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Lawsonia inermis Linn. Positively Improves the Expression of Various Inflammatory Cytokines and Apoptotic Cell Death Biomarkers of Streptozotocin-Induced Diabetic Wistar Rats

Lawsonia inermis Linn., Streptozotosinle İndüklenmiş Diyabetik Wistar Ratların Çeşitli İnflamatuar Sitokinler ve Apoptotik Hücre Ölümü Biyobelirteçlerinin Ekspresyonunu Olumlu Bir Şekilde Artırır

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ABSTRACT

Diabetes mellitus is a significant contributor to illness and death on a global scale. Interleukins have been identified as potential factors that can induce apoptosis of beta cells and diminish insulin secretion, resulting in various complications associated with diabetes mellitus. This study investigated the inflammatory cytokines, interleukins, and apoptotic cell death markers in streptozotocin-induced diabetic rats. *Lawsonia inermis* leaves were sequentially extracted using *n*-hexane, ethyl acetate, and methanol. Sixty-five male adult Wistar rats were grouped into 13, with 5 rats in each group. Streptozotocin-induced diabetic rats were treated as thus; 25, 50, and 100 mg kg^{-1} of each of the 3 partitioned extracts, metformin (500 mg kg^{-1}), glibenclamide (5 mg kg^{-1}), and untreated diabetic and nondiabetic rats were treated with distilled water for 28 days. Untreated diabetic rats showed an increased level of cytokines such as interleukin 1, interleukin 6, interleukin 12, and tumor necrosis factor alpha (TNF-α). The methanol fraction significantly decreased interleukin 1, interleukin 6, interleukin 12, interleukin 18, and TNF-α compared to other treatment groups and diabetic control. Methanol fraction of the extract showed a significant reduction in tTNF-α when compared to the 2 standard drugs and the controls. Nuclear factor-kappa beta increased nonsignificantly in both treated and untreated diabetic rats. There was a significant reduction in the expression of caspase-3, caspase-6, and caspase-9 in all the extract-treated groups. It was further noted that there is a slight increase level of B-cell lymphoma 2 in diabetic untreated rats. The study concluded that *Lawsonia inermis* Linn. has a significant positive modulatory effect on the expression of various cytokines, interleukins, and essential caspases that are implicated in the pathophysiology and pathogenesis of diabetes mellitus.

Keywords: *Lawsonia inermis* Linn., inflammation, cytokines, interleukins, diabetes

ÖZ

Diabetes mellitus, küresel ölçekte hastalığa ve ölüme önemli ölçüde katkıda bulunan bir durumdur. İnterlökinler, beta hücrelerin apoptozunu tetikleyebilecek ve insülin salgısını azaltabilecek potansiyel faktörler olarak tanımlanmıştır, bu da diabetes mellitus ile ilişkilendirilen çeşitli komplikasyonlara neden olmaktadır. Bu çalışma, streptozotosin ile indüklenmiş diyabetik ratlarda inflamatuar sitokinler, interlökinler ve apoptotik hücre ölüm belirteçlerini incelemeyi amaçlamaktadır. *Lawsonia inermis* yaprakları n-hekzan, etil asetat ve metanol kullanılarak sırasıyla ekstrakte edildi. Altmış beş erişkin erkek Wistar ratı, her biri 5 rat içeren 13 gruba ayrıldı. Streptozotosin ile indüklenmiş diyabetik ratlar şu şekilde tedavi edildi: metformin (500 mg kg^{-1}), glibenklamidin

(5 mg kg^{-1}) üçe bölünmüş ekstrelerin her birinden 25, 50 ve 100 mg kg^{-1} dozu ve tedavi edilmemiş diyabetik ve nondiyabetik ratlarda ise damıtılmış su 28 gün boyunca tedavide kullanıldı. Tedavi edilmemiş diyabetik sıçanlar, interlökin 1, interlökin 6, interlökin 12 ve tümör nekroz faktörü alfa (TNF-α) gibi sitokinlerde artış gösterdi. Metanol fraksiyonu, diğer tedavi grupları ve diyabetik kontrolle karşılaştırıldığında interlökin 1, interlökin 6, interlökin 12, interlökin 18 ve TNF-α'yı önemli ölçüde azalttı. Ekstrenin metanol fraksiyonu, 2 standart ilaç ve kontrol grupları ile karşılaştırıldığında tTNF-α'da önemli bir azalma gösterdi. Nükleer faktör-kappa beta, hem tedavi edilen hem de tedavi edilmemiş diyabetik sıçanlarda önemsiz bir şekilde arttı. Tüm ekstre tedavi gruplarında kaspaz-3, kaspaz-6 ve kaspaz-9'un ekspresyonunda önemli bir azalma gözlendi. Ayrıca, diyabetik tedavi edilmemiş sıçanlarda B-hücre lenfoma 2 seviyesinde hafif bir artış olduğu gözlendi. Bu çalışma ile, *Lawsonia inermis* Linn.'nin, diabetes mellitus'un patofizyolojisi ve patogenezinde rol oynayan çeşitli sitokinler, interlökinler ve esansiyel kaspazlar üzerinde önemli bir olumlu düzenleyici etkiye sahip olduğu sonucuna varıldı.

