

## Fractions of *Lawsonia inermis* Linn leaves improved the histomorphological appearance of major organs involved in diabetic complications

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### ABSTRACT

Diabetes mellitus (DM) is clinically known as diverse sets of biochemical syndromes with a basic symptom of glucose intolerance and is a major cause of morbidity and mortality worldwide. Diabetes mellitus exclusively disrupts glucose metabolism in various tissues and organs leading to complications such as retinopathy, nephropathy and brain micro-infarcts. This study investigated the positive modulatory effect of *Lawsonia inermis* Linn leaves on major organs involved in diabetic complications in Wister rats experimentally induced by type 1 diabetes using streptozocine. *Lawsonia inermis* leaves were partitioned using N-hexane, ethyl acetate and methanol. Fractions obtained were assessed for their modulatory potential. Seven groups of diabetic rats (n=5) were orally administered 100mg/kg of each of the three partitioned fractions, metformin (500mg/kg), glibenclamide (5mg/kg), while untreated hyperglycaemic and normoglycaemic rats received distilled water (*ad libitum*) daily for 28 days. Mean relative organ weight of the brain, heart, kidney, pancreas and liver increased significantly ( $p < 0.05$ ) in untreated diabetic rats when compared to *Lawsonia inermis* treated rats and non-diabetic control. Diabetic treated rats showed non-significant ( $p > 0.05$ ) increased weight in all the organs except the pancreas that decreased non-significantly when compared to normoglycaemic rats. The histopathology results showed that *Lawsonia inermis* improved the organ damage seen in diabetic complications through prevention of organomegaly and improvement in histoarchitectural appearance that is devoid of lesions when compared to untreated diabetic control with various lesions. Conclusively, *Lawsonia inermis* Linn leaves improve significant reduction in organ damage during course of Type 1 diabetes mellitus.

**Keywords,** *Lawsonia inermis*, diabetes mellitus, diabetes complications, relative organ weight.

### INTRODUCTION

Diabetes is acknowledged to be one of the oldest diseases ever suffered by mankind. Its occurrence was stated more than 3000 years in Egyptian manuscript (Ahmed, 2011). The distinctive difference linking the two types of diabetes was clearly made in 1936 and type II was initially ascribed as metabolic syndrome in 1988 (Ahmed, 2011).

Many countries have seen a surge in number of cases of diabetes attributed to lifestyle changes linked to increased cases of obesity (WHO, 2016). The metabolic outcome of constant high blood glucose and dyslipidemia increased the incidence of atherosclerosis, chronic kidney disease and

partial or total blindness (Li *et al.*, 2007). An estimate of 366 million individuals had diabetes in 2011 which is projected to increase to about 552 million by the year 2030 (Diabetic Atlas, 2011). The population living with diabetes is increasing around the world and 80% of people with this ailment are inhabitant of poor and developing countries. Epidemiological survey has shown that diabetes has been linked to about 4.6 million deaths (Diabetes atlas, 2011).

Diabetes leads to long-term complications which develop after several years of the disease (decades) but may not show initial sign at the early stage of the disease. Prolonged complications seen in diabetes are usually related to vascular

damage and these increase the chances of developing cardiovascular disease (Sarwar *et al.*, 2010). Reports have shown that more than 75% mortality usually seen in diabetic patients result from coronary artery related disorders such as stroke, macrovascular and periphery artery diseases (Sarwar *et al.*, 2010).

The basic complications of diabetes are due to injury to micro vasculature and these include injuries to kidneys, eyes and nerves. Injury to the eyes (diabetic retinopathy) is as a result of vessels damage in retina of the eye which usually leads to progressive loss of sight and anopsia. These complications also predispose patients to cataracts, glaucoma and other problems of the eyes (WHO, 2014). Injury to the kidney vasculature (diabetic nephropathy) can give rise to arrays of anomalies such as urine protein loss (UPL), tissue scarring and chronic kidney disease (CKD) which may need routine dialysis and renal transplant (WHO, 2014). Injury to nerves in diabetic neuropathy is the most prevalent diabetic complication leading to various anomalies such as tingling, weakness, muscle atrophy, numbness and pain sensation that leads to skin damage that is related to diabetic foot ulcer that may be amputated (WHO, 2014). These complications which are mostly seen in chronic diabetic patients increase burden on the entire public health care system. It has been suggested that adequate awareness about pathogenesis and complications of diabetes will help in prevention, management and treatment of the disease so as to meet the challenges in health care delivery system (Roy and Ray, 2009).

