was subsequently filtered. 2 mL of the filtrate was then added to another 10 mL of distilled water along with a drop of $FeCl_3$. The appearance of blue-green colouration indicates that tannins were present in the plant.

Phlebotannins

Three (3) drops of 40% formaldehyde were added to 5 mL of the filtrate from the tannin test. Then six (6) drops of dilute hydrochloric acid (HCl) were supplemented with the mixture. The temperature of the mixture was raised to a boiling point and cooled to a bulky precipitate that was washed with hot distilled water, ethanol, and a warm 5% potassium hydroxide. The appearance of coloured residues showed that phlebotannins were present in *Lawsonia inermis* Linn.

Flavonoids

0.5 g of the extract of the henna plant was added to some magnesium ribbons and 5 mL of concentrated hydrochloric acid. Red colouration confirms the presence of flavonoids.

Saponins

0.5 g of henna extract was added to 5 mL of hot water in a test tube and then shook vigorously. Persistent frothing indicates the presence of saponins.

Alkaloids

5 mL of distilled water was added to 0.5 g of henna plant extract. 1 mL of hydrochloric acid (HCl) was added in order to acidify the mixture and was subsequently filtered. Two (2) drops of Mayer's reagent were added to 1 mL of filtrate. The reaction formed a precipitate, which confirms that the henna plant contains alkaloids. In another test, Dragendorff's reagent was added to 1 mL of the filtrate. The reaction forming a turbid or precipitate further confirms that alkaloids were present.

Anthraquinones

500 mg of the extract was dissolved in distilled water. It was extracted after 5 minutes by heating it up. It was filtered, cooled, and extracted with 10 mL of carbon tetrachloride. The CCl_4 layer was washed with 5 mL of dilute ammonium hydroxide. A pink colouration in the ammonium layer confirmed that anthraquinones were present in *Lawsonia inermis* Linn.

Cardiac glycosides

500 mg of the extract of the henna plant was dissolved in 2 mL of chloroform, and 2 mL of concentrated H_2SO_4 was added carefully for it to form a layer. A reddish to brownish colouration at the interface showed that *Lawsonia inermis* possessed steroidal cardiac glycosides.

Experimental Animals and Ethical Consideration

Adult male Wistar rats were obtained from the Experimental Animal House, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, and were used for this study. This work was ethically approved by ACUREC, which is the regulatory body in charge of animal use at the University of Ibadan. ACUREC issued a full approval with assigned number: **UI-ACUREC/18/0063.** All stress factors, such as handling, feeding, housing, environmental conditions, etc., were adequately provided, and the animals were humanely handled.

Experimental Animals

Male Wistar rats weighing between 130 and 180 g (total of 50) were used for this experiment. Experimental rats were housed according to international standards and were maintained in ideal conditions under appropriate temperature and humidity. The experimental rats were fed with vital$^{(R)}$ feed (standard animal feed) with proximal analytical content of crude protein (16%), fat (5%), crude fibre (8%), calcium (1%), absorbable phosphorus (0.4%), and metabolic energy (2600 kcal/kg). Feed and water were provided *ad libitum*. The blood sugar of all the experimental rats was assessed using a fine test glucometer (United Kingdom) prior to the start of the experiments.

Diabetes induction

Experimental diabetes was induced using streptozotocin (STZ) (sigma® UK). STZ was dissolved in distilled water and injected at a single dose of 65 mg/kg intraperitoneally. This is the most frequently used procedure in diabetic models in mice and rats using STZ at 40–70 mg/kg, as described by Furman (2021)**.** The fasted blood glucose level (BGL) was measured 72 hours post-STZ induction, and BGL above 200 mg/dL signifies experimental diabetes. All rats used in this experiment were confirmed to be diabetic (> 200 mg/dL).

Animal grouping and treatment

Experimental rats were randomly grouped into ten (n = 5), and each group was treated with the extract and the drugs daily through oral gavage for 28 days, as shown in **Table 1.**

Table 1: Experimental groups.

Groups	Treatment
Neg Control	Normoglycaemic control treated with distilled water
POS Control	Untreated hyperglycaemic treated with distilled water
Diab+Li+Meth-50mg	Diabetic and treated at a dosage of 50 mg/kg methanol extract of *Lawsonia inermis* Linn. Leave
Diab+Li+Meth-100mg	Diabetic and treated at a dosage of 100 mg/kg methanol extract of *Lawsonia inermis* Linn. Leave
Diab+Li+*n*x-50mg	Diabetic and treated at a dosage of 50 mg/kg *n*-hexane extract of *Lawsonia inermis* Linn. Leave
Diab+Li+*n*x-100mg	Diabetic and treated at a dosage of 100 mg/kg *n*-hexane extract of *Lawsonia inermis* Linn. Leave
Diab+Li+EA-50mg	Diabetic and treated at a dosage of 50 mg/kg Ethyle acetate extract of *Lawsonia inermis* Linn. Leave
Diab+Li+EA-100mg	Diabetic and treated at a dosage of 100 mg/kg Ethyle acetate extract of *Lawsonia inermis* Linn leave
Diab+Metformin	Diabetic and treated at a dosage of 500 mg/kg metformin.
Diab+Gliben	Diabetic and treated at a dosage of 50 mg/kg glibenclamide.