Anahtar Kelimeler: *Lawsonia inermis* Linn, enflamasyon, sitokinler, interlökinler, diyabet

INTRODUCTION

Diabetes mellitus (DM) mainly affects how glucose is used, which results in side effects such as retinopathy, nephropathy, and brain micro-infarcts.[1] Reports have shown the contributory mechanism of tumor necrosis factor alpha (TNF-α) and other inflammatory mediators in the pathogenesis of insulin resistance seen with obesity and type II DM.[2] The inflammatory cytokines produced in β-cells contribute to the intrinsic pathway in apoptosis which can also enhance the release of FasL and TNF-α through the activation of the extrinsic pathway.[2] There are various biomarkers in the signaling machinery of apoptosis that enhance the development of DM.[3] When the apoptotic pathway is deficient, it gives rise to various abnormal conditions such as oncogenesis. On the other hand, excessive expression of apoptotic pathways leads to conditions such as DM and neuro-degenerative anomalies.[4] Many factors are implicated when the apoptotic pathway is triggered (physical, chemical, and biological), thereby activating complex systems that are mostly controlled by intracellular signal transduction.[4] In diabetic patients, hyperglycemia-induced β-cell apoptosis following stimulation of cytokines, leptin, glucose, and fatty acids, triggering a number of mechanisms that accelerate oxidative damage.[5]

Caspase families contributed to the development of DM as a result of their role in apoptosis.[6] There are about 14 types of caspases in vertebrates contributing to the activation of different pathways which may be intrinsic or extrinsic in nature.[7] The extrinsic pathway is more important and usually involves TNF-α superfamily receptors.[7] Reports have revealed that beta-cell apoptosis is vital to the pathogenesis of type I diabetes although this postulate did not show any standard practice in genetic approach to explain the mechanisms behind β-cell apoptosis using specific caspase knockouts in mice.[8]

Interleukin 1 (IL-1) is responsible for the inflammatory process and promotion of fever during sepsis.[9] It impairs insulin secretion and induces β-cell apoptosis in type II diabetes. Interleukin 6 (IL-6) has been linked to complex chronic inflammatory pathway and increased IL-6 level predispose to greater risk of type II diabetes.[9] Interleukin 21 (IL-21) appeared as an important pathway for chronic inflammation associated with atherosclerosis and autoimmune diabetes.[10] Reduction in the level of IL-12 will decrease the chance of diabetic complication as a result of atherosclerosis.[10] Increased level of IL-18 expression is seen in response to acute hyperglycemia in healthy patients with impaired glucose tolerance.[11] Increased expression of TNF-α has been associated with obesity-induced insulin resistance and the pathogenesis of type II diabetes.[12] By changing insulin signaling through serine phosphorylation, the increased expression causes insulin resistance in adipocytes and peripheral tissues, which results in the development of type II DM. Using anti-TNF-α therapy will lead to a reduction in the rate of insulin resistance and type II DM.[12]

Nuclear factor kappa beta (NF-kB) activation is a key event early in the pathobiology of diabetes and type II diabetic patients leading to pronounced higher expression of binding activity.[13] Beta-cell apoptosis is an important factor in the pathogenesis of DM, and caspase-3 is one of the major effector caspases involved in apoptotic pathways.[14] Beta-cell apoptosis leads to initiation of type I DM through antigen cross-presentation mechanisms.[15] Caspase 6 is directly linked to apoptotic pathways that leads to initiation of DM.[16] Caspase 9 is known as initiating caspase through activation of downstream executioner caspases and after initiation, it cleaves other caspases for initiating apoptosis in the target tissues.[17]

Because of these many elements implicated in the development of DM, this study was conducted to evaluate the modulatory effect of *Lawsonia inermis* Linn. extracts on the expression of various inflammatory cytokines, ILs, and apoptotic cell death biomarkers in streptozotocin (STZ)-induced diabetic rat model.

MATERIALS AND METHODS

Plant Collection, Identification, and Preparation

Lawsonia inermis Linn. leaves were collected from agricultural terrain in Kwara state, Nigeria's Ilorin East area council. At the University of Ibadan Herbarium, it was both recognized and verified, and a specimen was placed and given the voucher number UIH-22460. The leaves of *Lawsonia inermis* Linn. were dried at room temperature (25°C) under shade.

Extraction and Separation of *Lawsonia inermis* Linn.

Lawsonia inermis Linn. leaves weighing 5 kg were soaked for 72 hours in 4 liters of *n*-hexane, ethyl acetate, and methanol. Filter paper was used to gently decant to filter the mixture. A rotary evaporator was used to quickly evaporate the filtrate at a temperature of 40°C. The concentration (wet waste from various solvents) was dried and kept chilled at 4°C.

Fractionation of Crude Methanolic Extract of *Lawsonia inermis* Linn. Leaves

Lawsonia inermis Linn. crude methanol extract (200 g) was then extracted with *n*-hexane, ethyl acetate, and methanol in ascending polarity.

Phytochemical Screening

Following Trease and Evans' procedures, samples of *Lawsonia inermis* Linn. leaf crude extract was examined for phytochemical constituents.