Due to the aforementioned reasons and damage caused to various organs in the body, this study was aimed at assessing the modulatory effect of *Lawsonia inermis* on various organ damage and histomorphological changes observed during the course of type I diabetes mellitus in Streptozocine induced diabetic Wistar rats.

## MATERIALS AND METHODS

### PLANT HARVESTING, IDENTIFICATION AND PREPARATION

Leaves of *Lawsonia inermis* Linn were harvested from a farm land in Oke-oyi in Ilorin East area council of Kwara state, North Central, Nigeria. Taxonomically, it was both identified and authenticated at University of Ibadan Herbarium and a specimen was deposited and assigned a voucher number **UIH-22460**. The leaves of *Lawsonia inermis* Linn were dried at room temperature (25°C) under shade in a room for four weeks. The leaves were reduced to powdery form using a blender with brand name Panasonic<sup>(R)</sup> Japan. The powdery leaves of *Lawsonia inermis* Linn was used for crude and solvent partitioned fractions.

### EXTRACTION AND SEPARATION OF CRUDE LAWSONIA INERMIS LINN LEAVES

Two kilogrammes (2kg) of powdery leaves of the *Lawsonia inermis* were separately soaked in 5litres of N-hexane, ethyl acetate and methanol for 72 hours. Mixture was gently decanted and filtered using filtered paper. The filtrate was immediately evaporated at temp 40°C using a rotary evaporator with brand name Buchhi<sup>(R)</sup>. The concentrate (wet residue from different solvent) was dried and stored at 4°C in the refrigerator branded LG.

### PHYTOCHEMICAL SCREENING

The crude methanol extract was assayed for phytochemical content following the methods described by Trease & Evans, (1989).

### FRACTIONATION OF CRUDE METHANOLIC EXTRACT OF LAWSONIA INERMIS LINN LEAVES

The crude methanol extract of *Lawsonia inermis* Linn leaves (200g) was subsequently fractionated using 1 litter N-hexane, ethyl acetate and methanol in order of increasing polarity using 5cc column silica.

### EXPERIMENTAL ANIMAL AND ETHICAL CONSIDERATION

Adult male Wistar rats obtained from Experimental Animal House, Faculty of Veterinary Medicine, University of Ibadan were used for this study. This work was ethically approved by Animal care use and research ethical committee (ACUREC) which is the regulatory body in charge of animal use in 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 were adequately provided and the animals were humanly handled.

### CONSTITUTION AND ADMINISTRATION OF LAWSONIA INERMIS LEAVES EXTRACT

The stocks concentration of the three fractions were prepared separately by mixing 20ml of distilled water with 0.5g of extract. These preparations were administered orally at 100mg/kg of three different fractions for 28 days. The control groups were treated with purified water *ad libitum*.

### WEIGHING OF RATS AND THEIR ORGANS

All experimental rats were weighed before the start of the experiment and thereafter on weekly basis until last day of the experiment. The organs were weighed with electronic balance (Golden Metler<sup>(R)</sup>) and relative organ weight calculated

$$\text{Relative organ weight (\%)} = \frac{\text{Weight of the organ} \times 100}{\text{Final Body weight}}$$

### EXPERIMENTAL ANIMALS

Male Wistar rats between 130-180g, (total 65) were used for this experiment. Experimental rats were housed using plastic

cage and were maintained at appropriate temperature and humidity. The rats were fed with vital<sup>(R)</sup> feed (Nigeria). Feed and water were provided *ad libitum*. The blood glucose of all the experimental rats was assessed using fine test glucometer (United Kingdom) prior to the start of the experiments.

#### DIABETES INDUCTION

Experimental diabetes was induced using Streptozocine (STZ) (sigma®). STZ was dissolved in distilled water and injected intraperitoneally at 65mg/kg following the method earlier described by Deeds *et al.* (2011).

#### EXPERIMENTAL ANIMAL GROUPING

Experimental rats were randomly grouped into seven having 5 rats per group and each group was treated for 28 days as thus;

Control, Normoglycaemic control treated with distilled water  
Diabetic untreated, Hyperglycaemic control (diabetic and untreated).

