

Fractions of *Lawsonia Inermis* Linn Leaves Improves the Dynamics of Organ-Specific Oxidative Stress and Anti-Oxidant Biomarkers in Streptozotocin Induced Diabetic Wistar Rat

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Received January 12, 2023; Accepted January 22, 2023; Accepted January 25, 2023

ABSTRACT

Background and aims: Diabetes has been linked to over four million deaths and it is the seventh foremost cause of death worldwide. Oxidative stress contributes greatly to the onset and development of diabetes and associated complications. Diabetes mellitus is linked to higher formation of free radicals and reduced antioxidant activities. This study assessed the positive modulatory activities of *Lawsonia inermis* Linn leaves on the dynamics of oxidant and anti-oxidant biomarkers in various organs predisposed to diabetic complications in streptozotocin-induced diabetic rats.

Method: Crude methanol extract of *Lawsonia inermis* Linn leaves were partitioned into different fractions using N-hexane, ethyl acetate and methanol. Fractions obtained were assessed for their modulatory activities on oxidant and antioxidant biomarkers of streptozotocin-induced diabetic rats. Ten groups of diabetic rats (n=5) were orally administered 50 and 100 mg/kg of each of the three partitioned fractions, metformin (50 mg/kg), glibenclamide (5 mg/kg), while untreated hyperglycaemic and normoglycaemic rats received distilled water daily for 28 days.

Result: Kidney GPx increase significantly in Methanol fraction and glibenclamide-treated groups when compared to other treatment groups and diabetic control. GSH increases significantly in the heart especially in Methanol and N-hexane groups when compared with diabetic and non-diabetic control. *Lawsonia inermis* significantly increased all the anti-oxidant markers; GPx, GSH, GST, SOD and protein thiol while decreasing oxidant markers MPO, H₂O₂, NO and MDA in various organs (Brain heart, kidney and liver) that are predispose to diabetic complications.

Conclusion: Fractions of *Lawsonia inermis* possess a significant positive modulatory activity by improving the antioxidant system which may be helpful in delaying diabetic complications.

Keywords: *Lawsonia inermis*, Oxidative stress, Anti-oxidant system, Diabetes mellitus, Diabetic complication, Wistar rats

INTRODUCTION

Diabetes mellitus is a major problem affecting about 366 million individuals in the last decade but is projected to increase to about 552 million by 2030 [1]. The numerical population living with diabetes is increasing worldwide as 80% of people with this ailment are inhabitant of poor and developing countries [2]. Epidemiological survey has shown that diabetes has been linked to about 4.6 million deaths [1,2]. Reports have shown that oxidative stress contributed greatly to the onset and development of diabetes and its associated complications [3]. Persistent uncontrolled blood glucose level increases oxidative stress and decrease antioxidant system [4]. The body system is routinely exposed to various xenobiotics resulting into free radical

generation from the reacting oxidant which transfer the unpaired electron to the system thereby leading to oxidation of cellular system [5]. These dangerous effects are mostly

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Citation: Aremu A, Oridupa OA, Ahmed AO, Yusuf SK & Ibrahim RA. (2023) Diabetes and Gene Therapy. Int J Diabetes, 5(1): 187-196.

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counteracted by the antioxidant system which can either be endogenous or exogenous [6]. The imbalance between reactive species and the antioxidant system give rise to oxidative stress that contributed to development of various pathological anomaly such as diabetes [5,6]. Various reports have established a firm correlation between oxidative stress and diabetes through negative alteration in all the endogenous markers of the antioxidant system and positively increasing the oxidant markers [6].

There are reports from animal model research that affirm the contribution of oxidative stress in diabetes development (both type I and II). This was linked to formation of free radical ions from protein glycation, increased lipid peroxidation and glucose oxidation leading to enzymatic destruction of cellular mechanism and insulin resistance [7]. Oxidative stress observed in diabetes mellitus principally originated from the mitochondria. In the process of mitochondrial oxidative metabolism, one fraction from used oxygen is converted to water whereas leaving other portion changing to free radical (O^*) which is vital to reactive species formation, examples; OH , $ONOO^-$ and H_2O_2 [8]. Signaling of insulin is enhancing by two-way reaction (ROS/RNS). On first reaction, insulin responds positively in order to exercise the physiological effect [9]. This reaction leads to reactive species thus negatively modulating insulin signaling leading development of insulin resistance that is paramount to development of diabetes (mostly type II) [10].

Antioxidants reverse the oxidation triggered by reactive species resulting in cell injury [10,11]. Antioxidants like vitamins and thiols can disrupt this chain of reactions thereby preventing cell damage [11]. To counteract the damaging effect of oxidative stress, there is need to maintain both plant and animal source of antioxidants. Reports have shown that during prediabetic stage, the antioxidant system was able to mitigate injury pose by increasing free radicals, but during the diabetes proper, there is advancement in free radical generation and this was directly linked to hyperglycemia that have negative factor on the antioxidant pathway [12].

On this account, this study assessed the modulatory activities of *Lawsonia inermis* Linn leaves on the dynamics of oxidant and anti-oxidant biomarkers in various organs (Brain, heart, kidney and liver) predisposed to diabetic complications in STZ-induced diabetic rats.