Experimental Animal and Ethical Consideration

For this investigation, adult male Wistar rats were utilized, which were procured from the Experimental Animal House, Faculty of Veterinary Medicine, University of Ibadan. The regulatory organization in charge of animal use at the University of Ibadan, Animal Care and Use Research Ethics Committee (ACUREC), gave this work its ethical nod. Complete approval with the given number UI-ACUREC/18/0063 was issued by ACUREC. The animals were handled humanely and all stress-inducing factors, including handling, food, housing, and environmental conditions, were satisfactorily met.

Experimental Animals

Sixty-five male Wistar rats weighing between 130 and 180 g were used in this experiment. Rats used in experiments were housed in accordance with international standards and kept in perfect circumstances with the right humidity and temperature. Vital(R) feed, a common animal feed, was given to the experimental rats to eat. Water and food were freely available. Prior to the start of the research, the blood sugar levels of all the experimental rats were measured using a fine test glucometer (United Kingdom).

Diabetes Induction

Streptozocine (Sigma®) was used to create experimental diabetes. Streptozocine was given intraperitoneally at a dose of 65 mg/kg after being dissolved in distilled water. Streptozocine-induced rats with blood glucose above 200 mg/dL was considered diabetic, and these rats were used for the experiment

Animal Grouping

Experimental rats were divided into 13 groups of 5 rats each, and each group received treatment for 28 days as shown in Table 1.

Constitution and Administration of *Lawsonia inermis* Linn. Leaf Extract

By dissolving the 3 extracts in 2 mL of distilled water and 0.5 g of extract, the stock concentration of the 3 extracts was created. The rats in the test groups received these formulations orally for 4 weeks at various doses listed above. Distilled water was used to treat the control groups.

Evaluation of Inflammatory Cytokines

Using a commercial Elisa test kit and adhering to the manufacturer's recommended method and procedure, inflammatory cytokines were examined. The cytokines that were measured were TNF- α, IL-1, IL-6, IL-12, and IL-18.

Evaluation of Apoptosis Cell Death Markers

The commercial Elisa test kits were utilized to examine markers of cell death known as apoptosis. The analysis was conducted according to the manufacturer's specified standard method and procedure, using tissue homogenates. The markers that were assessed included caspase 3, caspase 9, B-cell lymphoma 2 (Bcl-2), and NF-Kβ.

Statistical Analysis

The data obtained were documented as the mean value accompanied by the SD (mean ± SD). Statistical analysis of the data involved using 1-way analysis of variance, followed by Dunnet's post hoc multiple comparison test. For all statistical analyses, GraphPad Prism (Boston, USA) software, version 5.03 (San Diego, Calif, USA) was utilized. Significance levels were determined with *P*-values of $P \leq .05$, $P \leq .01$, and $P \leq .001$ being considered as significant values.

Table 1. Experimental Rats are grouped into 13 (n=5) and each group are treated for 28 days as stated below

Control: Normoglycemic control treated with distilled water
Diabetic untreated: Hyperglycemic control treated with distilled water.
Diab+Li+Meth-25 mg: Diabetic and treated at a dosage 25 mg/kg methanol extract of *Lawsonia inermis* Linn. leave
Diab+Li+Meth-50 mg: Diabetic and treated at a dosage 50 mg/kg methanol extract of *Lawsonia inermis* Linn. leave
Diab+Li+Meth-100 mg: Diabetic and treated at a dosage 100 mg/kg methanol extract of *Lawsonia inermis* Linn. leave
Diab+Li+Nx-25 mg: Diabetic and treated at a dosage 25 mg/kg *n*-hexane extract of *Lawsonia inermis* Linn. leave
Diab+Li+Nx-50 mg: Diabetic and treated at a dosage 50 mg/kg *n*-hexane extract of *Lawsonia inermis* Linn. leave
Diab+Li+Nx-100 mg: Diabetic and treated at a dosage 100 mg/kg *n*-hexane extract of *Lawsonia inermis* Linn. leave
Diab+Li+EA-25 mg: Diabetic and treated at a dosage 25 mg/kg ethyl acetate extract of *Lawsonia inermis* Linn. leave
Diab+Li+EA-50 mg: Diabetic and treated at a dosage 50 mg/kg ethyl acetate extract of *Lawsonia inermis* Linn. leave
Diab+Li+EA-100 mg: Diabetic and treated at a dosage 100 mg/kg ethyl 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 glinbencamide.

RESULTS

Phytochemical Screening

The examination of *Lawsonia inermis* Linn. leaves for phytochemical content revealed the presence of diverse compounds including saponin, tannins, flavonoid, cardiac glycoside, terpenoids steroid, anthraquinones, and alkaloids (Table 2).

Interleukins

The untreated hyperglycemic control group exhibited a significant increase ($P < .001$) in IL-1 expression, whereas Meth 25 mg/kg, metformin, and glibenclamide demonstrated a significant decrease ($P < .001$) compared to the normoglycemic control group. The *n*-hexane and ethyl acetate fractions at different dosages did not show significant changes compared to the normoglycemic control group but exhibited an improvement compared to the hyperglycemic control group (Figure 1).

