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Original Article

In vivo antihyperglycaemic activities of different solvent partitioned extract of *Lawsonia inermis* leaves in streptozotocin-induced diabetic rat model

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Abstract

Diabetes mellitus is a global problem mostly characterized by abnormal elevation of blood glucose. The prevalence of diabetes mellitus is on the rise globally. Insulin and various synthetic oral antidiabetics are easily available to manage diabetes mellitus, but their use has been limited due to significant side effects, toxicity and ineffectiveness in chronic patients. This study aimed to assess the antidiabetic effect of *Lawsonia inermis* leaves in streptozotocin-induced diabetic rats. *Lawsonia inermis* leaves were partitioned using N-hexane, ethyl acetate and methanol. Extracts obtained were assessed for their antidiabetic activities on streptozotocin-induced diabetic rats. Thirteen groups of diabetic rats (n=5) were orally administered 25, 50 and 100 mg/kg of each of the three partitioned extracts, metformin (50 mg/kg), glibenclamide (5 mg/kg), while untreated hyperglycaemic and normoglycaemic rats received distilled water daily for 28 days. *Lawsonia inermis* solvent-partitioned extracts did not cause hypoglycemia in normoglycemic rats 24 hours postprandial. The extracts decreased blood glucose levels in diabetic rats with 100 mg/kg methanol extracts being the most potent. The glucose transport-4 (Glut-4) and pancreatic islet count increased significantly in extract-treated rats compared to untreated diabetic rats. Histopathology results of the pancreas of *Lawsonia inermis* treated rats showed reduced histoarchitectural abnormalities when compared to untreated diabetic control. The results obtained from this study confirmed the hypoglycaemic properties of the leaves of *Lawsonia inermis* Linn with basic mechanisms of enhancing Glut-4 mobilization and its ability to stimulate β-cells regenerating from the pancreas.

Keywords: *Lawsonia inermis*, antihyperglycaemic activity, Glut-4, β-cell regeneration.

Introduction

Diabetes mellitus (DM) is clinically known as different sets of biochemical syndromes with basic glucose intolerance symptoms [1]. It is a major cause of morbidity and mortality all over the world [1, 2]. Diabetes mellitus has a characteristic feature of insulin deficiencies leading to excessively high blood glucose, carbohydrate disturbance and disruption in lipid and protein metabolism [2]. Diabetes mellitus exclusively disrupt glucose metabolism leading to complications such as retinopathy, nephropathy and brain micro-infarcts [3]. Diabetes mellitus must be diagnosed early, followed by aggressive treatment to prevent complications and improve the prognosis, which is usually poor [3, 4]. Complications like retinopathy lead to cataracts and blindness, while nephropathy diminishes renal function and causes chronic kidney disease. Foot ulcers, amputations, tactile allodynia, impotence and stroke may be a sequel to neuropathic complications [4]. Diabetes is mostly linked with long-term cardiovascular damage in humans and the associated risk includes coronary artery disease, atherosclerosis, heart failure, peri-vascular disease and myocardial infarction [4, 5].

There are generally three main types of DM: I, II and gestational diabetes. Type I diabetes results when the pancreas does not produce sufficient insulin, while type II occurs from insulin resistance. Gestational diabetes is mostly seen in pregnant women without history of diabetes [1, 3]. The report showed that DM caused an estimated death of about 2.2 million in 2012 and 2016. The World Health Organization has declared that diabetes is the seventh leading cause of death worldwide. Between 2000 and 2016, there was a 5% increase in premature death linked to diabetes in less developed nations [4, 5].

Early diagnosis using a simple blood glucose test is a major breakthrough in the prevention and management of DM [5]. Treatment involves the use of insulin in type I while diet and lifestyle changes reduce the incidence of type II. Type II patients may also be treated with various oral hypoglycemic drugs such as metformin, glibenclamide and repaglinide, among others [5, 6].

Various medicinal plants have been used to treat all forms of diabetes. These plants are useful in preventing advanced glycated end products associated with diabetes and its complications [6]. Elhassan *et al*. stated that around 255 plants are currently being used for their antidiabetic activities. Leaves are the most preferred parts and several methods are used to make the formulations, including powdery, cooked, juice, maceration and infusions, mostly taken orally. The users often combine one or two plants for diabetes treatment, ranging from days to years [7]. Medicinal plants used to treat diabetes include: Argyreia nervosa Linn, *Brassica nigra* Linn, *Carum carvi* Linn, *Dorema aucheri* Linn and *Nigella sativa* Linn [7].

Reports have shown that herbal plants' antihyperglycemic effect is usually due to their ability to re-establish the function of pancreatic cells as a result of causing increased insulin production, inhibition of intestinal glucose absorption and facilitation of metabolites in insulin-dependent courses [7, 8]. These properties usually exhibited by herbal drugs ensure a protective effect on pancreatic cells leading to smooth fluctuation in glucose levels [8].

Lawsonia inermis Linn is a very useful plant employed worldwide and the powdered leaf has been used for staining different body parts, including hands, nails and beards [3, 9]. Many reports have shown that the leaves of *Lawsonia inermis* Linn are used in managing many diseases such as diabetes, poliomyelitis and measles [9]. Idowu *et al*. highlighted the multifaceted usage of *Lawsonia inermis* Linn when he reported its use as a blood tonic in South Western part of Nigeria [10]. He also reported that root extracts had been explored for their cosmetic and antimalarial activities. Aguwa emphasized the importance of the abortifacient properties of *Lawsonia inermis* Linn [11]. When combined with ginger oil, the powder from the roasted seed of *L. inermis* Linn can be used in treating ringworms, while leaf decoction is used for cleaning and healing infected wounds [12].