Diab+LiMeth-100mg, Diabetic and treated at a dosage 100mg/kg methanol fraction of *L. inermis* Linn leaves

Diab+LiNx-100mg, Diabetic and treated at a dosage 100mg/kg N-hexane fraction of *L. inermis* Linn leaves

Diab+LiEA-100mg, Diabetic and treated at a dosage 100mg/kg Ethyl acetate fraction of *L. inermis* Linn leaves

Diab+Metformin, Diabetic and treated at a dosage of 500mg/kg metformin.

Diab+Gliben, Diabetic and treated at a dosage 50mg/kg glibenamide.

#### CONSTITUTION AND ADMINISTRATION OF LAWSONIA INERMIS LEAVES EXTRACT

The stock concentration of the three fractions was prepared by mixing 20ml of distilled water to 0.5g of extract so as to dissolve it. These preparations were administered orally at different doses indicated above of the rats in the test groups for 28 days. The control groups were treated using distilled water.

#### ANIMAL SACRIFICE AND TISSUES SAMPLE COLLECTION

The entire rats in all the groups were sacrificed humanely using light chloroform anesthesia. To achieve this, a small quantity of chloroform was placed on cotton wool. The rat was placed in small air tight plastic container and the ether-soaked cotton wool was placed on the nostril of the rat and subsequently covered. The anaesthetized rats were humanly killed after five minutes and organs such as brain, kidney heart, and pancreas were harvested for the determination of the relative organ weight and histopathological features.

#### HISTOPATHOLOGICAL PROCEDURES

These organs were fixed with formalin (10%). All fixed organs were dried out by bathing them in graded mixture of both ethanol and water. Ethanol was replaced with

embedding standard solution. Embedded tissues were later infiltrated with xylene for clearing. Xylene impregnated tissues were placed in paraffin (embedding) inside an oven (Mermmet, Switzerland) and this was maintained at a temperature of 58 to 60°C.

The heat generated allowed the solvent to evaporate creating space within the tissues so as to allow paraffin to fill the space. The paraffin hardened the tissue following removal from the oven. 5µm of the tissue was sectioned, floated in water and then transferred on to a glass slide. The sectioned tissues were stained with H&E. Stained and washed slides of various organs were viewed using light microscope at x100 magnification.

#### DATA ANALYSIS

Data were recorded as Mean ±SD of all the measured values and analysed using ANOVA. Data were subjected to further test 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 analysis. P-values of ≤0.05, ≤0.01 and p≤0.001 were considered as significant.

#### RESULTS

##### PHYTOCHEMICAL SCREENING

Crude methanol extract yielded 20% W/V. It was dark-black in colour. Phyto-analysis of *L. inermis* Linn extract showed Saponin, Tannins, Flavonoid, Cardiac Glycoside, Terpenoids steroid, Anthraquinones, and Alkaloids (Table I).

**Table I, Phytochemical screening (qualitative) of methanol extract of *Lawsonia inermis* Linn leaves**

Test	Result
Saponins	Present
Tannins	Present
Flavonoids	Present
Cardiac glycosides	Present
Terpenoids	Present
Steroids	Present
Anthraquinones	Present
Alkaloids	Present

##### PERCENTAGE RELATIVE ORGAN WEIGHT OF TREATED DIABETIC WISTAR RATS

Mean relative organ weight of brain, heart, kidney, pancreas and liver presented non-significant (p>0.05) increased weight in untreated hyperglycaemic rats when compared to all treatment groups and normoglycemic control. All the treatment presented non-significant increased weight of all the organs except the pancreas that decreased non-significantly compared to normoglycemic control (Table II)

##### HISTOPATHOLOGY OF THE BRAIN

Various sections of the brain (cerebellum) are presented in Figure I. There was no visible lesion in normoglycemic rats, while ischaemic necrosis (black arrow) of Purkinje cell was

observed with untreated diabetic rats. Similar to the control, pancreatic acini (arrow) when compared with untreated

**Table II, Relative organ weight of diabetic Wistar rats treated with different solvent partitioned fractions of *Lawsonia inermis* Linn leaves and oral anti-diabetic drugs**