Note: Diab-(Diabetes); Li (*Lawsonia inermis*); Meth-(Methanol mg/kg); *n*x- (*n*-hexane mg/kg); Ea- (Ethyl acetate mg/kg); Gliben (Glibenclamide).

Constitution and Administration of *Lawsonia inermis* leaf extract and oral hypoglycaemic drugs

The stock concentration of the three extracts was prepared by mixing 2 ml of distilled water with 0.5g of extract so as to dissolve it. These preparations were administered orally at different doses indicated above to the rats in the test groups for 4 weeks. Metformin (500

mg) and Glibenclamide (5 mg/kg) (Emzor(R), Nigeria) were used as oral hypoglycaemic drugs and dosed accordingly using oral gavage. The two control groups were treated using distilled water (Aremu et al., 2022).

Blood sample collection

On the 29th day (fasted overnight), rats were anaesthesized using ether, and haematological samples were collected from the median canthus of the experimental animal for haematological and biochemical assays. Approximately 2 mL of each whole blood sample was collected into both plain and EDTA bottles (haematology screening). The serum was separated from the clot and centrifuged (3000 revolutions per minute (rpm) for 20 minutes) into Eppendorf tubes for biochemical assay.

Determination of haematological parameters

The whole blood in the EDTA bottles was used in the evaluation of haematological parameters. The parameters were evaluated using Cole's method (1986): PCV, Hb Conc, and RBC. Others include WBC, lymphocytes, monocytes, and neutrophils.

Serum Biochemical Parameters

The serum biochemicals were determined using commercial test kits (Randox® Netherlands), and this includes ALT, ASP, ALP, total protein (TP), albumin, globulin, creatinine, and total bilirubin (TB). Other are cholesterol, triglyceride, HDL, and LDL using various kits from Randox® Chemicals Netherlands.

Evaluation of osmotic fragility

Osmotic fragility was evaluated according to standard procedure as described by **Ufuk *et al.* (2020).** A series of test tubes containing a solution of NaCI with a carrying concentration ranging from 0.9–0.10% (0.9, 0.75, 0.6, 0.55, 0.5, 0.4, 0.35, 0.3, 0.2, and 0.10) were taken. Red cells were suspended in each tube and incubated for 30 minutes 20^0C (room temperature). The solution was then centrifuged for 15 minutes at 3000 revolutions per minute. The percentage of hemolysis in the supernatant solution of each tube (with various concentrations) was assessed by a by a photoelectric colorimeter using a spectrophotometer (UV/VIS spectrophotometer UV752, PEC Medical USA). The results were plotted in a graph, placing the percentage of NaCI solution on the X-axis and the percentage of lysis on the Y-axis. The reading optical density of the supernatant from tube 0.9% blank and the reading of the supernatant from tube 0.1% saline was 100%, as described by **Ufuk *et al.*, (2020).**

Evaluation of glycated haemoglobin *(HbA1c)*

Glycated haemoglobin was evaluated according to standard procedure as described by **Robin (2018).**

Biochemical analysis

The serum screening assays were conducted using commercial test kits (Randox® Netherlands), and this includes ALT, ASP, ALP, total protein (TP), albumin, globulin, creatinine, and total bilirubin (TB). Other are cholesterol, triglyceride, HDL, and LDL using various kits from Randox Chemicals Netherlands. The atherogenic index of plasma (AIP) was calculated using the following formula:

AIP = log Triglyceride ÷ HDL cholesterol

Data analysis

All generated data were recorded as mean ±SD. All the data were analysed using ANOVA and subjected to further testing using Dunnet's Post-Hoc multiple comparison test. GraphPad Prism software statistical package, version 5.03 (San Diego, U.S.A.), was used for all analyses. P-values of $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ were considered significant values.

RESULTS

Phytochemical screening

Phytochemical analysis of *Lawsonia inermis* Linn. leaves showed various phytochemicals such as Saponin, Tannins, Flavonoid, Cardiac Glycoside, Terpenoids steroid, Anthraquinones, and Alkaloids **(Table 2).**

Table 2: Phytochemical screening (qualitative) of methanol extract of *Lawsonia inermis* Linn leave.

