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Antidiabetic Investigations of Aqueous and Ethanol Extracts of *Terminalia macroptera* (Guill. & Perr.) Stem Bark in Streptozotocin-Induced Diabetic *Wistar* Rats

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ABSTRACT

Diabetes mellitus always-increasing prevalence has prompted investigations into plants traditionally extolled for antidiabetic properties. One of such is *Terminalia macroptera*, a member of the Combretaceae family that grows in the Savannah. Consequently, this study evaluated the antidiabetic potentials of the stem bark aqueous (AE) and ethanol extracts (EE). *Wistar* rats were allotted into 2 controls (nondiabetic and diabetic) and 2 treatment (diabetic) groups (n = 5). All diabetic groups were induced intraperitoneally with streptozotocin (55 mg/kg body weight) from which respective treatment groups were orally administered AE and EE (200 mg/kg/day), while controls received water for 70 days. Thereafter, the concentrations of glucose, lipids, insulin, C-peptide, testosterone, and α-amylase activities were measured in serum. Liver homogenates were evaluated for glycogen and antioxidant status, while mRNA expressions of PDX-1, insulin-1, TNF-α, and Il-1 β were examined in β-cell. AE and EE, respectively, caused 55.66% and 78.95% reductions in elevated glucose when compared to control. However, only EE-treated rats had glucose and glycogen levels normalized besides increased insulin concentration ($P < 0.05$ vs diabetic control, DC). Both extracts significantly decreased lipid profiles but caused increase in antioxidant levels ($P < 0.05$ vs DC). In contrast to AE, EE slightly increased C-peptide and testosterone concentrations but significantly enhanced α-amylase activity. Up-regulated insulin gene expressions remained unabated, but those of PDX-1 and inflammatory factors were downregulated by both extracts ($P < 0.05$ vs DC). *Terminalia macroptera* stem bark was revealed to possess antidiabetic properties that were better expressed by EE through insulin stimulation.

***Keywords*:** Glucose, C-peptide, insulin gene, pancreatic duodenal homeobox-1, tumor necrosis factor-α, interleukin-1β.

Introduction

Diabetes mellitus (DM) is an anomaly in sugar metabolism that is characterized by the destruction of blood tissues and peripheral organs following chronic high glucose concentration that are due largely to non-secretion of insulin by pancreas or the non-recognition of same by receptors.[1] The disease partly has its origin at the molecular level, and it is expressed physiologically in insulin changes as well as in sustained high glucose levels. These changes lead to alterations in serum lipid profiles (SLP) which result in serious complications and failure of various organs.[2] Oral synthetic hypoglycemic agents and insulin are usually used for the treatment of this metabolic derangement; however, their use over time has diminished due in part to their undesirable side effects such as the development of hypoglycemia, angiopathy, and induction of obesity.[3] The actions of these drugs have shown that they are useful for the management of the disease but not curative.[4] Consequently, scientists are in pursuit of more therapeutic agents that can permanently stop the plague.

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The search for a remedy has led to the investigation of *Terminalia macroptera*, a member of Combretaceae family which grows in the tropical savannah biome that is characterized by little rainfall and a long arid period.[5] The plant gives rise to a saucer-like fruit that is light green to purple tinge with its seed encased at the centre and bordered by a thin delicate wing.[6] The bark of the stem contains a high quantity of hydrolyzable tannins that are anti-*Bacillus subtilis*.[7] Also, extracts of the root bark and leaves have been shown to possess antiplasmodial and anti-bacterial activities.[8-10] *Terminalia macroptera* has been reportedly used in the treatment of rheumatism and diabetes.[11] It was revealed that useful bioactive molecules like flavonoids, tannins, saponins, alkaloids, and terpenoids are contained in the plant's stem bark which has also shown erythropoietic properties.[7,12,13] Previous toxicological studies of the stem bark extract in brine shrimp and *Wistar* rats showed a relatively nontoxic profile.[14,15] Despite the previous experiments and other claims by locals that the plant is utilized in the treatment of diabetes, it is, however, noted that the validity of this plant in lowering elevated blood sugar is yet to be scientifically certified. This study, therefore, investigated the antidiabetic properties of the aqueous (AE) and ethanol extracts (EE) of the plant's stem bark.