METHODOLOGY

Plant Harvesting, Identification and Preparation

Leaves of *Lawsonia inermis* Linn was 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 were dried at room temperature (25°C) under shade in a room for four weeks

and were blended to powdery form. The powdery leaves of *Lawsonia inermis* Linn were extracted.

Extraction and Separation of crude *Lawsonia inermis* Linn leaves

3 kg of powdery leaves of *Lawsonia inermis* were separately soaked in 5-litre methanol for 72 hours respectively. The mixture was gently decanted and filtered. The filtrate was immediately evaporated at temp 40°C with a rotary evaporator to yield 45% crude extract.

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

The crude methanol extract 500 g 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

This study was ethically approved by ACUREC which is the regulatory body in charge of animal use in the University of Ibadan. ACUREC issue a full approval the 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 humanly handled.

Experimental Animals

Male Wistar rats between 130-180g, (total 50) were used for this experiment. Experimental rats were housed using international standard and were maintained at ideal conditions under appropriate temperature and humidity. The experimental rats were fed with vital^(R) feed (standard animal feed). 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 streptozotocin (STZ) (sigma®). STZ was dissolved in distilled water and injected intraperitoneally at 65 mg/kg.

Animal grouping

Experimental rats were randomly grouped into thirteen having 4-5 rats per group and each group was treated for 28 days as thus;

Control: Normoglycaemic control treated with distil water

Diabetic untreated: Hyperglycemic controls treated with distil water.

- Diab+Li+Meth-50 mg/kg: Diabetic and treated at a dosage 50 mg/kg methanol extract of *Lawsonia inermis* Linn. leave

- Diab+Li+Meth-100 mg/kg: Diabetic and treated at a dosage 100 mg/kg methanol extract of *Lawsonia inermis* Linn. leave
- Diab+Li+Nx-50 mg/kg: Diabetic and treated at a dosage 50 mg/kg N-hexane extract of *Lawsonia inermis* Linn. leave
- Diab+Li+Nx-100 mg/kg: Diabetic and treated at a dosage 100 mg/kg N-hexane extract of *Lawsonia inermis* Linn. leave
- Diab+Li+EA-50 mg/kg: Diabetic and treated at a dosage 50 mg/kg Ethyle acetate extract of *Lawsonia inermis* Linn. leave
- Diab+Li+EA-100 mg/kg: Diabetic and treated at a dosage 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 50 mg/kg glibenclamide.

Constitution and Administration of *Lawsonia inermis* leave extract

The stock concentration of the three extract were prepared by mixing 2ml of distil 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 4 weeks. The control groups were treated using distill water.

The oxidative stress markers were analyzed using commercial Elisa test kits such as GST, GSH, GST, GPx, MPO, H₂O₂ MDA, protein thiol and SOD from the brain, liver, kidney, heart and serum.

Preparation of tissues homogenate (brain, heart, kidney and liver)

Brain, heart, kidney and liver were removed and cut from the fat and connective tissue. They were all weighed individually and immediately perfused with normal saline. The tissues were homogenized separately with Potassium Phosphate Buffer (KPB) using a homogenizer (Teflon, UK). The tissue homogenate from these various organs was subsequently centrifuged at 10000 rpm for 15 min using a cold centrifuge (Sipha USA) at 4°C. Post Mitochondrial Fraction (PMF) was obtained and decanted using a disposable pipette. The PMF supernatant was used for the assaying GSH, SOD, GPx, GST, Protein Thiol and Total Protein. Other assays were H₂O₂, NO, MDA and MPO content.

RESULTS

Glutathione Peroxidase (GPx)

The mean value of brain GPx in the untreated diabetic group decreased (201.77±76.26) significantly ($p<0.05$) when compared to all other treatment groups and normoglycaemic control. The brain GPx of group Meth-100 mg/kg (230.31±43.61) showed a non-significant decreased level while all other treatment groups increased non-significantly compared to normoglycaemic control (250.52±46.51) (**Table 1**).

Table 1. Effect of different solvent partition fraction of *Lawsonia inermis* Linn leave and oral hypoglycemic agent on Glutathione peroxidase (GPx) of different organs on diabetic treated rats.

GRP/Organ	Brain	Heart	Kidney	Liver
CONTROL	250.52±4 6.51	152.63±3 2.12	125.16±2 0.19	89.87±1 0.38
DIAB+UNTR EATED	201.77±7 6.26 ^a	116.26±2 2.15 ^b	89.21±28. 71 ^b	56.99±7. 25
DIAB+LI+ME TH-50MG	291.34±7 7.60	159.01±4 8.89	95.29±19. 65	78.49±1 5.50
DIAB+LI+ME TH-100MG	230.31±4 3.61	153.20±3 5.44	101.53±1 3.07	73.98±1 8.79
DIAB+LI+Nx -50MG	269.29±4 9.80	144.58±4 9.31 ^a	150.04±2 9.22 ^b	60.39±6. 27
DIAB+LI+Nx -100MG	281.62±3 2.13	138.68±3 0.15 ^b	146.69±2 2.91	62.35±2 1.47
DIAB+LI+EA -50MG	282.72±5 0.36	136.93±1 7.52	135.49±3 8.49	122.23± 44.24 ^b
DIAB+LI+EA -100MG	287.94±2 2.73	143.39±2 2.74	130.84±2 4.71	104.55± 46.82
DIAB+METF ORMIN	274.37±5 1.35	123.58±2 9.80	139.47±7 2.43	113.69± 11.74
DIAB+GLIBE N	278.55±5 6.91	119.58±3 9.75	126.44±1 08.15 ^b	120.47± 37.15 ^b

Results are shown as Mean ±SD: n=5

^a $p<0.05$ ^b $p<0.01$ ^c $p<0.001$

The GPx of the heart also followed a similar trend decreasing significantly ($p<0.01$) in groups Nx-50mg/kg (144.58±49.31), Nx-100 mg/kg (138.68±30.15) and diabetic untreated (116.26±22.15). All other treatment groups showed non-significant decreased levels except Meth-50 mg/kg (159.01±48.89) that increased non-significantly when compared to normoglycaemic control (**Table 1**).