In untreated hyperglycemic control, EA-50 mg/kg, and the 2 common medications (metformin and glibenclamide), IL-6 considerably increased ($P < .001$). When compared to the normoglycemic control, nx-25 mg/kg and nx-50 mg/kg both increase significantly ($P < .05$). When compared to normoglycemic control, other treatment groups showed nonsignificant changes in the expression of IL-6. When compared to the hyperglycemic control,

Table 2. Phytochemical Screening (Qualitative) of Methanol Extract of *Lawsonia inermis* Linn. Leaves

Test	Crude Methanol Extract
Saponins	Abundantly present
Tannins	Abundantly present
Flavonoids	Abundantly present
Cardiac glycosides	Abundantly present
Terpenoids	Present
Steroids	Present
Anthraquinones	Present
Alkaloids	Present

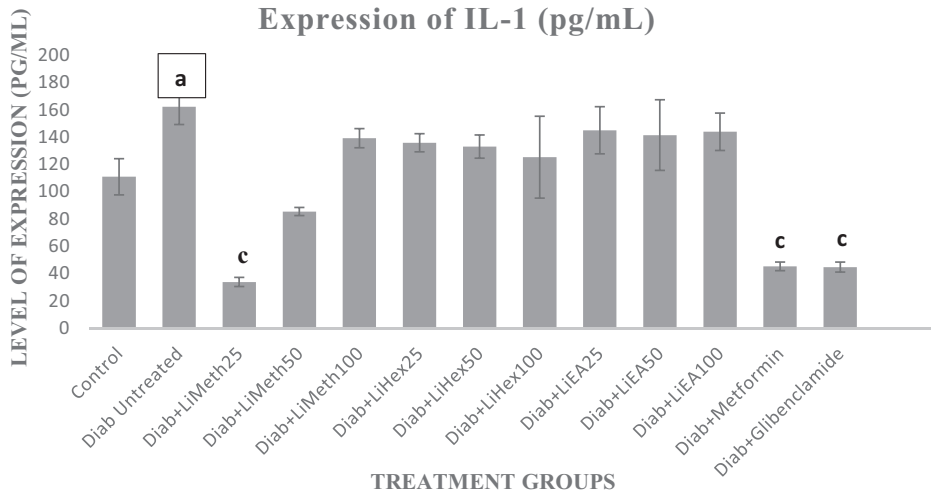

Figure 1. Expression of interleukin 1 (IL-1) of experimental rats induced with streptozotocin following 28 days of treatment with *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant [a]$P \leq .05$; [b]$P \leq .01$; and [c]$P \leq .001$. Note: Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

all extract-treated groups and metformin showed a substantial change in IL-6 expression (Figure 2).

Meth 100 mg/kg showed a significant ($P < .01$) reduction in IL-12 expression when compared with normoglycemic control. Metformin, glibenclamide, EA-50 mg/kg and EA-100 mg/kg did not show significant alteration in IL-12 expression when compared to normoglycemic control (Figure 3).

Normoglycemic control, metformin, and glibenclamide showed the highest level of IL-18 expression. Interleukin 18 decreased significantly in all the extract-treated groups when compared to normoglycemic control (Figure 4).

Tumor Necrotic Factors

In comparison to the other treatment groups and controls, Meth 25 mg/kg demonstrated a significant decrease in TNF-α expression. Conversely, the group treated with ethyl acetate exhibited a significant increase in TNF-α expression compared to the other treatment groups. The untreated hyperglycemic group displayed a significant ($P < .01$) elevation in TNF-α expression when compared to the normoglycemic control group. The 2 standard drugs (metformin and glibenclamide) showed minimal changes in TNF-α expression when compared to the groups treated with the extract (Figure 5).

Apoptosis Cell Death Markers

NF-kapa-B

In comparison to the normoglycemic control group, Meth 100 mg/kg exhibited a nonsignificant decrease in NF-κB expression. On the other hand, EA-50 mg/kg and glibenclamide demonstrated a significant ($P < .01$) increase in NF-κB expression compared to both the other treatment groups and the control group as seen in Figure 6.

Caspase 3

Methanol and ethyl acetate fraction showed a nonsignificant reduction in caspase 3 expression when compared to