Materials and Methods

Chemicals and instruments

All the chemicals used were of research standard (98–99.8% purity) purchased from registered dealers and were utilized as delivered without further purification. Equipment used for analysis included enzyme-linked immunosorbent assay (ELISA) microplate reader (Spectramax 340 PC molecular device), BIO-RAD thermocycler (model: *i*Cycler 96 Well Reaction Module, S/N 583BR005717, USA), Jen-way UV-VIS spectrophotometer (model 6305, UK),

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microcentrifuge (Model: 3531, Abbott Laboratories, United States), and Accu-chek active glucometer (Model: GC, Roche, Germany).

Collection and extraction of plant material
The fresh matured stem bark of the plant was harvested in August, 2015 from open forests between the axes of Ogbomosho in Oyo and Ilorin in Kwara State. Botanical authentication of plant samples was done in the Plant Biotechnology Department, University of Benin, Benin City with a voucher number of UBH_T 0232. AE and EE were obtained by a previously reported conventional maceration method.[12] Weighed powdered bark samples (200 g) were extracted in distilled water (placed at the lower compartment of a refrigerator, 10ºC) and in absolute ethanol (under room temperature, 25ºC) to obtain AE and EE, respectively, in a ratio of 1:4 of sample to solvent (w/v).[12]

Experimental animals
A total of twenty (20), twelve weeks old male *Wistar* rats (250 - 300 g body weight) were obtained from Biochemistry department, Faculty of Life Sciences, University of Benin. The rats were kept in wooden framed cages with a mean floor area of about 1,644 cm^2, domiciled in the Department of Biochemistry, University of Benin, Benin City. They were exposed to natural 12-hour light and 12-hour night conditions. The environmental temperature was observed to be within the range of 24 – 27 ºC by use of a mercury-in-bulb thermometer. The rats were acclimatized for two weeks before the commencement of the experiment and were fed with pelletized rodent chow and tap water throughout the period.

Ethical approval on the use of experimental animals
The care and handling of animals were by following internationally accepted Ethical Guidelines for Laboratory Animals, National Institutes of Health (NIH), publication No. 86–23 and revised 1985. Ethical clearance (approval code: LS 20011) for the research protocol involving laboratory animals was received from the Faculty of Life Sciences Research Ethics Committee, University of Benin, Benin City.

Induction of diabetes
The overnight fasted rats (12 hours) were injected intraperitoneally with a single dose (55 mg/kg b. w.) of streptozotocin, STZ (Sigma-Aldrich, St. Louis, MO, USA). It was dissolved in cold physiological saline (0.98%) to produce a concentration of 5 x 10^4 µg/mL with a pH maintained at neutrality and then placed on ice and used within 10 minutes of preparation. Diabetic conditions were confirmed in the STZ-injected rats by measuring fasted blood sugar (BG) levels from the caudal vein on the 7^{th}-day post-injection using glucose test strips. The success rate for the induction per time recorded after 1 week was 86.11%. Rats that showed BG levels above 200 mg/dL (for three consecutive readings) were chosen for the experiment. All rats with BG concentrations below 200 mg/dl were re-induced. New rats were also induced to make up for mortality before the commencement of the study.