The kidney GPx increased significantly ($p<0.05$) in groups Nx-50mg/kg (150.04±29.22) and glibenclamide (126.44±108.15) while it decreased significantly in diabetic

untreated (89.21±28.71) compared to normoglycaemic untreated (125.16±20.19).

All treatment groups showed significant ($p<0.01$) increased liver GPx level when compared to hyperglycemic control (56.99±7.25). There is a significant ($p<0.05$) increased GPx level in groups EA-50 mg/kg (122.23±44.24) and Glibenclamide (120.47±37.15) when compared to all other treatment groups and both Hyperglycemic (56.99±7.25) and normoglycaemic (89.87±10.38) controls (**Table 1**).

Glutathione (GSH)

The brain GSH presented significant ($p<0.01$) increased level in groups; Meth-50 mg/kg (122.98±29.51) and Meth-100 mg/kg (151.73±73.68) while groups Nx-50 mg/kg (100.29±27.15) Nx-100 mg/kg (98.06±1.76) and EA-50 mg/kg (89.58±16.23) increased non-significantly. All other treatment groups and hyperglycemic control showed non-significant decreased GSH level when compared to normoglycaemic control (81.66±5.16).

In heart, mean GSH level increased significantly ($P\leq0.05$) in Nx-50 mg/kg (88.69±18.24) while non-significant increased level is seen in groups Meth-100 mg/kg (96.38±13.03). All other treatment groups and hyperglycemic untreated control presented non-significant decreased GSH level when compared to non-diabetic untreated control (94.05±25.16).

The kidney GSH increased significantly ($p<0.01$) in groups Nx-50 mg/kg (134.52±24.68), Nx-100 mg/kg (130.27±61.29), EA-100 mg/kg (178.04±48.95) and metformin (109.58±32.59) while it increased non-significantly in other treatment groups where it decreased non-significantly when compared to normoglycaemic untreated (91.57±4.02).

All treatment groups showed significant ($p<0.01$) increased liver GSH level when compared to hyperglycemic control (89.93±22.45). There is significant ($p<0.05$) increased GSH level in groups Nx-100 mg/kg (128.26±78.73). All other treatment groups showed non-significant increased or decreased GSH level (liver) when compared normoglycaemic (122.98±11.18) controls (**Table 2**).

Glutathione s-transferase (GST)

GST of brain presented a significant ($p<0.01$) decreased level in all treatment groups except Nx-100 mg/kg that decreased (3.95±0.40) non-significantly increase compared to non-diabetic control (4.22±1.42). All treatment groups showed significant increased GST level when compared to hyperglycemic untreated control (2.39±2.07).

In the heart, GST followed the same trend as observed in the brain showing significant ($p<0.01$) decreased level in all treatment groups except Glibenclamide (2.15±1.93) that presented non-significant decreased level compared to non-diabetic control (3.62±1.35). Like in the brain, heart GST of

all treatment groups increased significantly when compared to hyperglycemic untreated control (1.03±0.51).

Table 2. Effect of different solvent partition fraction of *Lawsonia inermis* Linn leave and oral hypoglycemic agent on Glutathione (GSH) of different organs in diabetic treated rats.

GRP/Organ	Brain	Heart	Kidney	Liver
CONTROL	81.66±5.1 6	94.05±25 .16	91.57±4. 02	122.98±1 1.18
DIAB+UNTRE ATED	71.23±23. 44	81.45±11 .12	80.07±17 .01	89.93±22 .45
DIAB+LI+ME TH-50MG	122.98±2 9.51 ^a	91.59±12 .81	88.52±11 .02	101.80±1 4.96
DIAB+LI+ME TH-100MG	151.73±7 3.68 ^b	96.38±13 .03	95.94±19 .57	100.09±3 6.50
DIAB+LI+NX- 50MG	100.29±2 7.15	88.69±18 .24 ^b	134.52±2 4.68 ^b	144.26±9 4.29
DIAB+LI+NX- 100MG	98.06±1.7 6	87.00±31 .07	130.27±6 1.29 ^b	128.26±7 8.73 ^b
DIAB+LI+EA- 50MG	76.69±11. 43	70.38±6. 00	107.50±3 0.21	117.40±2 1.75
DIAB+LI+EA- 100MG	89.58±16. 23	74.64±9. 27	178.04±4 8.95 ^b	130.17±4 5.35
DIAB+METFO RMIN	74.88±3.4 5	83.81±15 .25	109.58±3 2.59 ^b	139.46±4 9.69
DIAB+GLIBE N	77.43±5.9 1	72.72±20 .18	98.61±4. 78	143.57±5 .37

Results are shown as Mean ±SD: n=5

^{a b c}Significant ^a $p\leq0.05$ ^b $p\leq0.01$ ^c $p\leq0.001$

GST of the kidney decreased significantly ($p<0.05$) in diabetic untreated control (2.01±2.37) but increased significantly ($p<0.05$) in groups Nx-50 mg/kg (5.39±1.07), EA-50 mg/kg (5.75±1.98) and metformin (5.99±5.10) while other treatment groups decreased non-significantly when compare to non-diabetic control (5.89±0.59). It is noted that all treatment groups showed significant improvement in the level of kidney GST when compared to diabetic untreated control (2.01±2.37).