Legends, Diab; diabetic-induced, LiMeth; methanol fraction of *Lawsonia inermis* Linn Leaves, LiNx; hexane fraction of *Lawsonia inermis* linn leaves, LiEA; Ethyl acetate fraction of *Lawsonia inermis* linn leaves

Grp/organ	Brain	Heart	Kidney	Pancreas	Liver
Control	0.79±0.11	0.29± 0.04	0.49±0.07	0.15±0.03	2.41±0.42
Diab-Untreated	1.06±0.26	0.47± 0.13	0.75±0.18	0.20±0.10	3.81±0.88
Diab+LiMeth-100mg	1.02±0.15	0.33±0.05	0.60±0.12	0.14±0.04	3.00±0.27
Diab+LiNx-100mg	1.02±0.43	0.31±0.03	0.58±0.10	0.14±0.02	2.61±0.33
Diab+LiEA-100mg	0.95±0.18	0.31±0.06	0.63±0.13	0.15±0.04	3.20±0.75
Diab+Metformin	0.77±0.06	0.37±0.04	0.58±0.02	0.14±0.01	2.80±0.31
Diab+Gliben	0.82±0.18	0.34±0.13	0.54±0.15	0.15±0.05	2.13±1.20

Data rep as Mean ±SD, n=5, <sup>a,b,c</sup> Significant <sup>a</sup>p<0.05 <sup>b</sup>p<0.01

there were no visible lesion in diabetic-induced rats treated with 100mg/kg methanol fraction of LI, 100mg N-hexane fraction of LI, 100mg/kg ethyl acetate fraction of LI, metformin, glibenclamide (H&E) without showing visible lesion.

#### HISTOPATHOLOGY OF HEART

Sections of the heart are shown in Figure II. There was no visible lesion in non-diabetic rats but untreated diabetic showed marked necrotizing myocarditis (arrow). Diabetic rats treated with 100mg/kg methanol fraction showed mild myofibre degeneration while necrotizing myocarditis and myocardial atrophy were seen in N-hexane and ethyl acetate fraction respectively. Both metformin and glibenclamide (H&E) are seen without visible lesion

#### HISTOPATHOLOGY OF KIDNEY

Various section of kidney was presented in Figure III. Normoglycaemic rat showed no visible lesion unlike untreated diabetic rats with tubular epithelial coagulation, necrosis and inflammation (Arrows and star). Diabetic rats treated with different fraction of LI showed reduced histopathological abnormalities when compared to untreated diabetic rats. N-hexane and methanol fraction at 100mg/kg showed reduced patchy tubular epithelial coagulation necrosis and peri-tubular inflammatory cells while ethyl acetate fraction was marked with diffuse tubular epithelial necrosis and ectasia (arrows). Metformin treated rats showed similar lesions like diabetic untreated rats with marked tubular epithelial coagulation necrosis, interstitial congestion and inflammation while glibenclamide showed no observable lesion like normoglycaemic rats (H&E).

#### HISTOPATHOLOGY OF PANCREAS

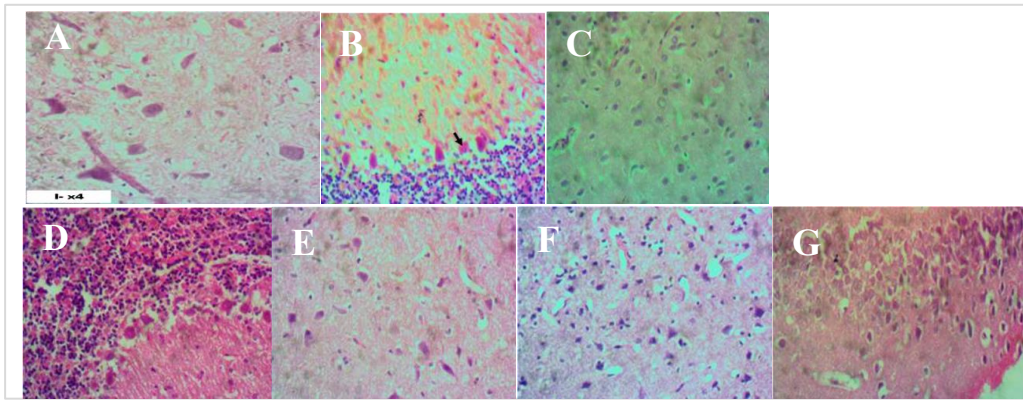
Various sections of pancreas were presented in figure IV. Normoglycaemic rat presented intact arrangement of acini without any noticeable lesion while diabetic untreated rats showed numerous numbers of scanty acini with focal area of necrosis (dotted). Diabetic rats treated with 100mg/kg methanol fraction of LI showed mild focal degeneration of

diabetic rats. N-hexane and ethyl acetate fraction of LI (100mg/kg) showed no visible lesion just like normoglycaemic rats. Metformin and glibenclamide that are standard drugs showed disruption of interlobular septae with moderate cellular depletion of pancreatic acini (dot) (arrow).