Experimental design
The rats were distributed into four groups of 5 rats each. Groups 1 and 2, respectively, contained normal and diabetic rats that were not treated but administered distilled water (4 ml/kg/day). Group 3 and 4 contained diabetically induced rats that were treated with 200 mg/kg b.w./day of AE and EE of *Terminalia macroptera* stem bark (TMSB), respectively. Choice of the administered dose was based on preliminary dose-response investigations. The animals were fasted by feed withdrawal for 12 hours overnight before the first day (being day 0, after diabetic conditions were established). Thereafter, appropriate doses were administered. Fasting BG was repeatedly monitored on the following days: 14, 28, 42, 56, and 70. Body weights were monitored concurrently with fasting BG levels throughout the experimental period. Total areas under the response curve (*t*AUC) were determined for the various doses. On the 70^{th} day, upon termination of the experiment, rats were immobilized with mild trichloromethane (70% chloroform-ethanol mixture). Blood was obtained by heart puncture using a 5 mL hypodermic syringe and placed in sterile bottles. The blood vials were positioned at room temperature for 15-30 minutes to allow clot formation, followed by centrifugation at 3000 x *g* for 10 minutes to obtain sera that were then stored at -20ºC for biochemical investigations. A weight of 0.5 g liver tissue/rat was excised, rinsed, homogenized, and boiled immediately in 10 ml double distilled water before centrifuging at 18,000 x *g* for 10 minutes. The resultant supernatant was decanted and stored at 4ºC for glycogen determination. For determination of antioxidant activities, 2 g of the liver tissue was extracted immediately and rinsed with cold saline. This was homogenized in 10 mL phosphate buffer saline (pH 7.4) and centrifuged for 15 minutes at 10,000 x *g*. Extracted pancreatic tissues were submerged in TRIzol at 4ºC for molecular analysis.

Biochemical analyses
Liver glycogen concentrations were determined based on direct enzyme hydrolysis method[16] following procedural steps stated in Abcam glycogen assay kit. Total cholesterol (T. CHOL) and triglyceride (TG) assays were done by the method described by Trinder.[17] High-density lipoprotein cholesterol (HDL-C) assay was carried out by the method described by Allain *et al.*[18] Low-density lipoprotein cholesterol (LDL-C) was determined following a modification of Friedewald's formula as reported by Chen *et al.*[19] Superoxide dismutase activity was measured by following the procedure as described by Marklund and Marklund.[20] Catalase (CAT) activity was estimated by the method described by Slaughter and O'brien[21], while Ellman's described method[22] was used to evaluate the reduced glutathione concentration. Insulin and c-peptide concentrations were quantified by the direct sandwich ELISA method described in Calbiotech insulin ELISA kit (catalog. No. IS130D). Testosterone assay was done by ELISA method developed from the protocol reported by Tietz[23]. Pancreatic amylase activity was determined following steps in Abnova pancreatic amylase ELISA diagnostics kit.

Genetic study
Total RNA was isolated from freshly excised pancreatic tissues and briefly homogenized in cold (4°C) TRIzol reagent (Zymo Research, USA, Cat: R2050-1-50, Lot: ZRC 186885). DNA contaminants were removed by treating with DNAse I (New England Biolabs, NEB, Cat: M0303S) following manufacturer's procedure. Total RNA (5 volumes) were mixed with (1 volume) chloroform and allowed to incubate for 15 minutes at room temperature. This was followed by centrifugation at 15,000 rpm/15 minutes using microcentrifuge. Upon centrifugation, RNA from the clear supernatant was precipitated using an equal volume of isopropanol for 10 minutes at room temperature. The above step was followed by centrifugation at 12,000 rpm for 8 minutes at 4°C. RNA pellets were rinsed twice with 70 ml ethanol in 30 ml of nuclease-free water. The pellets were dried under air current for 5 minutes and dissolved in RNA buffer (1 mM sodium citrate, pH 6.4). Prior to cDNA conversion, the concentration of total RNA (µg/ml) was calculated by 40 * A_{260}, and the quality was assessed using the absorbance ratio of A_{260}/A_{230}, where A represented the absorbance value. Quality ≥ 1.8 was accepted for further analysis. RNA was preserved at -80ºC until reverse-transcription was performed. Complemenatry DNA was synthesized using Moloney murine leukemia virus **(**M-MuLV) reverse transcriptase kit (NEB, Cat: M0253S). A volume of 2 µL solution containing 100 ng DNA-free RNA was converted to cDNA at room temperature using the reverse transcriptase in 20 µL final volume of Master Mix (2 µL, N^9 random primer mix; 2 µL, 10X M-MuLV buffer; 1 µL, M-MuLV RT, 200 U/µL; 2 µl, 10 mM dNTP; 0.2 µL, RNase inhibitor, 40 U/µL and 10.8 µL nuclease-free water). The reverse transcriptase degraded the original RNA template after the first strand of cDNA was synthesized. Its inactivation was done at 65°C/10 minutes and followed by incubation on ice for 5 minutes.
Amplification by PCR was achieved with OneTaq® 2X Master Mix (NEB). The amplification of the genes was done using primers designed with SnapGene software (PDX-1 [Genbank accession no.:NM_022852.3]-forward-primer-5'-CCTTTCCCGAATGGAACCGA-3'/reverse-primer-5'-AGGCTGTACGGGTCCTCTTA-3'; INS-1 [Genbank accession no.: NM_019129.3]-forward-primer-5'-CCAAGTCCCGTCGTGAAGT-3'/reverse-primer-5'-CTCCAGTTGGTAGAGGGAGC-3'; Il-1β-[Genbank accession no.: NM_031512.2] forward-primer-5'-GACTTCACCATGGAACCCGT-3'/reverse-primer-5'-GACTTCACCAT