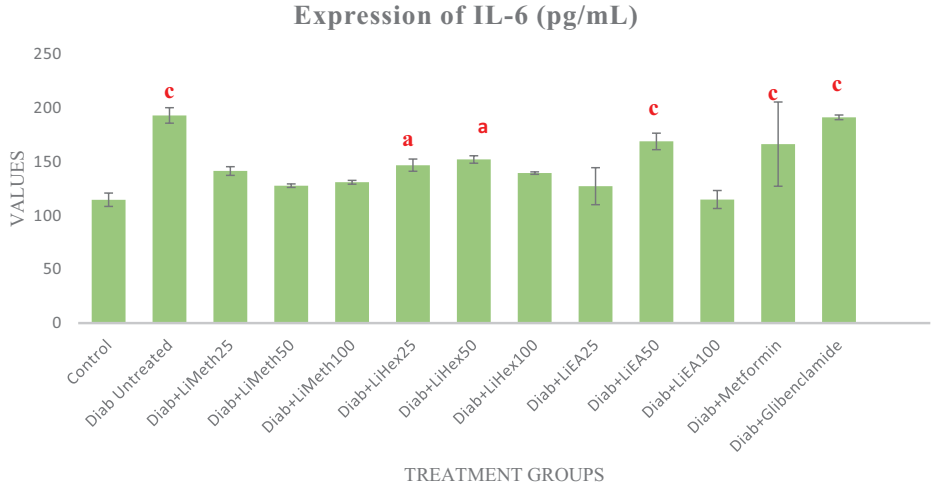

Figure 2. Expression of interleukin 6 (IL-6) of experimental rats induced with streptozotocin following 28 days of treatment with extract of *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant [a]$P \leq .05$; [b]$P \leq .01$; and [c]$P \leq .001$. Note: Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

Figure 3. Expression of interleukin 12 (IL-12) of experimental rats induced with streptozotocin following 28 days of treatment with extract of *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant $^{a}P \leq .05$; $^{b}P \leq .01$; and $^{c}P \leq .001$. Note: Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

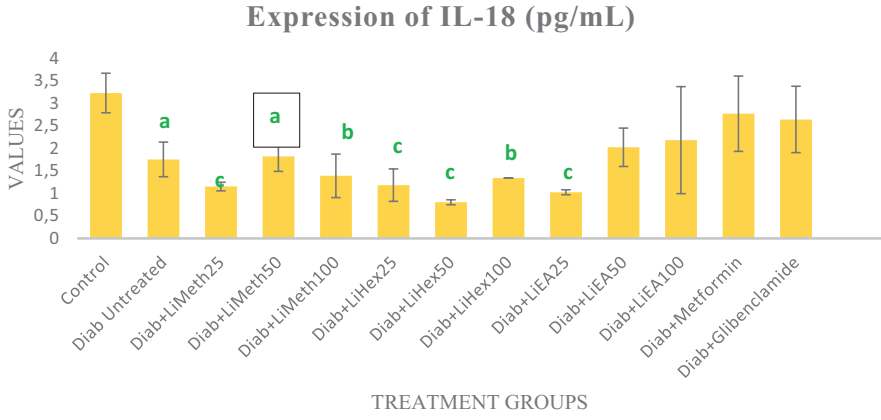

Figure 4. Expression of interleukin 18 (IL-18) of experimental rats induced with streptozotocin following 28 days of treatment of *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant $^{a}P \leq .05$, $^{b}P \leq .01$, and $^{c}P \leq .001$. Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

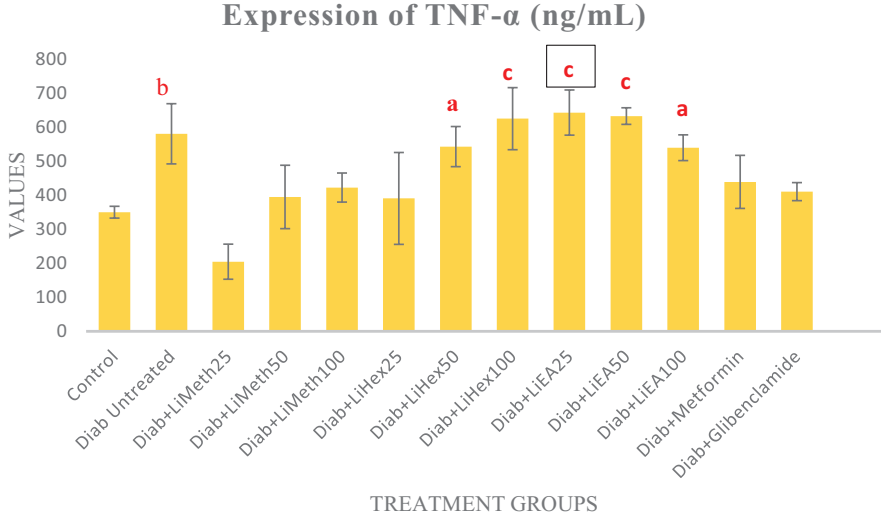

Figure 5. Expression of tumor necrosis factor alpha (TNF-α) of experimental rats induced with streptozotocin following 28 days of treatment with extract of *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant $^{a}P \leq .05$; $^{b}P \leq .01$; and $^{c}P \leq .001$. Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