Insulin and various synthetic oral antidiabetic drugs like biguanides and sulphonylurea are readily available for the management of diabetes mellitus, but their use has been restricted due to significant side effects, toxicity and ineffectiveness in chronic patients. Therefore, there is an increasing demand for natural herbs with significant antidiabetic activity and fewer side effects. This study was designed to assess the *in vivo* anti-hyperglycaemic activities of solvent partitioned extract of *Lawsonia inermis* Linn leaves in normoglycaemic and streptozotocin-induced diabetic rats.

Material and methods

Ethical consideration

This work was ethically approved by ACUREC, the regulatory body in charge of animal use at the University of Ibadan. ACUREC issued a full approval with the assigned number: UI-ACUREC/18/0063. All stress factors such as handling, feeding, housing, and environmental conditions were adequately provided and the animals were humanly handled.

Plant harvesting, identification and preparation

Lawsonia inermis Linn leaves were harvested from Kwara state, North Central, Nigeria Ilorin East area council. Taxonomically, it was both identified and authenticated at the 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) out of the sun for four weeks. The leaves were blended to powdery form using Panasonic(R) Japan blender.

Phytochemical screening of *L. inermis* leaf extract

The phytochemical analysis of *L. inermis* leaf was evaluated following the method described by Trease

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and Evans [13]. Quantitative analysis of methanol leaf extract of L. *inermis* leaf for tannins, terpenoids, flavonoids, total phenolics and steroids [13].

Extraction and separation of *Lawsonia inermis*

Three kilograms of powdery leaves of *Lawsonia inermis* were soaked in 5 liters of N-hexane, ethyl acetate and methanol successively for 72 hours. Each mixture was gently decanted and filtered using filtered paper. Each filtrate was evaporated at temp 40˚C using a rotary evaporator. The concentrate (wet residue of various solvents) was dried and stored in the fridge at 4˚C.

Experimental animals

Male Wistar rats between 130–160 g (n=65) were used for this experiment. Experimental rats were housed at ideal conditions under appropriate temperature and humidity. The experimental rats were fed with vital(R) feed. Feed and water were provided *ad libitum*. The blood glucose level (BGL) of all the experimental rats was assessed using a fine test glucometer prior to the start of the experiment.

Diabetes induction

An experimental diabetic model was carried out using Streptozocin (STZ) (sigma®). STZ was dissolved in distilled water and injected intraperitoneally at 65 mg/kg. Animals confirmed to be diabetic were selected and used for the experiment.

Treatment protocol

Experimental rats were randomly grouped into thirteen groups with 4–5 rats per group and each group was treated for 28 days according to the following:

- Control: Normoglycaemic control treated with distilled water;
- Diabetic untreated: Hyperglycaemic control treated with distilled water;
- Diab+Li+Meth-25 mg: Diabetic and treated at a dosage of 25 mg/kg methanol extract of *Lawsonia inermis* Linn leaf;
- Diab+Li+Meth-50 mg: Diabetic and treated at a dosage of 50 mg/kg methanol extract of *Lawsonia inermis* Linn. leaf;
- Diab+Li+Meth-100 mg: Diabetic and treated at a dosage of 100 mg/kg methanol extract of *Lawsonia inermis* Linn. leaf;
- Diab+Li+Nx-25 mg: Diabetic and treated at a dosage of 25 mg/kg N-hexane extract of *Lawsonia inermis* Linn. leaf;
- Diab+Li+Nx-50 mg: Diabetic and treated at a dosage of 50 mg/kg N-hexane extract of *Lawsonia inermis* Linn. leaf;
- Diab+Li+Nx-100 mg: Diabetic and treated at a dosage of 100 mg/kg N-hexane extract of *Lawsonia inermis* Linn. leaf;
- Diab+Li+EA-25 mg: Diabetic and treated at a dosage of 25 mg/kg Ethyl acetate extract of *Lawsonia inermis* Linn. leaf;
- Diab+Li+EA-50 mg: Diabetic and treated at a dosage of 50 mg/kg Ethyl acetate extract of *Lawsonia inermis* Linn. leaf;
- Diab+Li+EA-100 mg: Diabetic and treated at a dosage of 100 mg/kg Ethyl acetate extract of *Lawsonia inermis* Linn leaf;
- Diab+Metformin: Diabetic and treated at a dosage of 50 mg/kg metformin;
- Diab+Gliben: Diabetic and treated at a dosage of 5 mg/kg glibenclamide.

Constitution and administration of *Lawsonia inermis* leaf extract

The stock concentration of the three extracts was prepared by mixing 10 ml of distilled water with 0.5 g of the extract to dissolve it separately. These preparations were administered orally at different doses in the test groups for 4 weeks. The control groups were treated using distilled water.

Monitoring of blood glucose level (BGL)

The blood glucose level was monitored in the experimental test groups on Day 0 (24 hours), Day 1, 3, 7, 10, 14, 21 and 28 using the conventional fine test glucometer and strips.

Evaluation of glucose transporter (GLUT-4)

GLUT-4 was analyzed using commercial Elisa test kits following standard method and procedure as stated by the manufacturer from the serum and tissue homogenate.

Islet β-cell count

The β-cell count was carried out using a simple stereological method for Estimating the Number and

the Volume of the Pancreatic Beta Cells, following the method adopted by Ali *et al*. [14].