GST of the liver decreased significantly ($p<0.05$) in diabetic untreated control (2.48±2.21) compared to all treatment groups and normoglycaemic control. All treatment groups showed significant ($p<0.01$) increased GST level except groups Nx-50 mg/kg (3.02±1.65) and Nx-100 mg/kg (4.74±6.04) that decreased significantly ($p<0.01$) when compared to normoglycaemic untreated control (6.44±1.98) (**Table 3**).

Table 3. Effect of different solvent partition fraction of *Lawsonia inermis* Linn leave and oral hypoglycemic agent on Glutathione transferase (GST) of different organs in diabetic treated rats.

GRP/Organ	Brain	Heart	Kidney	Liver
CONTROL	4.22±1.4 2	3.62±1.3 5	5.89±0.5 9	6.44±1.9 8
DIAB+UNTREA TED	2.39±2.0 7 ^b	1.03±0.5 1 ^b	2.01±2.3 7 ^b	2.48±2.2 1 ^a
DIAB+LI+METH -50MG	4.14±1.4 8 ^b	2.29±0.8 8 ^b	3.97±1.0 5	8.14±0.9 7 ^b
DIAB+LI+METH -100MG	3.54±1.1 3 ^b	2.68±0.8 4 ^b	5.65±1.7 8	9.90±2.4 5 ^b
DIAB+LI+NX- 50MG	3.33±1.2 2 ^b	2.46±0.0 4 ^b	5.39±1.0 7 ^b	3.02±1.6 5 ^b
DIAB+LI+NX- 100MG	3.95±0.4 0	1.96±0.2 3 ^b	4.63±1.3 4	4.74±6.0 4 ^b
DIAB+LI+EA- 50MG	3.80±1.1 2 ^b	2.29±0.3 9 ^b	5.75±1.9 8 ^b	14.72±3. 97 ^b
DIAB+LI+EA- 100MG	2.43±0.8 5 ^b	2.88±1.1 7 ^b	4.19±1.8 1	13.80±7. 83 ^b
DIAB+METFOR MIN	3.70±1.0 4 ^b	2.76±0.4 8 ^b	5.99±5.1 0 ^a	8.79±2.2 2 ^b
DIAB+GLIBEN	2.43±2.4 9 ^b	2.15±1.9 3	4.66±2.1 2	7.11±2.6 3 ^b

Results are shown as Mean ±SD: n=5

^{a b c}Significant ^ap≤0.05 ^bp≤0.01 ^cp≤0.001

Superoxide Dismutase (SOD)

SOD in brain of hyperglycemic untreated rats (6.00±2.60) present a significant (p<0.05) decreased level when compared to all treatment groups and normoglycaemic control (8.30±1.50). All treatment groups showed non-significant increase SOD level in the brain compared to normoglycaemic control (8.30±1.50).

Like brain SOD, similar observation was noticed in the heart where the level of SOD decreased significantly (p<0.01) in untreated diabetic group (2.00±0.80) compared to all treatment groups and non-diabetic control. All treatment groups showed non-significant increased SOD level except Nx-100 mg/kg (3.10±0.30) that decreased significantly (p<0.01) compared to other treatment groups and normoglycaemic control (4.80±1.10).

SOD in kidney followed the same trend where it decreased significantly (p<0.01) in untreated diabetic group (3.00±0.60) when compared to all treatment groups and non-

diabetic control (6.30±0.50). Kidney SOD level in all treatment groups decreased non-significantly compared to normoglycaemic control (6.30±0.50).

Liver SOD increased significantly (p<0.01) in groups; EA-50 mg/kg (4.00±1.70) and glibenclamide (3.90±1.20). All other treatment groups increased non-significantly except Nx-50 mg/kg (2.00±0.20) that decreased non-significantly compared to normoglycaemic control (2.30±0.12) (Table 4).

Table 4. Effect of different solvent partition fraction of *Lawsonia inermis* Linn leave and oral hypoglycemic agent on Superoxide Dismutase (SOD) of different organs in diabetic treated rats.