#### DISCUSSION

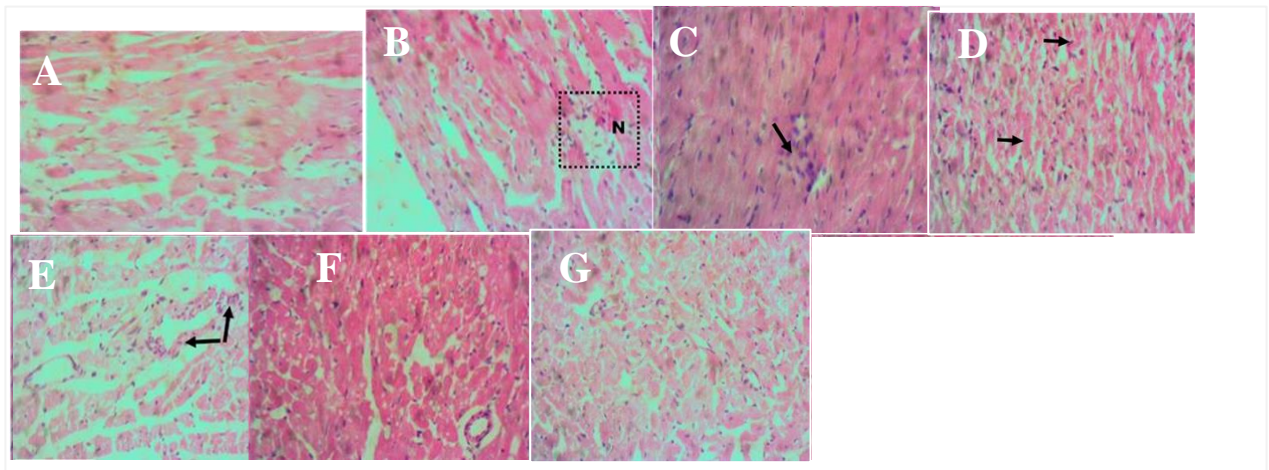
Medicinal effect of most sweet-smelling plants is due to presence of active phytochemicals like alkaloids, tannins, phenols and flavonoids. Different medicinal plants have their distinct set of secondary metabolites which are the basis for novel drug discovery. Extensive reports have shown that these constituents (glycosides, triterpenes, flavonoids, monoterpenes and glycosides) in various solvents are accountable for most pharmacological properties of the extracts (Hussain *et al.*, 2011). Phytochemical analysis of crude methanol extract of *Lawsonia inermis* Linn leaves used in this study showed presence of major constituents like Flavonoids, Anthraquinones, Alkaloid, Saponin, Tannins and Steroidal glycosides. These observed constituents agree with Khan and Nasreen (2010) that confirmed the phytochemical constituent of *L. inermis* Linn leaves. Phytochemical analysis of crude methanol extracts reveals that there exist a broad group of secondary constituents and this may be accountable for multifaceted activities of the plant. Saponin, tannins, flavonoids and cardiac glycosides were the four abundant phytochemical constituents observed in the crude extract (Menger *et al.*, 2013).

*Lawsonia inermis* Linn fractions treated diabetic rats showed non-significant increased weight of most organs except the pancreas that decreased significantly when compared to normoglycaemic rats. This observation was in contradiction to the report of Zafar and Naeem, (2010) that reported that there are no changes in the relative organ of pancreas in streptozocine induced diabetic rats (Zafar and Naeem, 2010).