Test	crude methanol extract
Saponins	Abundantly present
Tannins	Abundantly present
Flavonoids	Abundantly present
Cardiac glycosides	Abundantly present
Terpenoids	Present
Steroids	present
Anthraquinones	present
Alkaloids	present

Haematology

Red blood cell (RBC)

The packed cell volume (PCV) decreased non-significantly in all the treatment groups, but *n*-hexane at 50 mg/kg increased non-significantly ($p > 0.05$) compared to normoglycaemic control. The PCV of all treatment groups increased non-significantly except for EA-50 mg/kg, which decreased non-significantly compared to hyperglycaemic untreated control. Red blood cells (RBC) followed the same trend, showing non-significant decreased values across the treatment groups except *n*x-50 mg/kg, which increased non-significantly compared to the normoglycaemic untreated control. All treatment groups showed non-significantly increased RBC values compared to

hyperglycaemic untreated control. haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) values showed a similar trend in all the treatment groups, presenting non-significant decreased values compared to normoglycaemic untreated control, while the values increased non-significantly compared to hyperglycaemic untreated control **(Table 3).**

Table 3: Haematology of diabetic Wistar rats treated with different solvent portioned fraction of *Lawsonia inermis* Linn leave and oral hypoglycaemic agents.

GRP/INDEX	PCV (%)	RBC $\times10^6$/L	HB g/dl	MCV fl	MCH pg	MCHC g/dl
Control	45.25±1.89	6.65±0.57	14.33±0.79	67.75±2.63	21.23±0.68	31.58±0.94
Diabetic untreated	41.50±4.20	5.37±0.68	10.93±1.2*	62.75±1.50	20.85±0.76	29.60±0.52
Diab+li+Met-50mg	42.67±0.57	6.10±0.50	11.23±1.08	70.00±2.65	20.80±0.72	30.27±1.52
Diab+li+Met-100mg	43.33±2.08	6.20±0.66	11.37±0.40	68.67±2.89	21.47±1.27	29.53±4.92
Diab+li+Nx-50mg	47.00±2.71[a]	6.78±0.55	11.10±0.40	69.00±1.63	21.58±1.24	32.00±0.91
Diab+li+Nx-100mg	43.50±3.69	6.08±0.79	13.05±1.89	71.75±5.74	21.60±2.63	29.85±2.29
Diab+li+EA-50mg	40.80±5.89	5.58±1.13	12.36±2.03	73.40±4.45	22.22±1.22	30.78±0.73
Diab+li+EA-100mg	43.67±5.51	6.58±0.93	13.20±2.07	70.00±2.65	21.13±1.27	29.90±0.94
Diab+Metformin	44.25±2.63	6.50±0.48	13.75±1.05	69.50±2.38	21.10±1.12	30.50±0.38
Diab+Gliben	44.75±4.11	6.16±0.86	13.78±1.57	72.50±3.87	22.33±0.78	30.78±0.82

Results are shown as Mean ±SD: n=5, *Significant $P\leq0.05$

White blood cells (WBC)

Total white blood cells increased non-significantly in Meth-50 mg/kg, while other treatment groups presented a non-significant decrease in total white blood cells compared to normoglycemic untreated control. Neutrophil increased non-significantly in groups; diabetic untreated and 50 mg/kg, while all other treatment groups decreased non-significantly ($p > 0.05$) when compared to normoglycemic untreated control.

Lymphocytes in treatment *n*x-50 mg/kg increased non-significantly ($p > 0.05$), while other treatment groups showed a non-significant decrease in lymphocytes when compared to the normoglycemic untreated control. Lymphocyte values in the hyperglycemic untreated group decreased non-significantly ($p > 0.05$) compared to all treatment groups and the normoglycemic untreated control. Monocyte values increased non-significantly in the diabetic untreated group, but no significant alteration was observed in all other treatment groups compared to the non-diabetic untreated control **(Table 4).**

Table 4: Haematology of diabetic Wistar rats treated with different solvent portioned fractions of *Lawsonia inermis* Linn leave and oral hypoglycaemic agents.

Groups/index	WBC $\times10^3$/L	NEUT $\times10^3$/L	LYMPH $\times10^3$/L	MONO $\times10^3$/L	PLT $\times10^5$/dl
Control	7.45±1.97	4.50±1.51	2.70±1.16	0.15±0.10	2.09±0.10
Diabetic untreated	6.50±2.43	5.32±1.37	1.08±1.23	0.23±0.05	2.43±0.56
Diab+Li+Met-50mg	8.87±2.87	5.30±2.33	2.40±0.61	0.13±0.12	1.79±5.03
Diab+Li+Met 100mg	7.87±1.22	4.70±1.13	1.23±0.25	0.05±0.05	1.92±1.42
Diab+Li+Nx-50mg	7.58±3.01	4.08±1.89	3.30±1.27	0.15±0.19	1.78±0.67
Diab+Li+Nx-100mg	6.28±2.51	4.38±1.79	1.83±0.71	0.08±0.09	1.76±0.42
Diab+Li+EA-50mg	6.12±1.46	2.96±0.99	2.04±0.63	0.07±0.04	2.11±0.53
Diab+Li+EA-100mg	6.77±1.32	4.87±1.32	1.73±0.06	0.10±0.00	2.11±0.85
Diab+Metformin	6.45±1.26	3.97±0.92	2.33±0.48	0.10±0.08	1.93±0.16
Diab+Gliben	5.90±1.33	3.48±0.66	2.25±0.73	0.13±0.05	1.92±0.69

Results are shown as Mean ±SD: n=5 *Significant $P\leq0.05$.