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GGAACCCGT-3′; TNF-[Genbank accession no.: XM _008772775.2] forward-primer-5′-GGAGGGAGAACAGCAACTC C-3′/reverse-primer-5′-TCTGCCAGTTCCACATCTCG-3′; reference β-actin-[Genbank accession no.:NM_031144.3]forward-primer-5′-CTGGCTCCTAGCACCATGAA-3′/reverse-primer-5′-CGCAGCTCAGTAACAGTCCG-3′).

One-tenth of the first-strand cDNA was cycled in a thermocycler under the following 3-step PCR protocol: initial denaturation at 95°C for 60 seconds to activate the enzyme, 35 cycles of PCR (denaturation at 94°C for 30 seconds, annealing of primers at 55°C for 30 seconds and elongation at 72°C for 30 seconds) with a final extension for 5 minute at 72°C. In all experiments, negative controls were included where the reaction mixture had no cDNA. The amplicons were resolved in 1.5% agarose gel in Tris-borate-EDTA buffer (pH 8.4). Each sample was probed for target genes and *β*-actin gene in separate wells. Beta-actin mRNA was used as reference gene. Images of amplicon bands in gel were captured immediately after staining with ethidium bromide on camera and processed on the Keynote platform. The photographed gel images were quantified densitometrically with the aid of Image-J software. Target genes were normalized with *β*-actin gene before comparisons were made among samples. Relative gene expressions were determined by the formula: (test gene band intensity/ internal control band intensity)*100.

Statistical analysis

Data were expressed as mean ± SEM (n = 5). Results were statistically analyzed by one-way ANOVA followed by Tukey post hoc test for multiple comparisons using SPSS (Statistical Package for Social Sciences Inc., USA, version 23). Significance was set at $p < 0.05$. Microsoft Excel software (version 2013) was used to construct bars and line charts.

Results and Discussion

The antidiabetic claims of *Terminalia macroptera* were verified by administering the AE and EE of the plant's stem bark. The glucose concentration curves of the test rats are shown in figure 1A. They portray a tidal depression on the 28th day of the experiment which was replicated on the 70th day. AE caused a significant reduction ($p < 0.009$) in BG from the elevated level (55.66% reduction on the 70th day). It was, however, significantly higher than that of the normal rats ($p < 0.001$). Moreso, in figure 1B, the AUC of AE-treated group was not significantly reduced when compared with that of untreated diabetic group ($p = 0.365$). EE, on the other hand, caused a reduction in blood sugar from the elevated state to near normoglycemic level (78.95% blood glucose decrease on the 70th day; $p < 0.001$). No significant difference was found between the BG level of the EE-treated group and control rats ($p = 0.778$). Although EE-treated rats had AUC that was significantly lower than that of the untreated diabetic group; it, however, remained significantly higher than that of normal control rats (figure 1B).

At the end of the experiment, the normal control rats showed the usual increase in mean body weight, while AE-treated group had reductions that were similar to DC, as their weights remained well below the baseline. The inability of the AE of the stem bark to normalize the BG of the diabetic rats was reflected by the computed AUC, which was further buttressed by their reduced body weights, an indication of wastage at the tissues (figure 1C). EE-treated rats, on their own, exhibited gain in average body weight that rose to 13.27%. Although weight change remained well below that gained by normal control rats (figure 1C); it, however, indicated that EE moderated the weight alterations and a possible induction of obesity from continuous use could be ruled out.