**Figure I, Histology of the brain in the seven experimental animal groups**

**Plate A.** Section of the brain (cerebellum) of normoglycemic rats showing no visible lesion (NVL). **Plate B.** Section of the brain (cerebellum) of untreated diabetic rats showing ischaemic necrosis of purkinje cells (arrow). **Plate C.** Section of the brain of diabetic rat treated with 100mg/kg methanol fraction of LI showing no visible lesion (NVL). **Plate D.** Section of the brain of diabetic rats treated with 100mg N-hexane fraction of LI showing no visible lesion (NVL). **Plate E.** Section of the brain of rats induced with diabetes and treated with 100mg/kg ethyl acetate fraction of LI without showing any lesion. **Plate F.** Section of the brain of diabetic rats and treated with metformin) without showing visible lesion. **Plate G.** Section of the brain of diabetic rats and treated with glibenclamide without showing visible lesion. (A-G, H & E) H & E ( $\times 4$ )

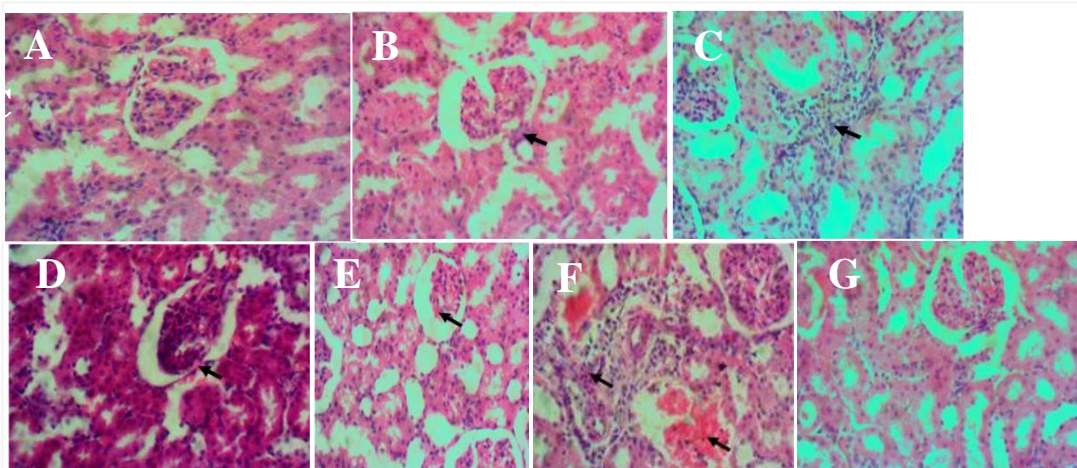


**Figure II, Histology of the heart in the seven experimental animal groups**

**Plate A.** Section of the heart of rats non-diabetic and untreated showing no visible lesion (NVL) (A). **Plate B.** Section of the heart of rats induced with STZ and untreated showing moderate necrotizing myocarditis (arrow). **Plate C.** The photomicrograph of the heart of rats induced with STZ and treated with 100mg/kg methanol fraction of LI showing multiple foci of myofibre degeneration (arrow). **Plate D.** Section of the heart of rats induced with STZ and treated with 100mg/kg N-hexane fraction of LI showing multiple foci of necrotizing myocarditis (arrow). **Plate E.** Section of the heart of rats induced with STZ and treated with 100mg/kg ethyl acetate extract of *L. inermis* showing myocardial atrophy (arrow). **Plate F.** Section of heart of rats induced with STZ and treated with metformin showing no observable lesion. **Plate G.** Section of the heart of rats induced with STZ and treated with glibenclamide showing no observable lesion. (A-G, H & E).