Note: Diab-(Diabetes); Li (*Lawsonia inermis*); Meth- (Methanol mg/kg); *n*x- (*n*-hexane mg/kg); Ea- (Ethyl acetate mg/kg); Gliben (Glibenclamide); PVC: Packed Cell Volume; RBC: Red Blood Cell; HB: Haemoglobin.

Platelet

Mean platelet counts increased non-significantly in EA-50 mg/kg, EA-100 mg/kg and diabetic untreated control compared to non-diabetic control. All other treatment groups presented non-significant ($p>0.05$) decreased platelet count compared to normoglycemic untreated control **(Table 4).**

Percentage osmotic fragility

The result showed that most of the extract treated group could not reverse the erythrocytic fragility except *n*x-50 mg/kg and EA-100 mg/kg compared to the standard drug and normoglycemic control. Other extract treatment groups showed significant improvement in erythrocytic fragility compared to hyperglycemic untreated group **(Table 5).**

Table 5: Percentage osmotic fragility of diabetic Wistar rats treated with different solvent partitioned fractions of *Lawsonia inermis* Linn leave and oral hypoglycaemic agents.

Conc. Of Nacl	Control	Diab-Untreated	Diab+ LiMeth50	Diab+ LiMeth100	Diab+ LiNx50	Diab+ LiNx100	Diab+ LiEA50	Diab+ LiEA100	Diab+ Metformin	Diab+ Gliben clamide
0	1.129	0.091	0.099	0.184	0.108	0.184	0.066	0.124	0.055	0.109
0.1	3.386	0.094	0.107	0.552	0.123	0.190	0.131	0.249	0.11	0.356
0.2	3.160	0.098	0.197	1.842	0.972	2.022	1.032	0.342	1.414	1.132
0.3	3.612	1.123	0.395	2.30	3.132	4.412	2.864	1.432	2.808	2.302
0.4	4.063	1.362	1.675	6.630	4.168	4.491	3.342	4.866	8.48	7.342
0.45	34.88	43.66	13.13	9.484	54.86	56.847	64.55	50.32	53.24	58.41
0.55	64.56	89.95	77.59	89.13	89.78	73.25	90.04	70.12	68.45	72.76
0.6	77.20	91.19	95.66	90.42	90.61	96.69	94.03	90.04	83.41	84.65
0.7	100.0	95.0	100.0	100.0	96.54	100.0	100.0	95.63	94.09	94.60
0.9	100.0	90.66	96.25	98.42	100	97.47	95.81	100.0	100.0	100.0

Note: Diab-(Diabetes); Li (*Lawsonia inermis*); Meth- (Methanol mg/kg); *n*x- (*n*-hexane mg/kg); Ea- (Ethyl acetate mg/kg); Gliben (Glibenclamide).

Glycated haemoglobin

The expression of glycated haemoglobin in hyperglycemic untreated individuals increased significantly (> 0.001), while those treated with various solvent partitioning fractions of *Lawsonia inermis* Linn and oral hypoglycaemic drugs presented a non-significant decrease in glycated haemoglobin across other treatment groups compared to non-diabetic control. Treatment groups Meth-50 mg/kg, Meth-100 mg/kg, *n*x-50 mg/kg, EA-50 mg/kg, EA-100 mg/kg, and metformin decreased significantly ($p<0.05$) compared to normoglycemic control **(Fig.1).** Glibenclamide showed a marginal level with normoglycaemic control.

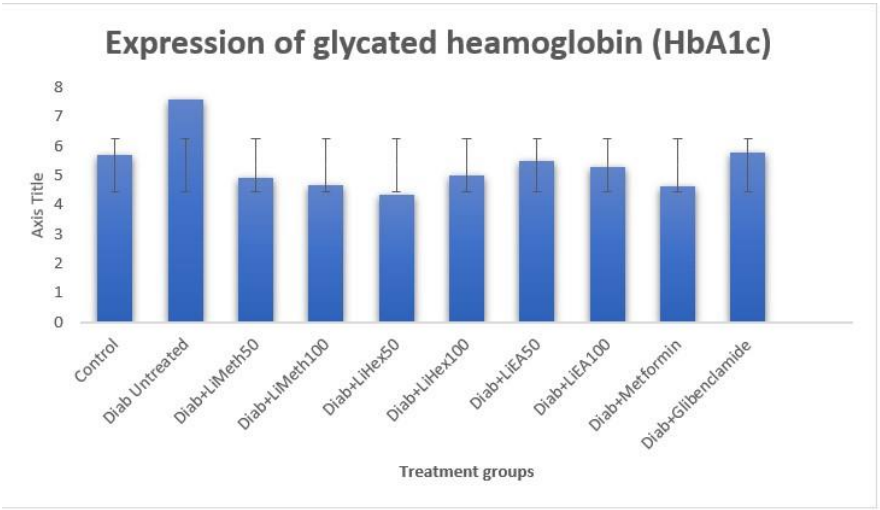

Fig.1: Expression of glycated haemoglobin of experimental rats induced with STZ following 28 days treatment with different extract of *Lawsonia inermis* and oral hypoglycemia agents. Results are shown as Mean ±SD: n=5 *Significant $P\leq0.05$.