GRP/Organ	Brain	Heart	Kidney	Liver
CONTROL	8.30±1.5 0	4.80±1.1 0	6.30±0.50	2.30± 0.12
DIAB+UNTREA TED	6.00±2.6 0 ^a	2.00±0.8 0 ^b	3.00±0.60 b	1.80±0 .20
DIAB+LI+MET H-50MG	10.00±2. 40	5.00±1.5 0	3.70±0.80	2.50±0 .40
DIAB+LI+MET H-100MG	8.40±1.9 0	5.00±1.3 0	4.80±0.80	2.40±0 .60
DIAB+LI+NX- 50MG	8.40±1.9 0	4.50±1.5 0	4.40±1.00	2.00±0 .20
DIAB+LI+NX- 100MG	8.50±0.8 0	3.10±0.3 0 ^b	4.30±0.80	2.30±0 .70
DIAB+LI+EA- 50MG	9.90±1.7 0	4.30±0.6 0	4.00±1.20	4.00±1 .70 ^b
DIAB+LI+EA- 100MG	10.52±1. 23	5.90±0.8 0	3.10±0.80	3.30±1 .40
DIAB+METFOR MIN	10.05±2. 34	4.82±0.6 0	4.40±2.40	2.70±0 .40
DIAB+GLIBEN	11.00±2. 40	4.90±1.3 0	5.30±3.50	3.90±1 .20 ^b

Results are shown as Mean ±SD: n=5

^{a b c}Significant ^ap≤0.05 ^bp≤0.01 ^cp≤0.001

Protein thiol

Protein thiol in brain of metformin treated group (74.71±18.58) showed significant (p<0.001) increased level compared to other treatment groups and normoglycaemic control (52.72±8.19).

All treatment groups showed significant (p<0.05) increased brain protein thiol level compared to hyperglycemic untreated control (40.14±26.61). Brain protein thiol level of groups treated with methanol fraction decreased non-significantly while other two fractions and glibenclamide

increased non-significantly when compared to normoglycaemic control.

Protein thiol level in the heart of glibenclamide treated group showed significant ($p < 0.001$) increased level when compared to other treatment groups and non-diabetic control (70.81 ± 19.0). EA-100 mg/kg (68.30 ± 4.91) showed significant ($p < 0.01$) decreased thiol level when compared to other treatment groups while other treatment groups did not show significant alteration compared to normoglycaemic control.

The protein thiol in the kidney presented significant ($P < 0.01$) increased level in groups Nx-50 mg/kg (111.68 ± 47.83), EA-50 mg/kg (108.28 ± 33.65), Metformin (111.15 ± 21.32) and Glibenclamide (119.01 ± 51.68) while other treatment groups increased non-significantly compared to diabetic (75.13 ± 16.49) and non-diabetic (83.79 ± 24.57) control.

Protein thiol in the liver increases in all treatment groups with significant ($p < 0.01$) increased level in groups; N-hexane treated groups when compared to other treatment groups, diabetic control (104.12 ± 45.09) and normoglycaemic control (115.06 ± 24.25) (Table 5).

Malondialdehyde (MDA)

MDA in brain increased significantly ($p < 0.01$) in diabetic untreated rats (1.83 ± 0.37) compared to all treatment groups and normoglycaemic control (0.63 ± 0.10). All treatment groups present non-significant decreased brain MDA level except Meth-50 mg/kg (0.30 ± 1.09) that decrease significantly ($p < 0.05$) when compared and normoglycaemic controls.

The MDA in heart of groups Glibenclamide (0.81 ± 0.53) and hyperglycemic untreated rats (1.74 ± 0.17) increased significantly ($p < 0.01$) when compared to other treatment groups and normoglycaemic control. MDA level in the heart of group; N-hex-100 mg/kg (0.53 ± 0.08) decreased significantly ($p < 0.05$) compared to other treatment groups and normoglycemic control. Other treatment groups did not show significant alteration in the heart MDA when compared to normoglycaemic control but decreased significantly compared to hyperglycemic control.

The MDA in the kidney showed similar trend increasing significantly ($p < 0.05$) in diabetic untreated rats (1.39 ± 0.0) when compared to all treatment groups and non-diabetic control (0.27 ± 0.07). There was significant alteration in kidney MDA in all treatment groups increasing non-significantly when compared to normoglycaemic control.

In liver; the MDA level significantly ($p < 0.01$) decreased in treatment groups Metformin (0.55 ± 0.28) and glibenclamide (0.63 ± 0.29) compared to other treatment groups and normoglycemic control (0.20 ± 0.08). Other treatment groups and hyperglycemic untreated group presented non-

significant decreased liver MDA level when compared to normoglycaemic control (Table 6).

Table 5. Effect of different solvent partition fraction of *Lawsonia inermis* Linn leave and oral hypoglycaemic agent on Protein thiol of different organs in diabetic treated rats.

GRP/Organ	Brain	Heart	Kidney	Liver
CONTROL	52.72±8.19	70.81±1.90	83.79±24.57	115.06±24.25
DIAB+UNTRE ATED	40.14±2.66	53.58±5.33	75.13±16.49	104.12±45.09
DIAB+LI+ME TH-50MG	43.76±5.52	60.27±9.98	89.51±12.26	164.59±73.03
DIAB+LI+ME TH-100MG	46.86±1.61	70.64±2.29	91.83±13.35	157.49±83.57
DIAB+LI+NX-50MG	59.77±8.10	68.63±8.65	111.68±4.78 ^b	202.04±11.40 ^b
DIAB+LI+NX-100MG	55.66±5.93	72.23±1.78	104.29±1.49	213.15±84.37 ^b
DIAB+LI+EA-50MG	64.57±2.82	77.29±1.70	108.28±3.65 ^b	149.06±69.59
DIAB+LI+EA-100MG	60.09±6.32	68.30±4.91 ^a	98.02±23.34	136.98±70.80
DIAB+METF ORMIN	74.71±1.85	82.87±9.16	111.15±2.13 ^b	166.93±28.38
DIAB+GLIBE N	51.61±7.18	91.66±9.75 ^c	119.01±5.16 ^b	187.66±28.98