Glycogen assay results in figure 1D reflected a high accumulation of the hepatic polysaccharide in diabetic rats that were treated with AE of TMSB; a change that was significantly higher in concentration than those of normal and DC groups ($p < 0.001$). It would be needful to state that the observed high glucose level that characterized the overnight fasted diabetic rats confirmed a high rate of gluconeogenesis in the liver.[24] As glucose continuously deposits in the blood stream, its concentration is raised until it becomes toxic. The elevation at its extreme is believed to make the liver adjust its metabolic route, forcing glucose secretion from liver to stop. It is suggested that the adjustment arises from the inhibition of glucose-6-phosphatase by excess α-ketoglutarate.[25] It is also of interest to note that the utilization of glucose-6-phosphate in the pentose phosphate pathway as an alternative route is not favored in the diabetic state because glucotoxicity inhibits glucose-6-phosphate dehydrogenase.[26] Normally, high concentration of cAMP in diabetic condition prompts the liver to produce glucose for starving peripheral cells, such that gluconeogenesis continues to yield glucose-6-phosphate. This is converted to UDP-glucose, and then to glycogen, as the only available route to evacuate accumulated phosphorylated glucose.[27] Based on the above mechanism, glycogen formation in the diabetic state remained unhindered and enhanced. This proposed mechanism explained the observed significant increase in basal hepatic glycogen of the untreated diabetic rats and its sustenance in the less effective AE treated group.

EE-treated rats showed no significant change in glycogen levels when compared with normal control. Obviously, their mean value was lower than those of the DC group. These observations indicated restoration of glucostasis and enzyme activities in the endoplasmic reticulum (ER). In effect, the extract reversed the excess glycogen accumulation associated with diabetic rats.

Elevated SLP is one of the indices of DM.[28] The lipid values of control rats were compared with those of AE and EE-treated in figure 2A. The data showed that both extracts-treated groups had lower lipid concentrations ($p < 0.05$) than those of untreated diabetic rats in their T. CHOL, TG, and LDL-C, respectively. However, the HDL-C concentrations of AE treated group were significantly lower than those of the normal control ($p = 0.001$). As expected, the DC rats showed significantly elevated SLP over control rats for T. CHOL, TG, and LDL-C, except for HDL-C whose values were significantly below normal concentration. Increased TG levels as observed in the control diabetic rats were in support of the postulate that increased oxidation of LDL-C induces high TG concentration in diabetes condition.[29] The decreased T. CHOL and TG concentrations observed with the SLP meant that the AE and EE possessed antihypercholesterolemic and antitriglyceridemic properties.

Figures 2B, 2C, and 2D, respectively, show the liver SOD, catalase, and reduced glutathione (GSH) levels in control, AE, and EE-treated rats after 70 days. The SOD activities in the DC group were significantly reduced when compared to normal control rats ($p < 0.05$). Conversely, both AE and EE restored the enzyme activities from the depressed diabetic state, but only AE-treated group showed significant restoration to near normal activity ($p < 0.001$). Also, both extracts redeemed catalase activities from the depressed diabetic states when compared with DC ($p < 0.05$). However, only AE-treated group had its activity brought within normal range. Similarly, both extracts caused an increase in the concentration of liver GSH well above that of the DC group ($p < 0.001$). The result revealed the GSH level of the treated rats compared well with those of normoglycemic rats ($p > 0.05$). By design, reactive oxygen species (ROS) play a notable role in the destruction of pathogens; however, in diabetic condition, their over-production becomes deleterious. In this study, the protection gained by the enzymes was scored on the supportive role provided by the extracts in mopping up the free radicals generated in the diabetic state. Our findings corroborated published studies that had shown similar properties of plant extracts in protecting antioxidant molecules and enzymes.[30]

Figure 3A shows the serum insulin levels in control, AE, and EE-treated rats after 70 days. The insulin concentrations were reduced in the untreated and AE-treated diabetic rats. The EE, in contrast, caused significant elevations in the pancreatic hormone when compared with those of the diabetic untreated group ($p = 0.001$); however, the values were below the insulin levels in control rats ($p < 0.001$).