Biochemistry results

Plasma protein and non-protein

The total protein of rats in groups *n*x-50 mg/kg metformin and glibenclamide increased significantly ($p<0.01$), while all other treatments increased non-significantly, with subsequent non-significant decreased levels in diabetic untreated rats compared to normoglycemic control. Treatment groups *n*-hexane 100 mg/kg, EA 100 mg/kg, and glibenclamide showed non-significant increased albumin, while other treatments and hyperglycemic untreated decreased non-significantly compared to normoglycemic control. Globulin increased non-significantly in groups Meth-50 mg/kg, *n*x-50 mg/kg, and EA-50 mg/kg while it decreased non-significantly in other treatment groups and untreated hyperglycemic control. The albumin-globulin ratio increased non-significantly in groups *n*x-100 mg/kg and metformin, while the ratio decreased non-significantly in groups meth-50 mg/kg and *n*x-50 mg/kg compared to the normoglycemic untreated control **(Table 6).**

Table 6: Biochemistry of diabetic Wistar rats treated with different solvent portioned fraction of *Lawsonia inermis* Linn leave and oral anti-diabetic agents.

Groups/values	T. Protein g/dl	Albumin g/dl	Globulin g/dl	Alb/Glob ratio (g/dl)
Control	6.50±1.72	4.32±0.95	2.18±1.52	1.98±0.63
Diab+untreated	5.63±0.77	3.95±0.45	1.68±2.88	2.35±0.16
Diab+li+Meth-50	6.20±0.99	4.00±1.41	2.20±1.13	1.82±1.25
Diab+li+Met-100mg	6.33±0.92	4.60±0.20	1.73±1.15	2.66±0.17
Diab+li+Nx-50mg	7.38±0.75*	5.50±4.72	1.88±2.73	1.17±1.72
Diab+li+Nx-100mg	7.53±0.51	5.75±5.73	1.78±2.25	3.23±2.55
Diab+li+EA-50mg	7.26±2.08	5.20±0.22	2.06±0.65	2.52±0.34
Diab+li+EA-100mg	7.30±1.15	5.00±0.53	2.30±0.40	2.17±1.33
Diab+Metformin	8.48±1.96*	6.75±0.89	1.73±0.29	3.90±2.54
Diab+Gliben	8.30±0.95*	6.00±0.10	2.30±1.26	2.61±0.08

Results are shown as Mean ±SD: n=5. *Significant $P \leq 0.01$

Note: Diab-(Diabetes); Li (*Lawsonia inermis*); Meth- (Methanol mg/kg); *n*x-(*n*-hexane mg/kg); Ea- (Ethyl acetate mg/kg); Gliben (Glibenclamide).

Serum enzymes

Alanine aminotransferase (ALT) level presented significant ($p<0.05$) decreased ALT level in groups metformin and glibenclamide, while the extract-treated groups presented non-significant ($p>0.05$) decreased ALT compared to the normoglycemic untreated group. Aspartate aminotransferase (AST) decreased significantly ($p<0.01$) in treatment groups Meth-50 mg/kg and EA-50 mg/kg, while other treatment groups decreased non-significantly compared to normoglycemic control. AST of *n*x 100 mg/kg did not show any significant alteration compared to the other treatment groups and normoglycemic control. Alkaline phosphatase (ALP) decreased non-significantly in both treated and untreated groups compared to normoglycemic control. The bilirubin level did not show any significant alteration in all the treatment groups when compared to the non-diabetic control but the level decreased non-significantly compared to untreated diabetic control. Urea level increased non-significantly in hyperglycemic untreated group while other treatment groups decreased, especially EA-50 mg/kg and glibenclamide, with a significant ($p<0.05$) reduced level compared to other treatment groups and normoglycemic control. Creatinine in diabetic untreated rats increased non-significantly, while treatment groups decreased non-significantly compared to the normoglycemic untreated control **(Table 7).**

Serum lipid profile

The cholesterol of hyperglycemic untreated rats increased non-significantly ($p > 0.05$), while all treatment groups presented a non-significant decrease ($p > 0.05$) in total cholesterol compared to normoglycemic control. Triglyceride and high-density lipoprotein (HDL) decreased non-significantly in both treated and untreated diabetic rats compared to normoglycemic rats. Low-density lipoprotein (LDL) increased non-significantly ($p > 0.05$) in diabetic untreated rats, while all treatment groups presented non-significant ($P > 0.05$) decreased LDL-C compared to normoglycemic control. Atherogenic index of plasma (AIP) showed that group Meth-50 mg/kg (0.09±1.25) had the lowest risk ($PI<0.1$), groups and metformin (0.15±0.00) had intermediate risk (PI 0.1-.0.24), while other treatment groups and both controls (hyperglycemic and normoglycemic control) are at the highest risk ($PI>0.24$) of developing cardiovascular problems **(Table 8).**

Table 7: Biochemistry of diabetic Wistar rats treated with different solvent portioned fraction of *Lawsonia inermis* Linn leave and oral anti-diabetic agents.