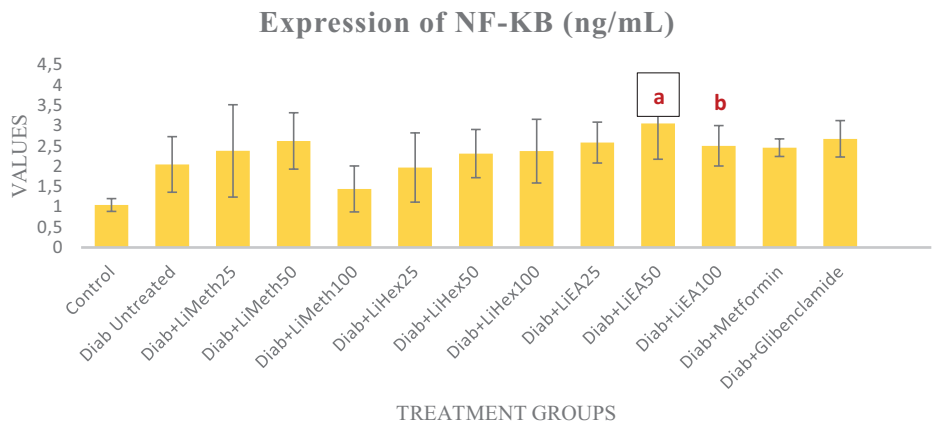

Figure 6. Expression of NF-Kβ of experimental rats induced with streptozotocin following 28 days of treatment with *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant [a]$P \leq .05$; [b]$P \leq .01$; and [c]$P \leq .001$. Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

normoglycemic control. Comparing the glibenclamide-treated group with the other treatment groups and the 2 controls revealed substantial ($P < .001$) caspase 3 expression (Figure 7).

Caspase 6

When compared to the normoglycemic control, the level of caspase 6 expression was nonsignificantly reduced by methanol and nx-25 mg/kg. When compared to other treatment groups and nondiabetic controls, nx-50 mg/kg, 100 mg/kg ethyl acetate fraction, and the 2 conventional medications increased significantly ($P < .01$). When compared to the diabetic untreated control group, all therapy groups demonstrated a significant decrease in caspase 6 expression (Figure 8).

Caspase 9

The group treated with glibenclamide displayed a significant ($P < .001$) increase in caspase 9 expression compared to the other treatment groups and the 2 control groups. Ethyl acetate fraction, nx-25 mg/kg, and nx-100 mg/kg exhibited a significant ($P < .01$) increase in caspase 9 expression compared to the normoglycemic control group. However, Meth 25 mg/kg and Meth 50 mg/kg did not show a significant change in caspase 9 levels when compared to the normoglycemic control group (refer Figure 9).

B-cell lymphoma 2

When compared to other treatment groups and the normoglycemic control, nx-25 mg/kg and 50 mg/kg did not significantly lower Bcl-2 expression. Comparing other treatment groups to the normoglycemic control group revealed a substantial ($P < .01$) increase in Bcl-2 expression (Figure 10).

DISCUSSION

Phytochemical screening of *Lawsonia inermis* Linn. leaf extract used in this study showed some important phytoconstituents like flavonoids, anthraquinones, alkaloids, saponins, tannins, and steroidal glycosides. These observed constituents are in agreement with Aremu et al[18] who confirms the phytochemical constituent of *Lawsonia inermis* Linn. leaves. *Lawsonia inermis* Linn. plants had high concentrations of cardiac glycosides, flavonoids, tannins, and saponins. Interestingly, saponins are known to improve nutrient absorption and smooth digestion in animals while having a bitter taste that often reduces palatability.[18] Due to their antioxidant properties associated with functional hydroxyl groups that scavenge free radicals and chelate metallic ions, flavonoids have been shown to have substantial positive effects on health.[18] Natural medications called cardiac glycosides primarily

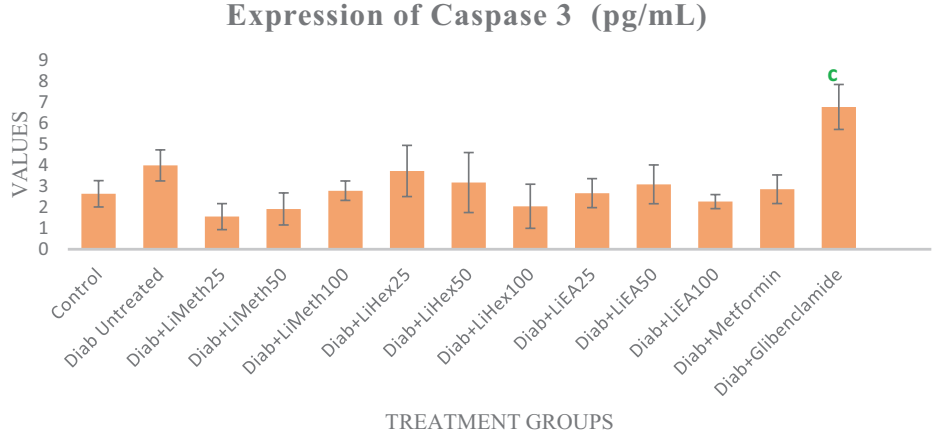

Figure 7. Expression of caspase-3 of experimental rats induced with streptozotocin following 28 days of treatment with *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant [a]$P \leq .05$; [b]$P \leq .01$; and [c]$P \leq .001$. Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