Results are shown as Mean ±SD; n=5

^{a b c}Significant ^a $p \leq 0.05$ ^b $p \leq 0.01$ ^c $p \leq 0.001$

Hydrogen peroxide (H₂O₂)

The brain of diabetic untreated rats (56.09 ± 12.91) had significant ($p < 0.01$) increased H₂O₂ level compared to other treatment groups and non-diabetic control (47.31 ± 15.31). All treatment groups showed non-significant decreased H₂O₂ level except groups; Nx-50mg/kg (48.53 ± 6.49) and glibenclamide (48.45 ± 21.27) that increased non-significantly compared to normoglycaemic control.

H₂O₂ in heart of diabetic rats increased non-significantly while it decreased in other treatment groups compared with non-diabetic control (60.03 ± 16.07). All other treatment groups did not show significant alteration when compared to both diabetic and non-diabetic controls.

H₂O₂ in kidney followed the same trend increasing non-significantly in diabetic untreated rats (105.23 ± 13.35), metformin (98.84 ± 22.51) and glibenclamide (96.89 ± 37.15) while it decreased non-significantly in other treatment

groups when compared to normoglycaemic control (94.25±19.11).

Table 6. Effect of different solvent partition fraction of *Lawsonia inermis* Linn leave and oral hypoglycemic agent on Malondialdehyde (MDA) of different organs in diabetic treated rats.

GRP/Organ	Brain	Heart	Kidney	Liver
CONTROL	0.63±0.1 0	0.75±0.4 7	0.27±0. 07	0.20±0.0 8
DIAB+UNTREAT ED	1.83±0.3 7	1.74±0.1 7 ^b	1.39±0. 0 ^b	0.85±0.0 6
DIAB+LI+METH- 50MG	0.30±1.0 9 ^a	0.69±0.2 4	0.32±0. 15	0.32±0.1 8
DIAB+LI+METH- 100MG	0.79±0.1 7	0.82±0.3 6	0.47±0. 21	0.30±0.3 1
DIAB+LI+NX- 50MG	0.40±0.3 8	0.59±0.3 1	0.57±0. 28	0.39±0.2 1
DIAB+LI+NX- 100MG	0.39±0.1 1	0.53±0.0 8 ^b	0.80±1. 13	0.31±0.2 7
DIAB+LI+EA- 50MG	0.48±0.1 0	0.54±0.2 1	0.46±0. 08	0.39±0.0 7
DIAB+LI+EA- 100MG	0.50±0.1 2	0.60±0.2 8	0.44±0. 09	0.40±0.2 2
DIAB+METFOR MIN	0.62±0.2 9	0.80±0.3 9	0.58±0. 16	0.55±0.2 8 ^a
DIAB+GLIBEN	0.61±0.1 2	0.81±0.5 3 ^a	0.61±0. 36	0.63±0.2 9 ^b

Results are shown as Mean ±SD: n=5

^{a b c} Significant ^ap≤0.05 ^bp≤0.01 ^cp≤0.001

In the liver, H₂O₂ level of diabetic untreated rats (127.51±19.83) increased non-significantly compared to non-diabetic control and all other treatment groups. There is

non-significant increased or decreased level of liver H₂O₂ in all the treatment groups when compared to normoglycaemic control (102.28±44.83) (Table 7).

Table 7. Effect of different solvent partition fraction of *Lawsonia inermis* Linn leave and oral hypoglycemic agent on Hydrogen peroxide (H₂O₂) of different organs in diabetic treated rats.

GRP/Organ	Brain	Heart	Kidney	Liver
CONTROL	47.31±1 5.31	60.03±1 6.07	94.25±19 .11	102.28±4 4.83
DIAB+UNTR EATED	56.09±1 2.91 ^b	76.39±4 .39	105.23±1 3.35	127.51±1 9.83
DIAB+LI+ME TH-50MG	42.69±5. 31	58.01±1 2.93	90.73±25 .77	109.95±4 0.27
DIAB+LI+ME TH-100MG	40.45±2 1.69	60.17±1 5.74	88.45±20 .45	110.48±3 4.24
DIAB+LI+NX -50MG	48.53±6. 49	50.34±5 .46	71.59±10 .46	108.14±4 8.99
DIAB+LI+NX -100MG	40.89±3. 58	57.53±9 .12	82.01±16 .58	112.23±4 2.37
DIAB+LI+EA- 50MG	31.95±1 3.06	51.84±8 .01	85.78±41 .78	100.28±7 4.49
DIAB+LI+EA- 100MG	34.19±3. 69	48.67±7 .19	89.34±18 .23	99.39±42 .30
DIAB+METF ORMIN	44.06±3. 85	61.57±1 6.44	98.84±22 .51	107.34±1 5.05
DIAB+GLIBE N	48.45±2 1.27	68.98±1 6.81	96.89±37 .15	101.89±4 4.63

Results are shown as Mean ±SD: n=5

^{a b c} Significant ^ap≤0.05 ^bp≤0.01 ^cp≤0.00

Nitric oxide (NO)

Untreated hyperglycemic group showed a significant (p<0.001) increased NO expression when compared to all other treatment groups and the normoglycemic control. EA-100mg/kg presented a significant reduction in No expression when compared to other treatment group and hyperglycemic control. Methanol fraction reduced No expression just like the two standard drugs (Figure 1).