Histopathological procedures

The pancreas was carefully removed from experimental rats. It was fixed with formalin (10%). The fixated pancreas was dried out by bathing them in a graded mixture of ethanol and water. Ethanol was replaced with a standard embedding solution. Embedded tissues were later infiltrated with xylene for clearing. Xylene-impregnated tissues were placed in paraffin (embedding) inside an oven (Mermmet, Switzerland), and that was kept at a temperature of 58 to 60˚C

The generated allowed the solvent to evaporate, creating space within the tissues to allow paraffin to fill the space. The paraffin hardened the tissue following removal from the oven. 5 µm of the tissue was sectioned, floated in the water and then transferred onto a glass slide. The sectioned tissues were stained with H&E. Stained and washed slides of various organs were viewed using a light microscope at X100 magnification.

Data analysis

All generated data were recorded as Mean±SD of all the measured values. All data were analyzed using ANOVA and were subjected to further tests 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

The yield of crude extract from the crude methanol extract was 35% W/V. It was a dark-black color. Phytochemical analysis of *Lawsonia inermis* Linn leaf crude methanol extract showed that the plant contains Saponin, Tannins, Flavonoid, Cardiac Glycoside, Terpenoids steroid, Anthraquinones, and Alkaloids (Table 1).

Table 1: Phytochemical screening (qualitative) of methanol extract of *Lawsonia inermis* Linn leave.

Test	Crude methanol extract
Saponins	++ve
Tannins	++ve
Flavonoids	++ve
Cardiac glycosides	++ve
Terpenoids	+ve
Steroids	+ve
Anthraquinones	+ve
Alkaloids	+ve

Note: Absent; +ve – Present; ++ve – Abundantly present.

Hypoglycaemic effect of *Lawsonia inermis* Linn leaves in normoglycemic rats

Normoglycaemic rats were administered different solvent portioned extracts of *Lawsonia inermis* and showed significant ($p<0.001$) increased blood glucose in the treatment groups and untreated control 1 hour post-treatment except in group EA-25 mg/kg (98.20±18.57 mg/dl) and 50 mg/kg (110.60±14.72 mg/dl) that increased non-significantly compared to initial blood glucose (0 hours).

2-hour post-administration, untreated control and met-25, 50 mg/kg and Nx-100 mg/kg increased significantly ($p<0.001$) till 6 hours post-treatment, while groups met-25 mg/kg, EA-50 and 100 mg/kg decreased non-significantly at these hours when compared to initial blood glucose (0 hour).

12-hour post-administration, blood glucose decreased non-significantly in most treated groups and the control except for the higher dosage (100 mg/kg) in all the fractions that increased significantly ($p<0.01$) when compared to initial blood glucose (0 hour).

24-hour post-treatment, untreated control and most treatment groups showed normal blood glucose just like 0 hours except group Nx-50 mg/kg (102.60±5.09 mg/dl), 100 mg/kg (102.00±2.21 mg/dl) and EA-100 mg/kg (98.80±10.33 mg/dl) that showed significant ($p<0.05$) increased blood glucose when compared to initial blood glucose (0 hour) (Table 2).

Antihyperglycaemic effect of *Lawsonia inermis* Linn leaves in diabetic rats

Blood glucose level decreased non-significantly in all treatment groups while it increased significantly ($p<0.01$) across 28 days of treatment in the hyperglycaemic untreated control. All treatment groups presented non-significant decreased blood glucose compared to untreated hyperglycemic control. On day 28, all treatment groups presented non-significant decreased blood glucose compared to normoglycaemic untreated control. All the solvent partitioned fractions

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Table 2: 24 hours hypoglycaemic activities of solvent portioned fraction of *Lawsonia inermis* Linn leaves in normoglycemic rats.

Group/hours	0 hour	1 hour (after extract)	2 hours	3 hours	6 hours	12 hours	24 hours
Control	90.75±8.42	112.30±7.13 [c]	116.50±5.00 [c]	118.30±5.25 [c]	120.30±5.50 [c]	102.80±7.042	92.50±4.04
Meth-25 mg	83.00±9.95	100.60±6.10 [a]	93.80±10.92	91.80±7.49	83.60±9.73	95.60±8.96	91.20±3.03
Meth-50 mg	78.60±5.41	104.60±10.33 [b]	105.80±15.17 [b]	95.80±3.70	92.20±15.42	95.60±14.36	83.20±8.43
Meth-100 mg	72.80±3.89	103.80±20.95 [a]	97.00±15.22 [b]	104.20±3.56 [b]	109.00±6.67 [c]	107.60±15.85 [c]	90.60±7.82
N-hex 25 mg	99.60±11.26	123.20±11.28 [c]	107.00±8.09	104.20±9.68	118.60±4.33 [b]	87.00±7.96	85.60±5.32
N-hex 50 mg	81.80±7.46	109.00±7.77 [c]	96.60±8.79	93.60±11.63	97.60±11.89 [a]	88.60±5.45	102.60±5.09 [c]
Nx-100 mg	77.20±3.34	106.60±11.63 [c]	96.20±14.89 [b]	92.00±12.73	95.20±10.28	102.60±11.80 [b]	102.00±2.21 [b]
EA-25 mg	79.80±6.22	98.20±18.57	97.60±19.05	93.40±21.81	92.80±25.40	99.00±14.27	98.80±11.37
EA-50 mg	80.60±11.89	110.60±14.72	105.20±11.01	95.80±22.43	105.40±26.76	101.00±10.34	92.80±7.56
EA-100 mg	69.80±8.58	110.40±11.26 [c]	98.80±9.33 [c]	95.20±9.06 [b]	97.20±12.38 [b]	96.20±11.73 [b]	98.80±10.33 [c]

Note: Data rep. as Mean±SD: n=5; [a b c] Significant: [a] – p≤0.05, [b] – p≤0.01, [c] – p≤0.001.