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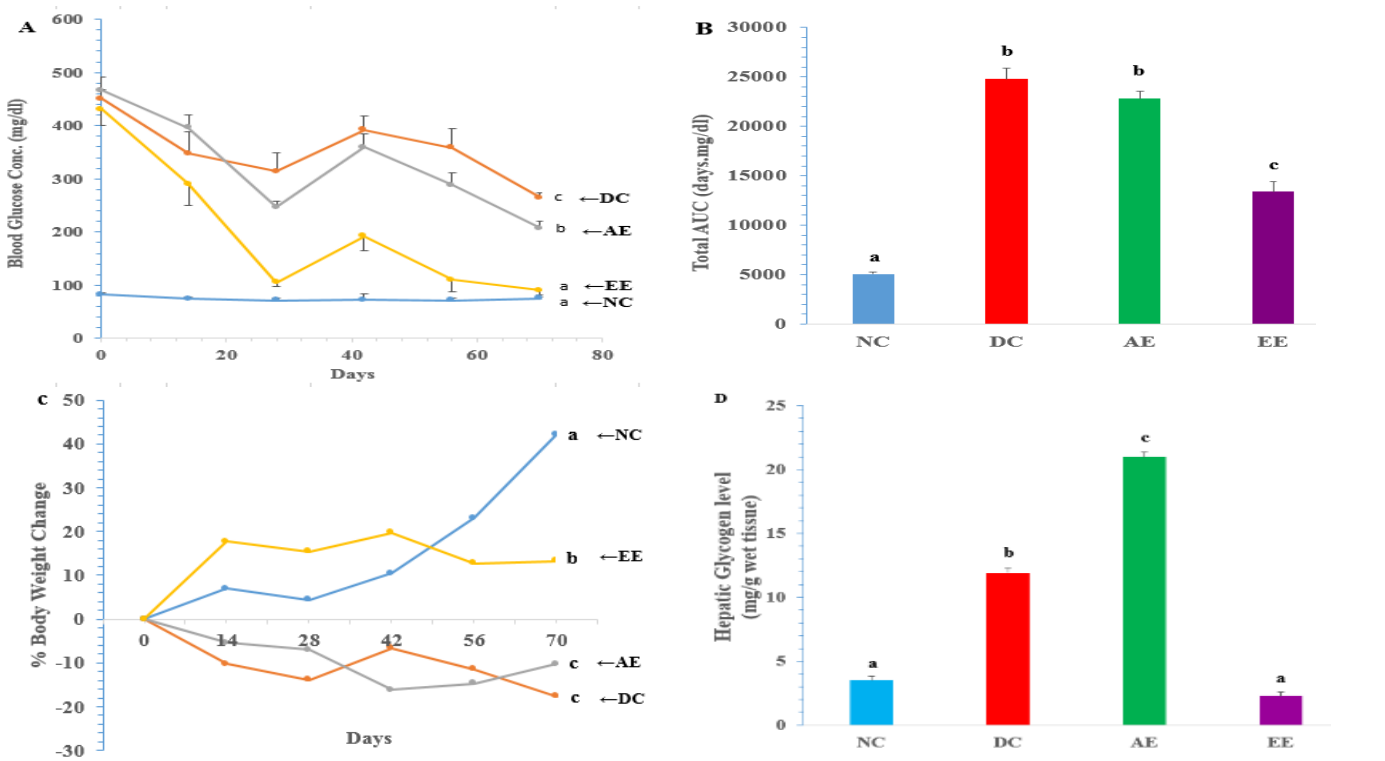

Figure 1: A. Glycemic profile, **B**. Total area under glucose curve (AUC_{gluc}), **C**. Body weight changes, and **D**. Glycogen levels in control and treatment rat groups administered water and *Terminalia macroptera* stem bark (TMSB) extracts, respectively. NC = normal control; DC = diabetic control; AE = aqueous extract-treated diabetic rats (200 mg/kg TMSB), and EE = ethanol extract-treated diabetic rats (200 mg/kg TMSB). Coordinates on curves are mean values of y-axis parameters ± SEM vs days of extract administration while height of bars represents mean values of y-axis parameters ± SEM ($n = 5$). Mean values with different lower case alphabets (ab) differ significantly ($p < 0.05$).