Groups/values	ALT (u/l)	AST (u/l)	ALP (u/l)	Bilirubin µmol/L	Urea mmol/L	Creatinine µmol/L
Control	38.75±23.41	6.21±3.94	10.55±4.15	1.12±0.25	3.82±0.92	80.25±18.70
Diab+untreated	34.75±6.70	5.70±2.54	7.80±3.74	2.82±0.23	4.10±0.50	85.50±10.34
Diab+li+Meth-50	36.50±2.12	3.40±0.28**	8.20±5.51	1.45±2.19	3.40±0.28	70.50±4.95
Diab+li+Meth-100mg	37.67±11.72	4.30±2.20	8.66±5.84	1.63±0.35	3.23±0.90	75.67±19.30
Diab+li+Nx-50mg	29.75±5.12	6.15±2.45	7.27±1.53	0.95±0.61	3.45±0.65	72.00±14.76
Diab+li+Nx-100mg	29.00±9.93	6.20±2.27	7.57±3.52	0.90±0.52	3.72±0.22	80.25±10.90
Diab+li+EA-50mg	24.00±5.61	3.78±0.52**	9.44±3.58	1.07±0.16	2.94±0.37	58.20±12.17
Diab+li+EA-100mg	24.33±7.57	4.62±1.07	8.33±5.54	1.03±0.25	2.86±0.35*	56.33±7.02
Diab+Metformin	19.75±2.50[a]	4.02±1.10	7.05±3.98	0.70±0.00	3.17±0.35	67.00±6.00
Diab+Gliben	20.50±1.91[a]	4.67±1.16	8.45±3.11	0.55±0.33	2.72±0.25*	58.25±11.18

Note: Diab-(Diabetes); Li (*Lawsonia inermis*); Meth- (Methanol mg/kg); *n*x- (*n*-hexane mg/kg); Ea- (Ethyl acetate mg/kg); Gliben (Glibenclamide) ALT: Alanine aminotransferase, AST: Aspartate aminotransferase; ALP: Alkaline phosphatase. Significant **P*≤0.05, ***P*≤0.01.

Table 8: Serum lipid (mmol/l) of diabetic Wistar rats treated with different solvent portioned fraction of *Lawsonia inermis* Linn leave and oral hypoglycaemic agents.

Groups/values	Chol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	AIP
Control	3.10±0.20	0.95±0.49	2.57±0.40	0.35±0.30	0.48±0.39
Diab+untreated	3.20±0.46	1.77±0.22	1.62±0.41	1.93±0.25	0.59±1.48
Diab+li+Meth-50	2.39±0.35	0.70±0.14	1.75±0.31	0.50±0.14	0.09±1.25
Diab+li+Met-100mg	2.20±0.30	0.73±0.46	1.70±0.57	0.45±0.05	0.38±0.23
Diab+li+Nx-50mg	2.19±0.22	0.62±0.17	1.82±0.44	0.55±0.23	0.52±0.11
Diab+li+Nx-100mg	2.48±0.38	0.62±0.20	1.97±0.17	0.40±0.21	0.20±0.05
Diab+li+EA-50mg	2.24±0.47	0.64±0.20	1.88±0.28	0.36±0.23	0.58±0.05
Diab+li+EA-100mg	2.37±0.10	0.50±0.26	1.90±0.20	0.53±0.30	0.59±0.41
Diab+Metformin	2.30±0.45	0.60±0.29	1.67±0.45	0.58±0.05	0.15±0.00
Diab+Gliben	2.19±0.31	0.70±0.34	1.65±0.30	0.60±0.28	0.26±3.52

Results are shown as Mean ±SD: n=5.

Note: Diab-(Diabetes); Li (*Lawsonia inermis*); Meth- (Methanol mg/kg); *n*x- (*n*-hexane mg/kg); Ea- (Ethyl acetate mg/kg); Gliben (Glibenclamide); Chol: Cholesterol, TG: Triglycerides; HDL: High-density Lipoprotein; LDL: Low-density Lipoprotein AIP: Atherogenic index.