Table 3: 24 hours hypoglycaemic activities of solvent portioned fraction of *Lawsonia inermis* Linn leaves in normoglycemic rats.

Groups/days	Day 1	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28
Control	81.00±7.52	93.50±13.70	94.25±10.63	114.50±36.85	96.25±7.50	120.80±12.63	99.50±5.50
Diabetic untreated	255.80±40.99	264.00±45.71	394.80±95.05b	416.50±55.07b	358.30±43.12b	423.80±110.50b	435.80±54.02b
Diab+li+Met-25 mg	138.50±50.56	184.50±55.89	204.0±80.54	204.80±50.43	193.50±54.59	128.30±66.03	164.30±35.07
Diab+li+Met 50 mg	119.30±27.54c	135.00±86.81	218.80±51.66	182.80±57.71	186.00±18.55	118.70±63.52	160.00±62.23
Diab+li+Met100 mg	109.00±13.36b	184.40±50.57	184.40±74.35	211.00±27.65	191.6±71.16	196.70±7.37	124.30±36.61
Diab+li+Nx-25 mg	133.00±52.59a	182.00±40.35	189.60±73.45	168.60±30.75	165.50±21.17	179.00±54.09	156.60±46.17
Diab+li+Nx-50 mg	150.30±63.54a	182.00±55.72	233.00±27.07	117.00±63.22	159.50±56.04	197.50±66.21	181.00±24.01
Diab+li+Nx-100 mg	138.20±34.68c	149.80±73.73	200.80±18.84	193.80±52.86	189.40±45.85	166.80±60.34	146.00±48.60
Diab+li+EA-25 mg	148.20±48.37b	201.2±47.36	227.00±61.58	206.00±40.18	196.60±48.99	180.00±70.53	171.00±41.46
Diab+li+EA-50 mg	155.00±13.80b	150.60±57.00	182.80±62.41	181.00±49.33	179.80±54.59	139.80±40.80	166.00±42.06
Diab+li+EA-100 mg	169.80±72.53	162.2±111.50	178.30±34.47	178.00±74.36	169.90±70.12	169.80±41.43	139.30±55.73
Diab+Metformin	144.50±46.32	187.50±99.79	235.50±111.30	199.30±70.15	186.40±54.89	153.00±63.25	167.30±77.32
Diab+Gliben	133.50±62.41	117.71±71.38	139.00±103.50	169.00±76.71	168.56±74.67	161.30±83.75	165.00±45.33

Note: Results are shown as Mean±SD: n=5; [a b c] Significant: [a] – p≤0.05, [b] – p≤0.01, [c] – p≤0.001.

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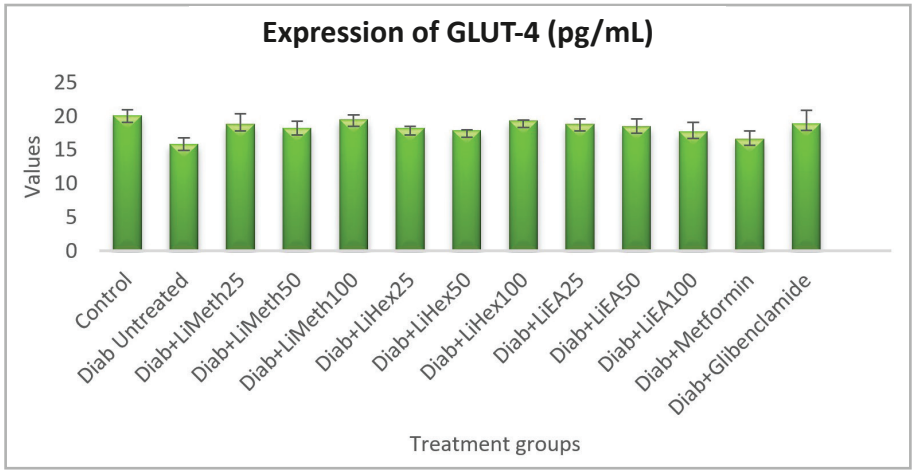

Figure 1: Expression of Gluth-4 of experimental rats induced with STZ following 28 days of treatment with solvent partitioning of *Lawsonia inermis* extract and oral hypoglycemic agents. Results are shown as Mean±SD: n=5.

of *Lawsonia inermis* Linn extract decreased blood glucose across 28 days of treatment, just as metformin and glibenclamide. Methanol fraction at 100 mg/kg has the lowest fasting blood glucose (124.30±36.61 mg/dl) on day 28, even lower than the two conventional drugs (Table 3).

GLUT-4

Expression of glucose transport 4 (GLUT-4) in hyperglycemic untreated and metformin treatment decreased significantly ($p<0.001$), while those treated with various solvent partitioning fractions of *Lawsonia inermis* Linn extract and glibenclamide presented a non-significant increase or decrease GLUT-4 expression compared to normoglycaemic untreated control (Figure 1).

Islet cell counts

Islet cell counts increased significantly ($p<0.001$) in those treated with various solvent partitioning fractions of *Lawsonia inermis* Linn while decreased in metformin and glibenclamide treatment (Figure 2).