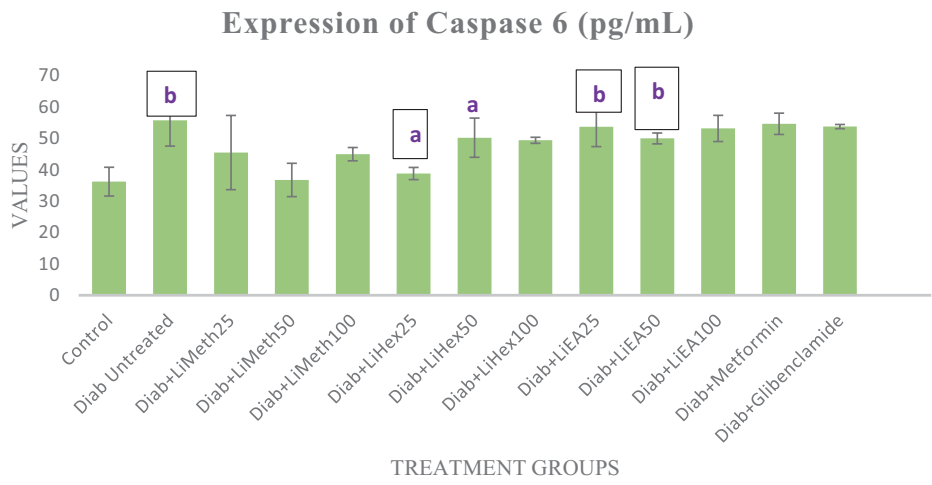

Figure 8. Expression of caspase 6 of experimental rats induced with streptozotocin following 28 days of treatment with *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant $^{a}P \leq .05$; $^{b}P \leq .01$; and $^{c}P \leq .001$. Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

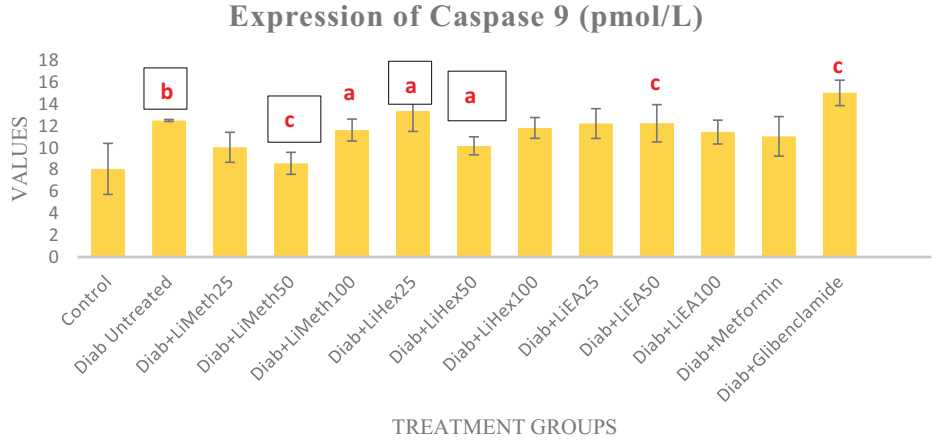

Figure 9. Expression of caspase 9 of experimental rats induced with streptozotocin following 28 days of treatment with *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant $^{a}P \leq .05$; $^{b}P \leq .01$; and $^{c}P \leq .001$. Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

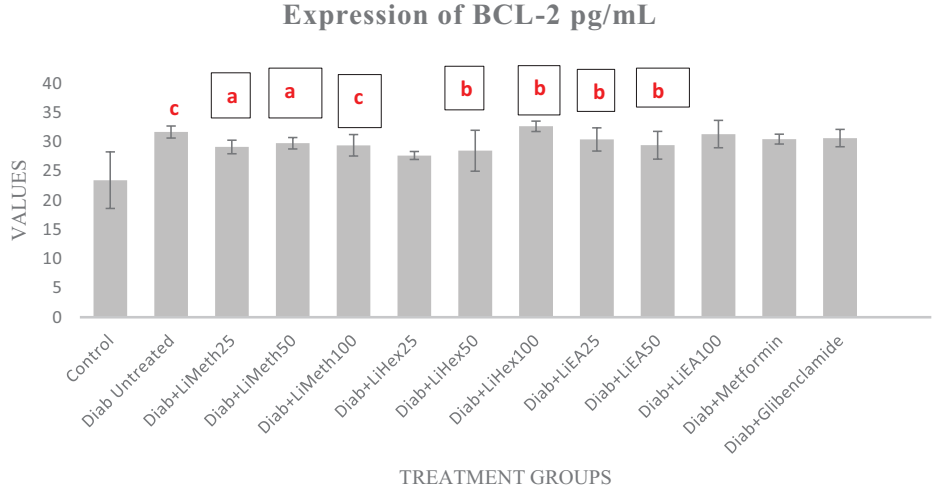

Figure 10. Expression of B-cell lymphoma 2 of experimental rats induced with streptozotocin following 28 days of treatment with *Lawsonia inermis* Linn. extract and oral hypoglycemic agents. Results are shown as mean ± SD: n = 5. Significant $^{a}P \leq .05$; $^{b}P \leq .01$; and $^{c}P \leq .001$. Diab, diabetic; Li, *Lawsonia inermis;* Meth, methanol; Hex, *n*-hexane; EA, ethyl acetate.

affect the heart in a cardiotonic (beneficial) and toxic (heart poisoning) way.[19]