DISCUSSION

The majority of aromatic plants have medicinal properties because of their active ingredients, which include flavonoids, phenols, tannins, and alkaloids. Novel drug discovery relies on the unique collection of secondary metabolites found in various medicinal plants, and numerous studies have demonstrated that the majority of the modulatory and pharmacological activities of the extracts are caused by these ingredients **(Hussain *et al.*, 2011).** Phytochemical screening of *Lawsonia inermis* leaves used for this study showed some important phytoconstituents like flavonoids, anthraquinones, alkaloids, saponins, tannins, and steroidal glycosides. These observed constituents are in agreement with **Aremu *et al.*, (2022)** who confirm the phytochemical constituents of *Lawsonia inermis* Linn leaves, and the presence of these various phytoconstituents could be the reason for the modulatory activities observed in this present study.

One of the prevalent pathophysiological characteristics and consequences of diabetes mellitus has been identified as anaemia, and reports have shown that the immune system and a number of blood parameters significantly change as diabetes progresses **(Alba-Loureiro *et al.*, 2007).** The haematological results from this study showed increased PCV, RBC, and Hb, and this agrees with **Elwan *et al.*, (2022)** who reported that metformin reversed the abnormal changes seen in the haematological parameters of treated diabetic patients. Decreased MCV, MCH, and MCHC in diabetic untreated rats are linked to RBC destruction without compensatory reticulocytosis, and this was reversed in all treated rats with increased MCV and MCH as observed in responsive reticulocytes **(Oridupa *et al.*, 2012).** This result confirmed the orthodox claims of using *L. inermis* Linn as a blood tonic in the treatment of non-regenerating anaemia, and this observation agrees with **Pereira *et al.*, (2017)** who stated that most

medicinal plants that have haemopoietic stimulatory potential are useful in treating anaemia.

Diabetes mellitus is usually characterized by defective neutrophilic, phagocytic, and microbicidal actions **(Alba-Loureiro *et al.*, 2007).** Differential white blood cells of *L. inermis* Linn-treated diabetic rats showed increased lymphocytes, monocytes, and neutrophils, thereby validating the immunostimulatory activities of the plant because increased lymphocytes are associated with improved immunological actions in the biological system **(Ufuk *et al.*, 2020).** The increased neutrophil count observed in various treated diabetic rats may be linked to the augmented inflammatory response seen in diabetes mellitus. This observation can be attributed to the anti-bacterial potential of *Lawsonia inermis*, which is believed to reduce or eliminate bacteria due to its neutrophilic mobilization **(Tomori *et al.*, 2017).**

Although the mean circulating platelet counts in diabetic patients have gotten less attention and have not been thoroughly researched in both human and animal models, numerous investigations have demonstrated that most diabetic individuals have lower platelet counts **(Ferguson *et al.*, 2009).** The outcome of this present study showed that fractions of *L. inermis* Linn. increased platelet count and this inference pointed to the fact that *L. inermis* Linn possesses coagulatory action. This result disagrees with **Muhamed *et al.* (2017)** who reported decreased platelet survival in diabetic patients. Serum proteins are important in the haemopoietic process for the maintenance of osmotic pressure and also serve as carriers for various substances like drugs, metabolites, and lipids during transportation across plasma membrane **(Diószegi *et al.*, 2019).** Serum protein from this study showed statistically non-significant decreased levels in untreated diabetic rats that were reversed in *L. inermis*-treated diabetic rats. The decreased level observed in untreated diabetic rats could be attributed to a hepatic abnormality linked to diabetes because plasma proteins are mostly produced by the hepatic cells, and this forms the basis of decreased plasma protein seen in untreated diabetic rats **(Muhamed *et al.*, 2017).** It can be deduced from this study that *L. inermis* Linn leaves possessed protein synthesizing ability, thereby conferring increased immunity to treated diabetic rats.

Liver-specific enzymes often exhibit elevated values in relation to hepatic damage in the majority of illness situations. The slight increases in the liver enzymes AST, ALT, and ALP observed in diabetic rats that were not treated with *L. inermis* were reversed. Reports have shown that diabetes-induced hepatic damage is usually linked to cellular necrosis and fibrosis (**Muhamed *et al.*, 2017; Diószegi *et al.*, 2019).** This observation agrees with the work of **El-Demerdasha (2017)** who found marginally increased liver enzymes in treated diabetic rats. Fractions of *L. inermis* Linn-treated diabetic rats showed decreased total bilirubin when compared to untreated diabetic rats.

Increased total bilirubin may be attributed to reduced hepatic uptake of conjugated bilirubin or hemolysis of RBC **(Rana *et al.*, 2012).** Persistently uncontrolled blood glucose levels (BGL) lead to abnormally increased serum urea and creatinine, which are considered important renal biomarkers and signify renal dysfunction **(Masood *et al.*, 2021).** Diabetic rats treated with fractions of *L. inermis* Linn leaves decreased the level of urea and creatinine by more than 20% compared to untreated diabetic rats. Diabetic, untreated rats had significantly increased creatinine and urea levels when compared to all treated and normoglycemic rats. This observation follows the reports of **Badr El-and Ezzat (2010)** that noted increased urea and creatinine levels in diabetic rats. The inference from this study suggested that *L. inermis* Linn had modulatory effects on the kidneys, and this agrees with the previous work of **Badr El-and Ezzat (2010)** which observed improvements in urea and creatinine levels in diabetic rats when treated with *Lawsonia inermis* and *Panax ginseng* Linn.