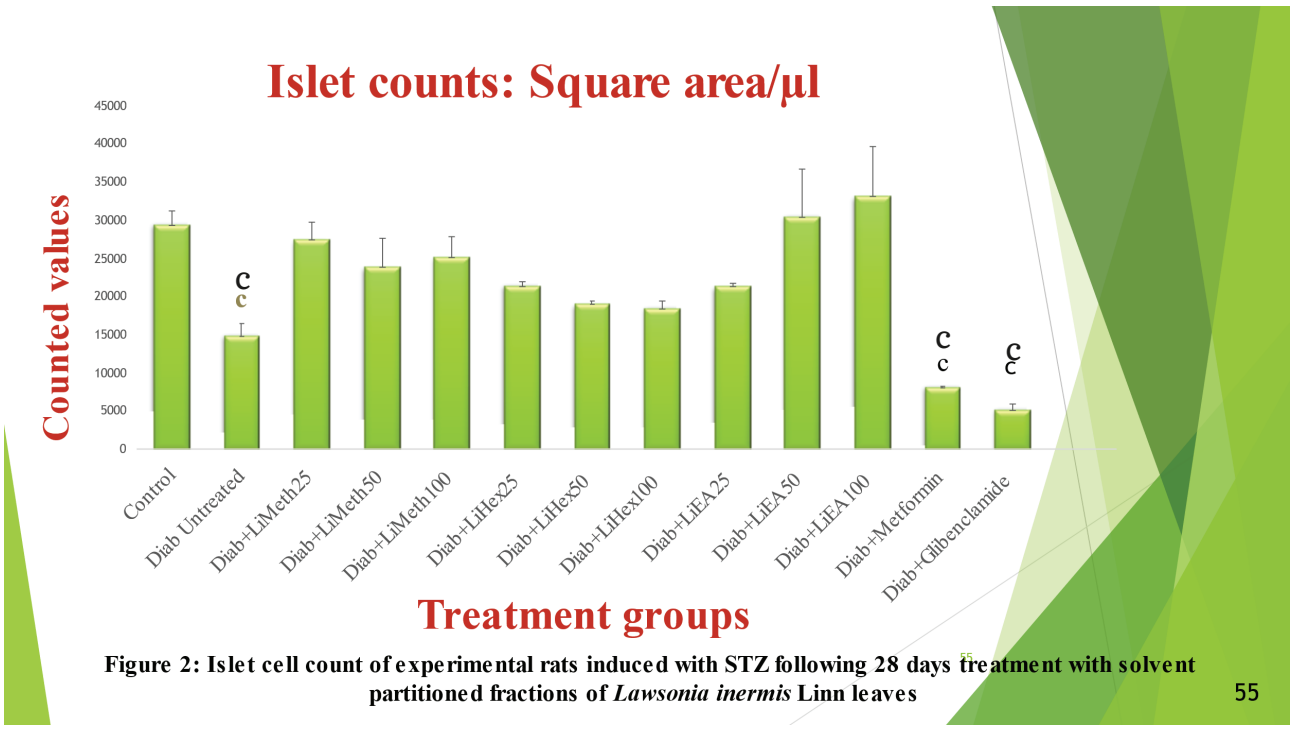

Figure 2: Expression of pancreatic islet cell count of experimental rats induced with STZ following 28 days of treatment with solvent partitioning of *Lawsonia inermis* extract and oral hypoglycemic agents. c – statistically significant.

Discussion

Phytochemical analysis of crude methanolic extract of *Lawsonia inermis* Linn leaves used for this study signifies the presence of major constituents like Flavonoids, Anthraquinones, Alkaloid, Saponin, Tannins and Steroidal glycosides. These observed constituents agree with the work of Khan and Nasreen, who confirms the phytochemical constituent of *Lawsonia inermis* Linn leaves [15]. Thus, the phytochemical analysis of methanol extracts reveals that a broad group of secondary constituents exist, which may be responsible for the plant's multifaceted activities. Saponin, tannins, flavonoids and cardiac glycosides were the four abundant phytochemical constituents observed in the crude extract of *Lawsonia inermis* Linn. Saponin is often bitter to taste, reducing palatability, but it is interestingly known to enhance nutrient absorption and smooth digestibility in animals [15]. Tannins are plant polyphenols with extensive antioxidant activities. Tannins have been reported for their anti-inflammatory potential, while flavonoids possess significant health benefits due to their antioxidant activities linked to functional hydroxyl groups that scavenge free radicals and chelation of metallic ions [16]. Cardiac glycosides are natural drugs with immediate effects on the heart, informing of benefit (cardiotonic) and toxicity (heart poisons). Cardiac glycoside is beneficial when it increases the cardiac muscle's contraction force during arrhythmias and cardiac failure [17].

In this present study, hypoglycemic effects of *Lawsonia inermis* Linn were assessed in normoglycemic rats and the outcome showed a significant reduction in blood glucose level (BGL) in normoglycemic rats. It was noted that 3 and 6 hours post-administration to normoglycemic rats, BGL reduces significantly with methanol fraction when compared with untreated normoglycemic rats. This result contradicts the report of Agabna *et al.*, who stated that *Lawsonia inermis* Linn does not reduce the BGL of normoglycemic rats, and this variation may be linked to the seeds used in that experiment [18].

It is pertinent to note that hyperglycemia is a prominent feature of diabetes, leading to glycation of tissue proteins which enhance diabetic complication [19]. Blood glucose of diabetic rats treated with *Lawsonia inermis* Linn studied over twenty-eight reduced significantly when compared with untreated hyperglycaemic control. The basic explanation for this observation is that many herbs with blood glucose-reducing potentials act by increasing circulating insulin concentration in normal subjects [20]. The methanol extract of *Lawsonia inermis* Linn reduces blood glucose significantly compared to metformin and glibenclamide.