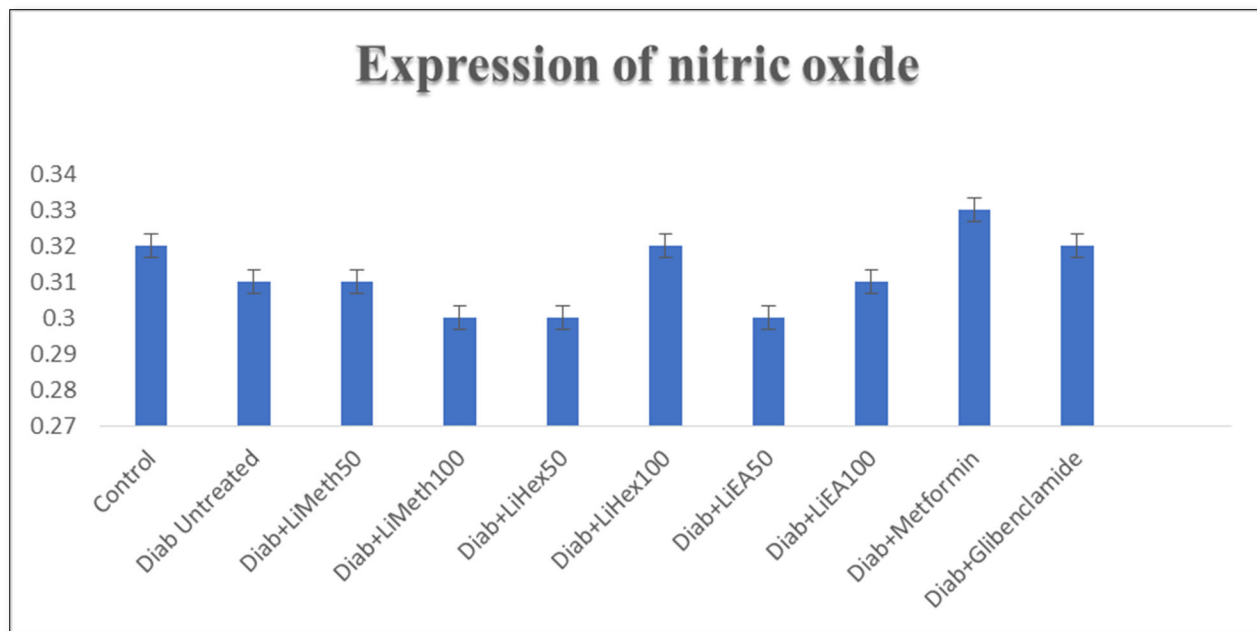


Figure 1. Values of nitric oxide (NO) of experimental rats induced with STZ following 28 days treatment with different extract of *Lawsonia inermis* and oral hypoglycemic.

Results are shown as Mean ±SD: n=5

Myeloperoxidase (MPO)

Increased expression of MPO usually increase the risk of cardiovascular complication in DM. Untreated hyperglycemic group showed a significant (p<0.001)

increased MPO level when compared to all other treatment groups and the normoglycemic control. All extract treated groups presented a significant decreased in the level of MPO when compared to normoglycemic control (**Figure 2**).