Cytokines are immune-regulatory molecules that control pro-inflammatory response.[19] It has been shown that most of the inflammatory processes contribute to the pathogenesis of DM and progression to diabetes complications.[20] Reports have confirmed the contributory mechanism of TNF-α and other inflammatory mediators in the pathogenesis of insulin resistance seen with obesity and type II diabetes.[21] Result from this present study showed that untreated diabetic rats exhibited a marked increased inflammatory cytokine such as IL-1, IL-6, IL-12, and TNF-α. This result explained the assumption that inflammatory cytokines are strong predictors of DM.[22] This observation agrees with the report of Alexandraki et al [23] who stated that IL-1, IL-6, and TNF-α are main cytokines involved in the pathogenesis of diabetes. Previous report showed abnormal increase in the expression of IL-1, TNF-α, interferon-α, and interferon-7, IL-6, and IL-12 mRNAs in insulitis; infiltrate intracellular nucleotide binding and oligomerization domain in animal model.[24] The production of TNF-α by activated immune cells in animal models of insulin dependent DM is higher than nondiabetic and prediabetic mice.[25] The methanol fraction of *L. inermis* Linn. showed significant decreased IL-1, IL-6, IL-12, IL-18, and TNF-α when compared to other treatment groups and hyperglycemic control. Both metformin and glibenclamide showed marked decrease in IL-1. The basic explanation for this development is that interleukin-1 is involved in the development of abnormalities in intraglomerular hemodynamics which is mostly linked to prostaglandin synthesis from the mesangial cells and treatment of these cells with IL-1 leads to the synthesis and release of prostaglandin (E2), which will increase the activities of phospholipase A_2.[26] All the fractions showed a nonsignificant reduction to IL-6 when compared to the 2 standard drugs. Suzuki *et al*[27] reported that there exists a relationship between the severity of diabetic glomerulopathy and expression of IL-6 mRNA in glomerular cells, which showed that IL-6 affects the dynamics of extracellular matrix surrounding those cells.[27] Interleukin 18 showed a contradicting perception by presenting a significantly lower level in the expression in both diabetic untreated and other treatment groups when compared with nondiabetic control. Reports showed that IL-18 concentrations in the serum and urinary tract are correlated with albumin excretion rate, leading to albuminuria.[26,27] Tumor necrosis factor alpha have been reported to enhance Reactive Oxygen Species (ROS) generation that results in barrier alteration and function, thereby leading to oxidative stress that leads to diabetes complication.[28] The methanol fraction of the extract showed a significant reduction in TNF-α expression when compared to both the standard drugs and the controls.

Activation of NF-kB pathway due to release of cytokines and its mediator from adipose cells results in resistance of insulin and non-insulin-dependent form of diabetes.[29] Results from this study showed nonsignificant increase in the expression of NF-kB in both treated and untreated diabetic rats. Results observed a varying expression of NF-kB in all the groups but methanol fraction of *L. inermis* Linn. at 100 mg/kg reduced the expression significantly. This inference disclosed that reduced NF-kB expression in treated diabetic rats will prolong the life span of the cell.

Several members of the caspases have been implicated to be directly involved in the initiation and execution phases of physiological cell termination. Caspase 3 is the executioner and plays an important part in proteolytic process during apoptosis.[30] The detection of activated caspases is a reliable way to identify cells that will die even before the identification of other morphologic characteristics like DNA fragmentation.[31] Reports have shown that increased oxidative stress will lead to the induction of apoptosis that contributed to the development of diabetes complications as a result of induced vasculitis.[32] This present study showed a significant reduction in the expression of caspase 3, caspase 6, and caspase 9 in all the extract-treated groups. It was noted that methanol fraction reduced this expression significantly when compared to other groups. Glibenclamide has the highest expression of all the caspases even more than the diabetic control, and this observation could be attributed to the drug toxicity mostly seen with many synthetic drugs. Overexpression of the essential caspase family infers that the drug would promote aging of different cells in diabetic patients. Basic explanations for the expression of apoptosis death markers in diabetic patients have received little attention in recent times. The molecular mechanisms of diabetes-induced cell death are not well studied in animal experimental models. The result of this work follows the report of Ho et al[33] who noted that diabetic apoptosis is induced through initiation of caspase activation.[33]

One of the mechanisms through which apoptosis is induced by DM is through regulation of the Bcl-2 signaling molecule in the apoptosis pathway.[34] B-cell lymphoma 2 has a primary function of blocking apoptosis by inhibition of free radicals and ROS formation.[34] Previous reports[35] noted overexpression of Bcl-2 in the endothelial cells of diabetic ulcers. The results of this study showed a slight increase in the expression of Bcl-2 in untreated diabetes. Treated groups did not show significant variations. The inference in the positivity of Bcl-2 expression in most cells and connective tissue induced apoptosis development, and this is mostly considered as an important pathological induction during diabetic complication.[35]

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

Results obtained from this study displayed that fraction of *Lawsonia inermis* Linn. had a significant positive modulatory activity on the expression of several cytokines, interleukins, and essential caspases implicated in the pathophysiology and pathogenesis of DM. The methanol fraction had the best modulatory activities on the measured cytokines when compared to *n*-hexane and ethyl acetate fraction.

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