One of the pointers for diagnosing diabetes is the use of fasting blood glucose and reports showed that a value higher than 126 mg/dL (7mmol/L) signifies diagnostic diabetes [21]. The present study showed that different solvent partitioned extracts of *Lawsonia inermis* presented non-significant decreased blood glucose in all the treated groups. The blood glucose rises across 28 days of treatment in the diabetic untreated control. This study showed that *Lawsonia inermis* reduces BGL after 28 days of treatment, just as metformin and glibenclamide. The methanol extract at 100 mg/kg has the most potent anti-hyperglycaemic activity (124.30±36.61) at day 28, even lower than the two conventional drugs. This observation is in line with reports of Singh *et al.*, who reported that the ethanolic fraction of *Lawsonia inermis* Linn. causes a significant reduction in the level of blood glucose in alloxan induced diabetic experimental rat model [22]. It also agrees with the report of Ankur *et al.*, who concluded that *Lawsonia inermis* Linn significantly lowered the blood glucose of STZ-induced diabetic rats [22, 23]. The mechanism for this action may be attributed to certain biomolecules in the plant, which stimulated β-cells of Langerhans, thereby releasing insulin that improves the carbohydrate's metabolizing enzymes, bringing the blood glucose to normal level [24].

GLUT-4 is known as a major glucose transporter available in various tissues like the heart, liver, skeletal muscle and adipose cells. Many studies reported that the expression of GLUT-4 mRNA decreased significantly in diabetic rats induced with STZ [25]. Further studies showed that GLUT-4 is an important component of glucose regulation during homeostasis as its expression is much needed during glucose homeostasis [26]. The outcome of this study showed a non-significant reduction in the expression of GLUT-4 in the untreated hyperglycemic control. All treatment groups showed improvement in the level of GLUT-4 expression, but it is noteworthy that both methanol and hexane extract of *Lawsonia inermis* Linn showed a better expression of GLUT-4. Glibenclamide showed noticeable improvement in GLUT-4 expression compared to metformin. Reduction in GLUT-4 content in diabetic untreated rats is directly linked to a general decrease in the transcription of mRNA of GLUT-4. Extracts of *Lawsonia inermis* Linn. restored GLUT-4 to almost normal expression in the treatment groups and possibly contributed to the antihyperglycemic effect of the plant. This observa-

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tion is in line with the reports of Askar *et al*. They confirmed that GLUT-4 increases in skeletal muscle of diabetic rats after treatment with Vanadium, which also possess significant antidiabetic activities [27].

Reports on diabetes studies showed that oxidative stress destroys β-cells leading to altered insulin production, release and functions [28]. Many reports have shown that reduced islet cell count diminished the volume of the β-cell mass in the pancreas of the untreated diabetic patient [29, 30]. In this present study, diabetic rats treated with L. *inermis* Linn showed a promising improvement in islet cell count regeneration when compared to untreated diabetic rats and standard drugs [30]. This result can be supported by the histopathological result of the pancreas showing improved histoarchitectural properties when compared to untreated diabetic rats showing scanty acini with a focal area of necrosis (Figure 3 A–M; normal histopathological