Osmotic fragility is the proportion of red blood cells that lyse when subjected to osmotic or metabolic stress (such as diabetes) in a hypotonic solution **(Robin, 2018).** The result of this study showed a significant increase in red blood cell (RBC) fragility, which was noted at concentrations of 0.3, 0.5, 0.7, and 0.9% in diabetic untreated rats compared to *L. inermis* Linn-treated diabetic and non-diabetic rats. This result agrees with the reports of **Ejike *et al.*, (2018)** which state that osmotic fragility increased significantly in diabetic rats experimentally induced with streptozotocin. Reports have shown that the membrane of RBC is sensitive to oxidative stress because of the high concentration of fatty acids and oxygen **(AlKurd *et al.*, 2022).** Treated diabetic rats had a significant decrease in fragility, thus concluding that *L. inermis* showed a certain stability of RBC in the treated rats compared to normoglycemic rats. The outcome agrees with **Adenkola *et al.*, (2017)** who concluded that ascorbic acid improves the osmotic fragility of swine due to the antioxidant activities shown by most medicinal plants.

It is important to note that hyperglycaemia is a prominent feature of diabetes mellitus, leading to glycation of tissue proteins, which develops into diabetic complications **(Sherwani Lizardi-Cervera *et al.*, 2018).** Glycated haemoglobin is formed from a non-enzymatic combination of the aldehyde group of glucose and amino β-chain, and its estimation has provided a more dependable method of diagnosing diabetes mellitus **(Chandalia and Krishnaswamy, 2012).** The level of glycation of haemoglobin is an

important diagnostic tool in diabetes **(Sherwani Lizardi-Cervera *et al.*, 2018).** In this study, expression of glycated haemoglobin increased significantly in untreated diabetic rats, and this increased expression was linked to auto-oxidative glycosylation which explains the mechanisms linking excessive blood glucose with vascular complications seen in diabetes mellitus **(Hall *et al.*, 2004).** Treatment with *L. inermis* showed a significant reduction in the expression of glycated haemoglobin especially the methanol fraction when compared to *n*-hexane, ethyl acetate, and the two standard drugs. Higher expression of glycated haemoglobin observed in untreated diabetic rats may be linked to poor glycemic control while *L. inermis*-treated rats (decreased expression) have good glycemic control. This result agrees with **Koti *et al.*, (2018)** who state that plants having antidiabetic effects significantly reduce the level of glycated haemoglobin.

High blood lipid levels are a constant symptom of diabetes mellitus, and they are primarily caused by an increased mobilization of free fatty acids from surface fat depots **(Bopama *et al.*, 2007).** Persistently high blood glucose levels have been reported to consistently increase the risk of developing dyslipidemia, a characteristic feature of diabetes mellitus **(Oyedepo *et al.*, 2018).** In this present study, fractions of *L. inermis* Linn showed decreased triglycerides, cholesterol, and low-density lipoprotein while increasing high-density lipoprotein in all treated rats, whereas diabetic untreated rats followed the opposite trend, showing a high level of triglycerides, cholesterol, and low-density lipoprotein and subsequent decreased high-density lipoprotein (HDL). Methanol and ethyl acetate fractions of *L. inermis* increased the level of high-density lipoprotein when compared to both metformin and glibenclamide, thereby validating the hypolipidemic effect of *L. inermis* because reports have shown that glycemic control is directly linked to decreased low-density lipoprotein and triglyceride **(Selvan *et al.*, 2016).** Many controlled experimental studies have validated that decreased low-density lipoprotein and triglyceride concentrations are very important in the efficacy of any anti-diabetic agent, as *L. inermis* Linn has shown when compared with standard drugs **(Stein *et al.*, 2016).** This outcome agrees with the report of **Ojewumi *et al.*, (2016); Annie *et al.*, (2017)** which confirmed a significant alteration in lipids level of diabetic rats following treatment with *Lawsonia inermis* and anti-diabetic drugs **(Fuller *et al.*, 2000).**

CONCLUSION

Although several studies have reported the trend of haemobiochemical parameters and lipid profiles in diabetic patients, information on the modulatory effect of medicinal plants when treating the disease is scarce. Various results obtained from this study showed that *Lawsonia inermis* has significant modulatory activity on various haemobiochemical changes, osmotic fragility, glycated haemoglobin and lipid profiles of STZ-induced diabetic rats.

Limitations of the study

The male rat was used for this study because of their sensitivity to streptozotocin, unlike female rats that were relatively resistant. Streptozotocin also caused significant mortality in the experimental rats.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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