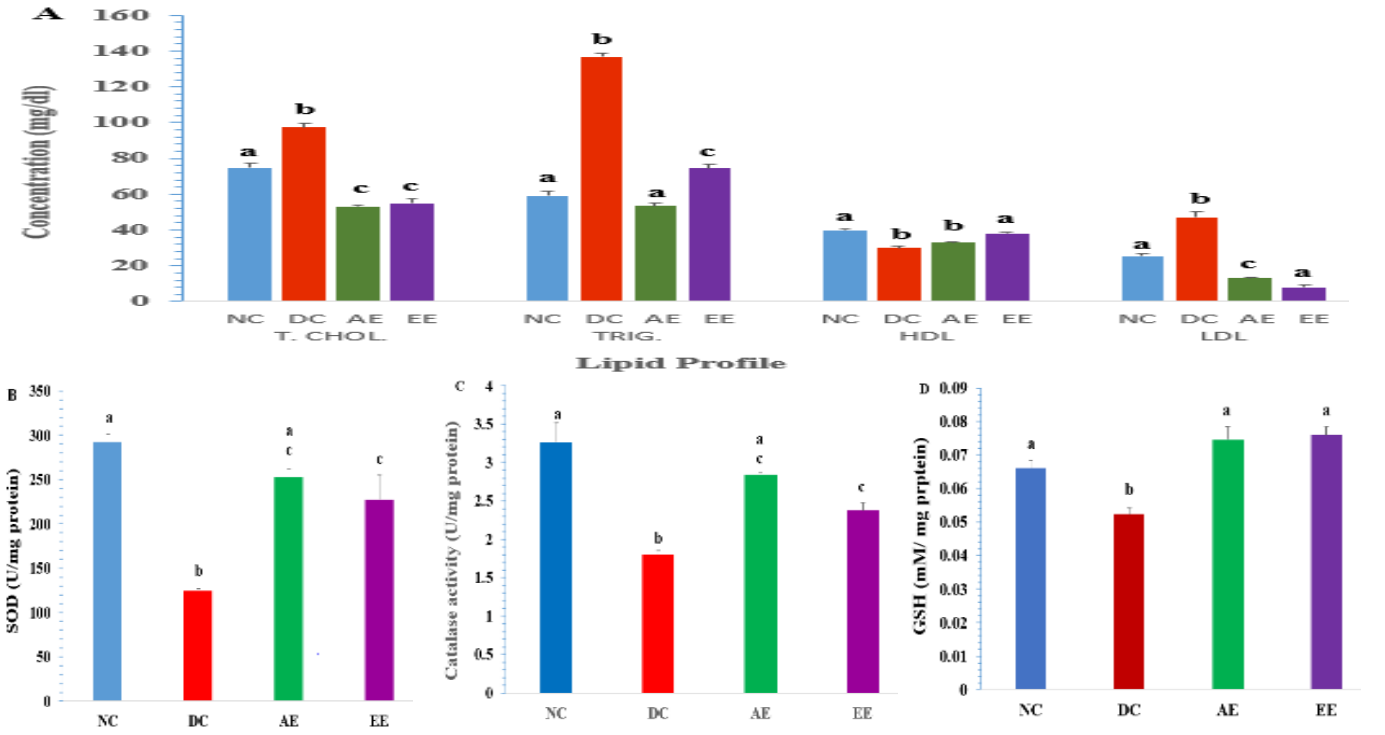

Figure 2: A. Serum lipid levels, **B**. Superoxide dismutase (SOD) activities, **C.** Catalase activities, and **D**. Reduced glutathione (GSH) levels in control and treatment rat groups administered water and *Terminalia macroptera* stem bark (TMSB) extracts, respectively. T. CHOL = total cholesterol; TRIG. = triglyceride; HDL = high density lipoprotein cholesterol; LDL = low density lipoprotein cholesterol; NC = normal control; DC = diabetic control; AE = aqueous extract-treated diabetic rats (200 mg/kg TMSB), and EE = ethanol extract-treated diabetic rats (200 mg/kg TMSB). Height of each bar represents mean value ± SEM. Statistical differences among groups ($n = 5$) were significant at $p < 0.05$, and indicated by different lower case alphabets.