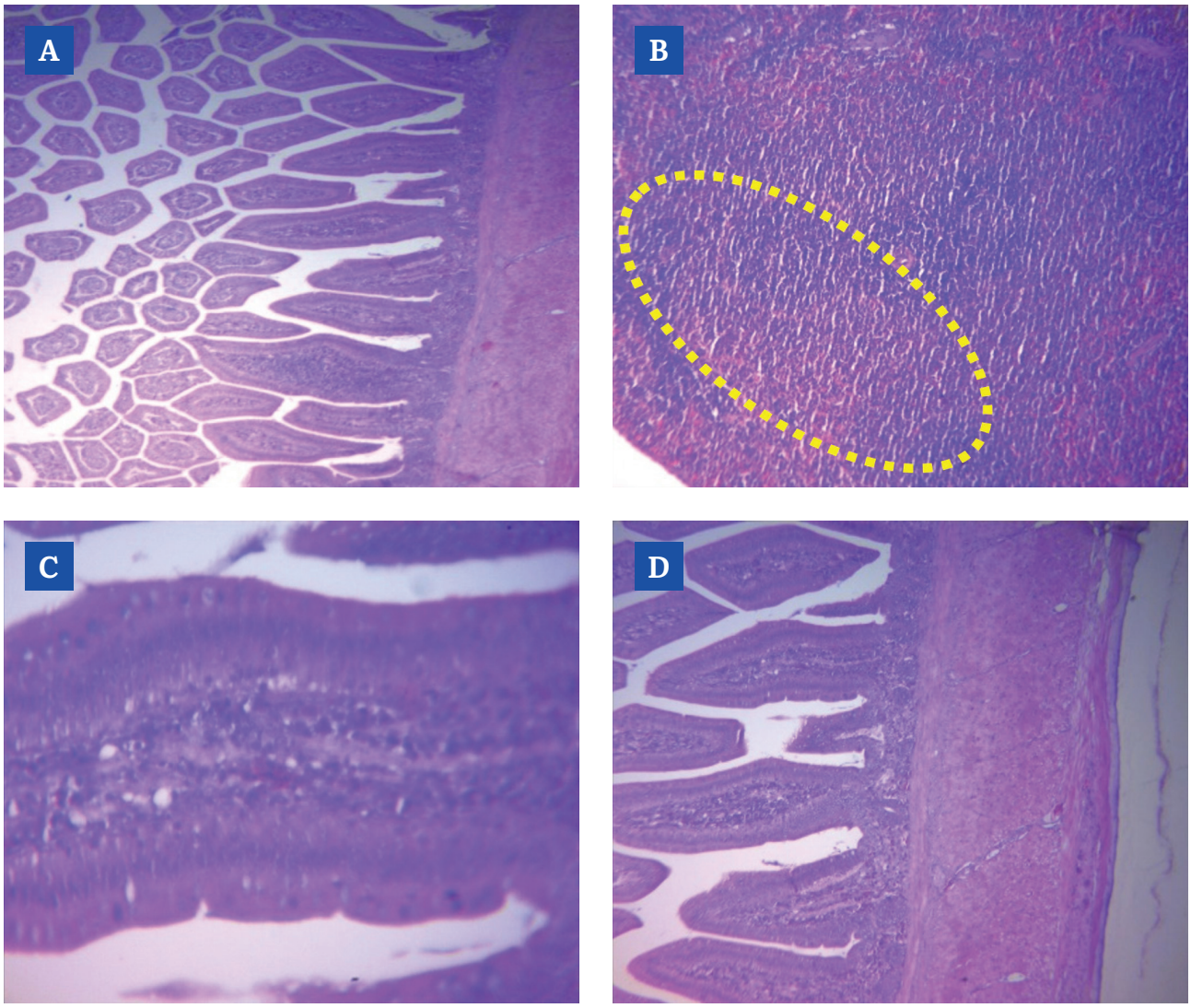

Figure 3: **A** – Non-diabetic rats (H&E) showed an intact arrangement of acini with no visible lesion (NVL). **B** – Diabetic untreated rats (H&E) showing several scanty acini with a focal area of necrosis (doted). **C** – Diabetic rats treated with 25 mg/kg methanol fraction *Lawsonia inermis* (LI) Linn leaves (H&E) showed no visible lesion. **D** – Diabetic rats treated with 50 mg/kg methanol fraction of LI (H&E) showed acini characterized of cell depletion. **E** – Diabetic rats treated with 100 mg/kg methanol fraction of LI (H&E) showed focal degeneration of pancreatic acini (arrow). **F** – Diabetic rats treated with 25 mg/kg N-hexane fraction of LI (H&E) showed degeneration of pancreatic acini (arrow). **G** – Diabetic rats treated with 50 mg/kg N-hexane fraction of LI (H&E) showed mild pancreatic acini (arrow) degeneration. **H** – Diabetic rats treated with 100 mg N-hexane fraction of LI (H&E) showed no visible lesion (NVL). **I** – Diabetic rats treated with 25 mg/kg Ethyl acetate fraction of L. *inermis* (H&E) showed no visible lesion (NVL). **J** – Diabetic rats were treated with 50 mg ethyl acetate fraction of LI (H&E) without showing visible lesions. **K** – Diabetic rats were treated with 100 mg/kg ethyl acetate fraction of LI (H&E) without showing any lesion. **L** – Diabetic rats treated with metformin (H&E) without showing disruption of interlobular septae with moderate cellular depletion of pancreatic acini (dot). **M** – Diabetic rats treated with glibenclamide (H&E) with acini showed infiltration of inflammatory cells (arrow).