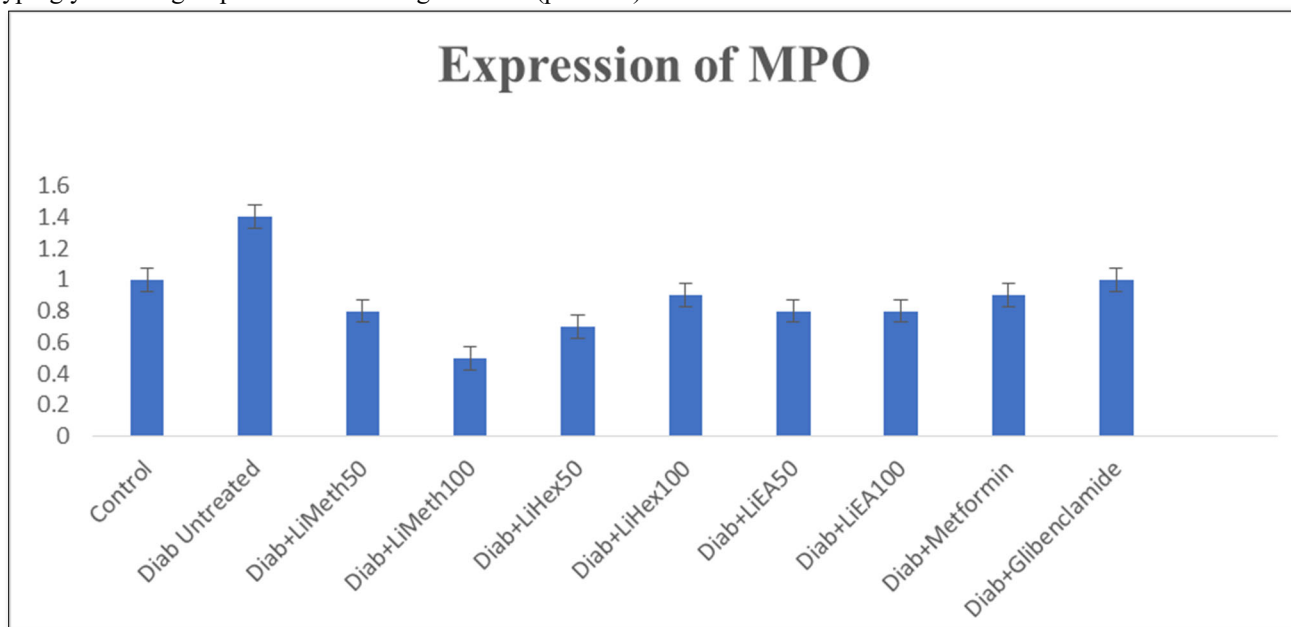


Figure 2. Values of myeloperoxidase (MPO) of experimental rats induced with STZ following 28 days treatment with different extract of *Lawsonia inermis* and oral hypoglycemic agents.

Results are shown as Mean ±SD: n=5

DISCUSSION

Streptozotocin (STZ) is a cytotoxic compound that is mediated by reactive oxygen species (ROS) which penetrates the β -cells via glucose transporter. The resultant oxidative stress leads to DNA strand breakage in β -cells causing decreased endogenous insulin release [13].

Oxidative stress which is imbalance between oxidant (reactive oxygen species) and anti-oxidant cascade [14]. Free radicals are mostly generated in many metabolic diseases including diabetes and this result in oxidative stress which leads to ineffective natural antioxidant system that subsequent result to free radical accumulation [15]. Reports on diabetes studies showed that oxidative stress destroys β -cells leading to altered insulin production, release and functions [16]. The oxidant markers used in this study are Malondialdehyde (MDA), Hydrogen peroxide (H_2O_2), Nitric oxide (NO) and myeloperoxidase (MPO). The MDA and H_2O_2 were assayed in the brain, heart, kidney and liver while MPO and NO were assayed from the serum. The anti-oxidant markers include GPx, GSH, SOD, and Protein thiol. The GPx, GSH, GST, SOD and PT were assayed from various organs predisposed to diabetic complications. The result from this study showed that diabetic untreated rats have significant increased oxidant markers (MDA, H_2O_2 , NO and MPO) and significant decreased anti-oxidant markers (GPx, GSH, GST, SOD and PT) in all the tissues (brain, heart, kidney and liver) when compared to treatment groups and normoglycemic control. Reports have shown that the rate of oxidative stress in diabetes patients is high when compared to normal metabolic conditions [17].

The groups treated with *Lawsonia inermis* Linn showed a promising improvement to both oxidant and anti-oxidant markers by decreasing and increasing them respectively across different fractions in all the tissues. The MDA of the heart and kidneys in N-hexane at 100 mg/kg showed a significant reduction when compared with other treatment groups and non-diabetic control. The H_2O_2 of the rats treated with oral hypoglycemic drugs increases non-significantly while it decreases in other treatment groups with ethyl acetate fraction which reduced significantly when compared with normoglycaemic control group. MPO reduced significantly in both the extract and standard drugs treatment compared with both diabetic and non-diabetic control. The result obtained from this study followed consistently with the report of [18] who confirms significant decreased free radical level in alloxan induced diabetic rats treated with *Lawsonia inermis* Linn [18].

Glutathione S-transferases; an enzyme that catalyzes addition of tripeptide glutathione to endogenous and exogenous substrates with electrophilic functional group. It plays a crucial role in metabolism and detoxification of xenobiotic pathway acting as potent endogenous anti-oxidant [19]. This present study showed *Lawsonia inermis* Linn improved the anti-oxidant system in all the marker of

both serum and tissues. The body defense mechanisms used anti-oxidant system as remedy to mop up free radicals [20]. GSH of liver was higher in Ethyl acetate treatment when compared to other treatment groups. GST followed the same trend as observed in all the tissues showing significant increase level. SOD increased across all the tissues when compared with hyperglycemic control. This agrees with work of [18,20] who report the increment in the SOD of diabetic *Rattus norvegicus* when treated with *Lawsonia inermis* Linn [18]. The protein thiol in the kidney also increased in treatment both methanol and ethyl acetate fraction when compared with other treatment groups. NO that can both act as oxidant and anti-oxidant presented the reverse case in the treatment groups decreasing significantly in all the fractions when compared to diabetic untreated control.

Increased activities of the various anti-oxidants in the serum, brain, heart, kidney and liver serves as a potent defense mechanism that detoxify or neutralize toxic metabolites in form of ketone bodies that is produced by hepatic cell in diabetes mellitus. Surge in the activities of the anti-oxidant effectively normalize the abnormal impairment posed by STZ induced-diabetes [21]. Effective antioxidant system may be helpful in delaying diabetic complication such as retinopathy, nephropathy and neuropathy which arise due to imbalance between the oxidant and antioxidant system in the body [22].

In conclusion, fractions of *Lawsonia inermis* possess a significant positive modulatory activity by improving the antioxidant system which may be helpful in delaying diabetic complications.

COMPETING INTEREST

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors

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