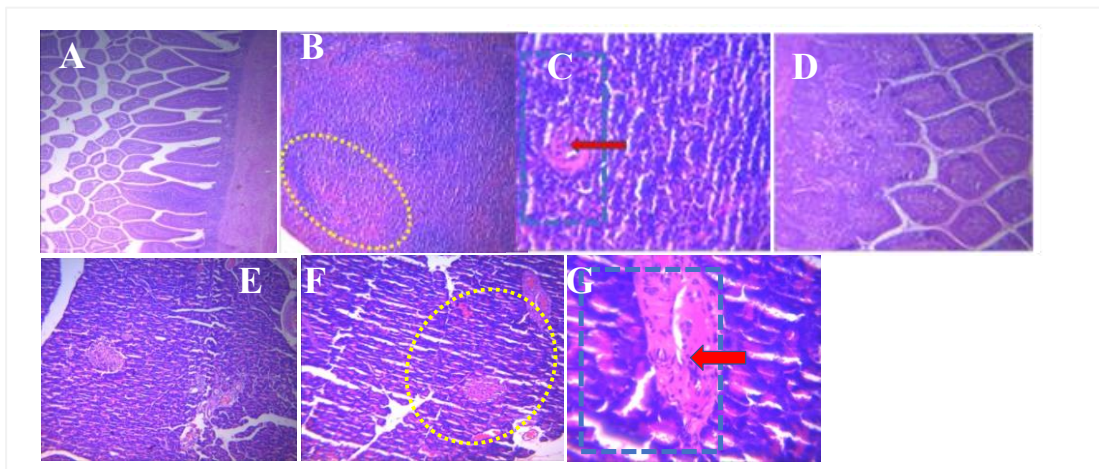
Relative mean weight of brain and heart decreased significantly. Organ weight of methanol fraction of *Lawsonia inermis* Linn leaves showed a close relation to normoglycemic control thereby showing a promising modulatory potential when compared to other fractions and the conventional agents. Increased organ weight is a prominent feature of diabetes especially in those organs that are involved in diabetic complications (Philip *et al.*, 2006).

Brain injury as a result of diabetic complication (neuropathy) seen in uncontrolled blood glucose level (BGL) is usually linked to neuronal damage due to the presence intracellular glucose within neurons (Ye *et al.*, 2011). Results from this study showed reversed brain damage in *L. inermis* treated rats without apparent lesion (Figure I-C, D & E). Fractions of *L. inermis* Linn treated diabetic rats showed few This outcome is in accordance with reports of Alice *et al.* (2013)



**Figure III, Histology of the kidney in the seven experimental animal groups**

Plate A. Section of rat kidney of rats un-induced and untreated showing no visible lesion (NVL). Plate B. Section of kidney induced with STZ and untreated showing tubular epithelial coagulation, necrosis and inflammation (Arrows and star). Plate C. Section of rat kidney induced with STZ and treated with 100mg/kg methanol fraction of LI showing patchy tubular epithelial coagulation necrosis and peritubular inflammatory cells (arrows). Plate D. Section of rat kidney induced with STZ and treated with 100mg/kg N-hexane fraction of LI showing patchy tubular epithelial necrosis (arrow). Plate E. Section of rat kidney induced with STZ and treated with 100mg/kg ethyl acetate fraction of LI showing diffuse tubular epithelial necrosis and ectasia of the lumen (arrow). Plate F. Section of rat kidney induced with STZ and treated with metformin showing tubular epithelial coagulation necrosis, interstitial congestion and inflammation (arrows). Plate G. Section of rat kidney induced with STZ and treated with glibenclamide showing no observable lesion. (A-G, H&E)



**Figure IV, Histology of the Pancreas in the seven experimental animal groups**

Plate A. non-diabetic rats showing intact arrangement of acini with no visible lesion (NVL). Plate B. Diabetic untreated rats showing number of scanty acini with focal area of necrosis (dotted). Plate C. Diabetic rats treated with 100mg/kg methanol fraction of LI showing focal degeneration of pancreatic acini (arrow). Plate D. Diabetic rats treated with 100mg N-hexane fraction of LI showing no visible lesion (NVL). Plate E. Diabetic rats treated with 100mg/kg ethyl acetate fraction of LI without showing any lesion. Plate F. Diabetic rats treated with metformin) without showing disruption of interlobular septae with moderate cellular depletion of pancreatic acini (dot). Plate G. Diabetic rats treated with glibenclamide with acini showing infiltration of inflammatory cells ( red arrow) (A-G, H & E).

that confirmed the nano-protective effects of *L. inermis* Linn in the brain of streptozocine-induced diabetic rat model. Brain damage in diabetic rats is pathognomically lesions when compared to untreated diabetic rats (Fig-1B). linked to factors such as the production of free radical and metabolic dysfunction (Hasanein & Shahidi, 2010). In this study, untreated diabetic rats had marked brain damage with numerous ischemic necrosis of cerebellum when compared to the treated and normoglycaemic rats. This result agrees with the claims of Kuhad and Chopra (2007) that showed pronounced neuronal damage to in the hippocampus (brain) of streptozocine induced diabetic rats.