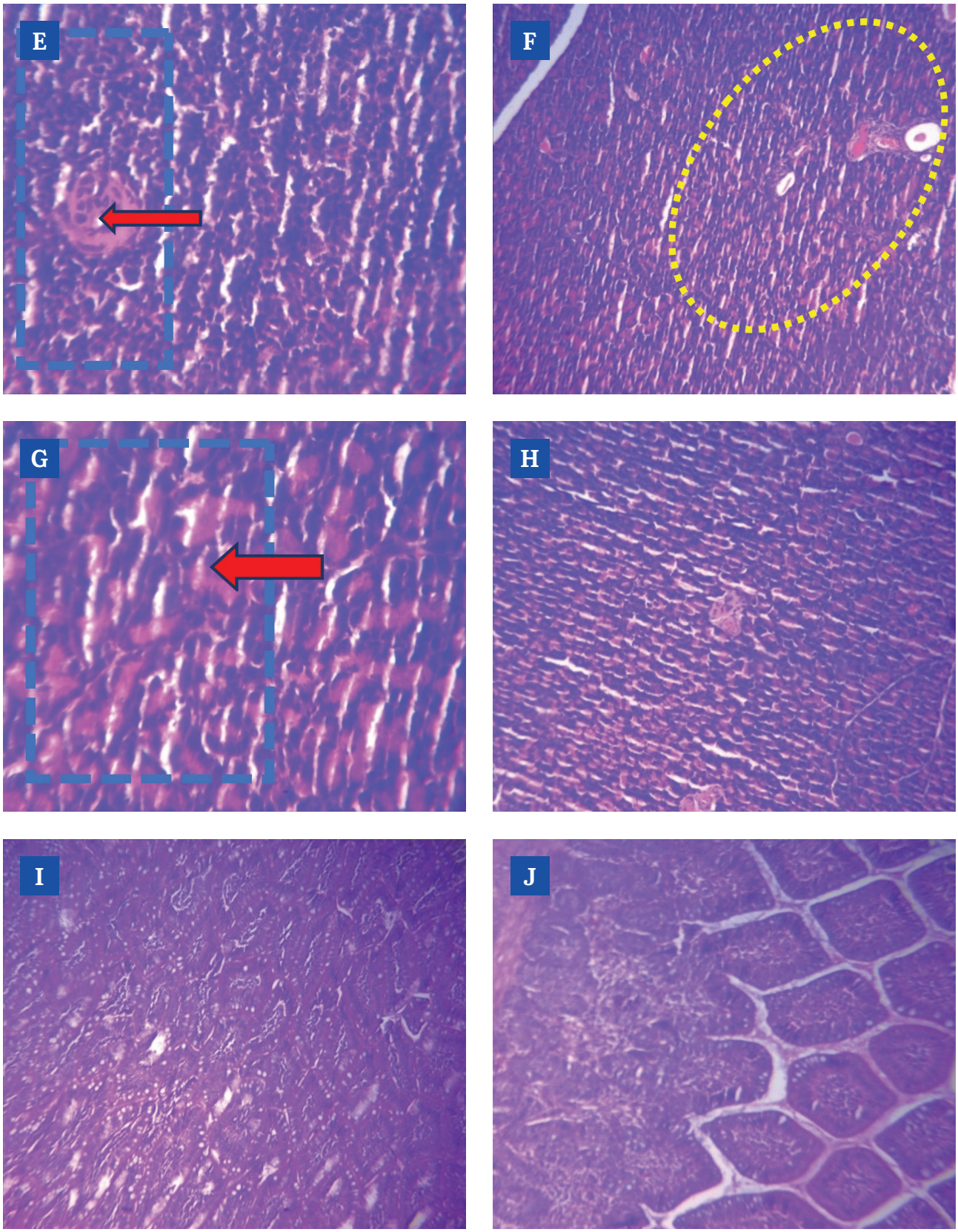

Figure 3: Continued.

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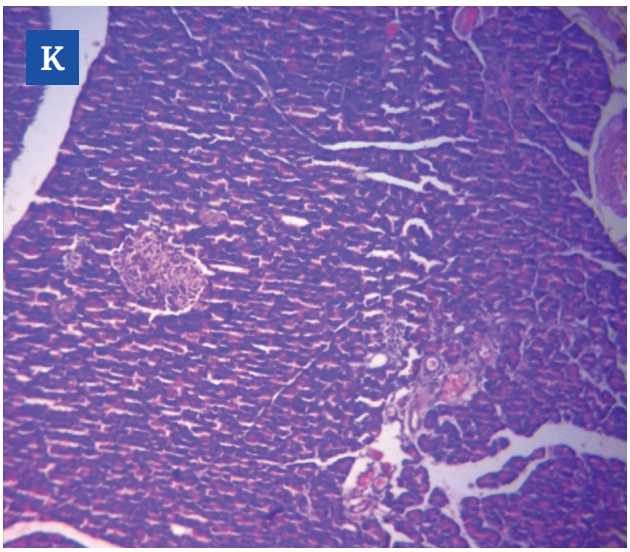

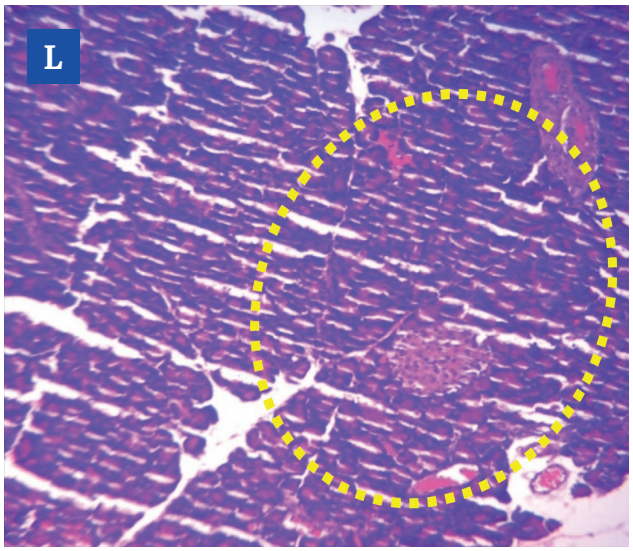

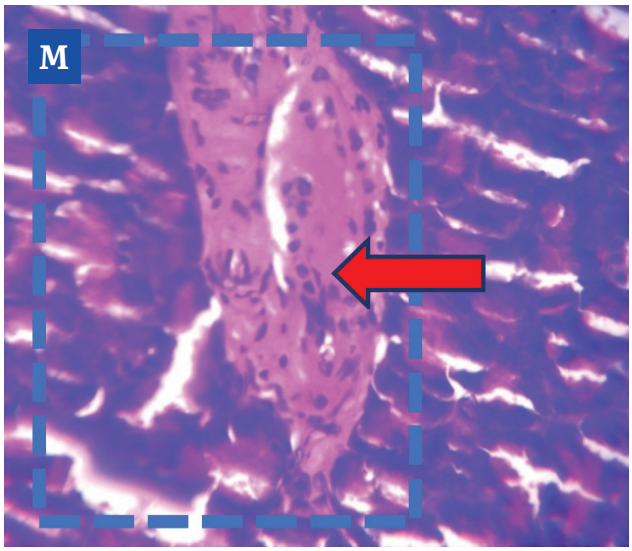

Figure 3: Continued.

appearance is shown in A, C, D, H, I and J). The extract-treated rats showed reduced pancreas abnormalities compared to glibenclamide, which showed marked acini infiltration (Figure 3 A–M).

Conclusion

The outcome of this study further showed that untreated diabetic rats had a marked abnormality in the form of degenerated seminiferous tubules compared to normoglycemic rats. Conclusively, partitioned solvent extract of *Lawsonia inermis* in diabetic rats showed significantly decreased BGL. The effect was of both solvent and dose-dependent. Methanol extract at 100 mg/kg showed better activity than 25 and 50 mg/kg of both N-hexane and ethyl acetate. Methanolic extract (100 mg/kg) was found to be better than the two conventional oral hypoglycaemic drugs, Metformin (50 mg/kg) and Glibenclamide (5 mg/kg). Antidiabetic activity of this plant may be linked to its ability to increase GLUT-4 and stimulate pancreatic β-cell regeneration. The outcome of this study suggests that L. *inermis* possess significant antidiabetic activity.

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Conflict of interest

The authors declare no conflict of interest.

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