Excessive and uncontrolled high blood glucose for long period of time will predispose patients to cardiovascular complications. Cardiac tissues of diabetic rats usually showed disrupted histoarchitectural change leading to inflammation of cardiac muscles indicating myocardial damage (Soltani *et al.*, 2007). Result from this present study showed moderate necrotizing myocarditis and myofiber degeneration untreated diabetic rats which were slightly reversed in all fractions treated rats (Figure II A-G). Cardiovascular anomaly is one of the complications observe in diabetic patient and it was reported that more than 29% of diabetic patients may develop diabetic cardiomyopathy (Soltani *et al.*, 2007). Result of this study showed marked improvement of cardiac tissues damage in *L. inermis* Linn treated rats when compared to untreated diabetic rats. This outcome agrees with Thent *et al.* (2012) stating that damage to cardiac tissues is an important marker for developing cardiovascular complication in diabetic rats.

In this study, weight of the kidney increased non-significantly in untreated diabetic rats when compared with the treated and normoglycemic rats. This observation agrees with report of Safar and Naeem (2010) that noted increased weight of the kidneys in diabetic patient. Abnormality of kidney due to nephropathy in diabetic patient leads to hypertrophy of the glomerulus during early episode of diabetes before progression to a more specific complication that usually occurs in the absence of mesangial expansion (Spellberg *et al.*, 2008). Nephritis is the end stage condition usually encountered in diabetic patient with higher incidence in uncontrolled hyperglycaemia. Reports showed that excessive BGL over long period of time is directly linked to nephron damage which later result to diabetic complication (Fioretto and Mauer, 2007).

Histologically, kidneys of diabetic patients usually show structural derangement of vacuolar and interstitial tubules of the nephron (Mohsen *et al.*, 2014). This present study is similar to previous report of Moshen *et al.* (2014) showing abnormality such as tubular epithelial coagulation, necrosis and inflammation of the nephron in untreated diabetic rats (Figure III). All *L. inermis* treated rats showed reduced

abnormalities in histomorphological appearance of kidney tissue. Glibenclamide treated rats present a good histoarchitectural property just like non-diabetic rats without observable lesion. The result observed in this present study is similar to previous study of Mutiara *et al.* (2018) stating that *Lawsonia inermis* Linn restored the histoarchitectural properties of the kidney of treated diabetic rats (Mutiara *et al.*, 2018).

It has been established that diabetes mellitus with uncontrolled hyperglycaemia leads to destruction in the microarchitecture of the  $\beta$ -cells of the pancreatic islets of Langerhans (Donath *et al.*, 2009). Pancreatic histological result from this study showed enormous pathological changes in diabetic untreated rats when compared to the normal structure observed in the non-diabetic rats (Fig IV A-B). The histoarchitecture of  $\beta$ -cells in untreated rats were observed with varying degree of degeneration with focal area of necrosis. The histopathological changes of untreated diabetic rat agree with Balamash *et al.*, (2018) who reported varying degree histological abnormalities in the pancreas of STZ-induced diabetic rats.

The pancreatic tissue of methanol fraction treated rats showed mild focal degeneration of pancreatic aceni while N-hexane and ethyl acetate fraction showed intact histoarchitectural arrangement devoid of any lesions (Fig IV- C, D and E). It was observed that fractions of *Lawsonia inermis* Linn leaves possess positive modulatory effect on the lesion observed in pancreatic islet cells when compared to the two standard drugs (Metformin and glibenclamide (Fig IV- F, G)) with histoarchitectural disorganization of the endocrine islets showing depletion and aceni infiltration of inflammatory cells. Pancreatic tissue of *Lawsonia inermis* treated rats showed normal population of islets just like non-diabetic control. This finding is in agreement with the report of Al-Janabi *et al.* (2013) which reported improved histoarchitecture of islets and acinar cells following treatment with extracts of *L. alba* and olive oil.

Conclusively, this study revealed that *Lawsonia inermis* improves the organ damage seen in diabetic complications through prevention of organomegaly and improvement in histoarchitectural appearance devoid of lesions. The significant reduction in organ damage during course of Type 1 diabetes mellitus may be linked to anti-diabetic and antioxidant activities of *Lawsonia inermis* reported by Chauhan (2011).

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#### CONFLICT OF INTEREST

There is no any conflict of interest to report

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