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β-cells destruction in the diabetic rats was responsible for their reduced insulin values.[31] In previous work, following insulin treatment of diabetic rodents, neogenic cells assumed functionality and survived.[32] However, the high glucose concentration that prevailed in the diabetic rats without exogenous insulin treatment must have eliminated any chance of neogenic cell continuous survival. That possibility left behind only matured *β*-cells that survived the STZ destruction upon extract treatment.[32]

Interestingly, the significant rise in insulin concentration observed with the EE-treated group indicated restoration of synthetic function in the mature survivor cells. However, a similar attempt made by the AE-treated group was insignificant. C-peptide concentrations were all reduced ($p < 0.001$) in the extract-treated groups when compared to normal rats (figure 3B). The result shows its decrease in the AE-treated rats below those of DC, whereas those of the EE-treated group were increased but not significant (AE, $p = 0.720$; ethanol extract, $p = 0.439$). The peptide has been published to suppress the release of chemokines in hyperglycemic condition.[33] However, the destruction of *β*-cells by STZ, which was confirmed in the significant reduction of c-peptide concentration, must have given room for the overt up-regulated expressions of proinflammatory factor genes in the diabetic rats. The low concentrations of insulin and c-peptide of AE-treated diabetic rats indicated that their pancreatic *β*-cells were not restored before the experiment ended. Although the notable increase in insulin concentrations of EE-treated group was not supported by the insignificant rise in c-peptide, it, all the same, registered an early sign of *β*-cell restoration.

Decreased concentrations of serum testosterone were shown in the DC rats which remained below those of normal control (figure 3C); an observation that was in line with previous publications.[34] These also corresponded with the decreased levels recorded for HDL-C.[35] The sex hormone concentrations were significantly below those of control rats in both extract-treated groups ($p < 0.001$). However, the slight increase shown above DC group indicated a possible restoration that could occur if the extracts (particularly EE) were to be administered beyond the experimental duration. A boost in the pancreatic amylase activity was caused by the administration of EE extract of TMSB at the end of the experiment (figure 3D). This was significantly higher than the activities of the enzyme in normal ($p = 0.001$) and DC groups ($p < 0.001$). AE-treated group did not show any significant change in the enzyme activity when compared to the DC group ($p = 1.00$). In order to control excess sugar, amylase activities are usually low in diabetic subjects, but these become increased after insulin administration.[36] In this study, increased activity of amylase in the EE-treated rats indicated induced secretion of insulin by the extract.

The expressions of the PDX-1, insulin, IL-1*β*, and TNF-*α* genes of the diabetic rats were all significantly up-regulated when compared to those of the control group, but they became downregulated in extract-treated groups, except for insulin genes that had expressions that were close to the status of diabetic control ($p < 0.05$) (figures 4A, 4B, 4C, and 4D). Translation of genetic information to proteins is strongly dependent on a host of associated factors that make up the cytoplasmic trans-molecular factory. A possible vacuolation of pancreatic *β*-cells by STZ most likely led to the accumulation of insulin mRNA as seen in this study. The factor most likely implicated in this occurrence was the inositol-requiring transmembrane kinase/endoribonuclease 1α (IRE1α).[37] When IRE1α is inhibited, insulin mRNA is prevented from being degraded and in the presence of prolonged high glucose concentration, its expression becomes up-regulated and retained.[38, 39] Obviously, in this study, significantly high insulin mRNA expressions must have resulted from the genetic machinery of survived *β* cells that were fired up by the prevailing high glucose level via enhancement of the PDX-1 transcription factor. The observation was in agreement with the *in vitro* cell experiment conducted by Welsh and his/her team of researchers.[40]

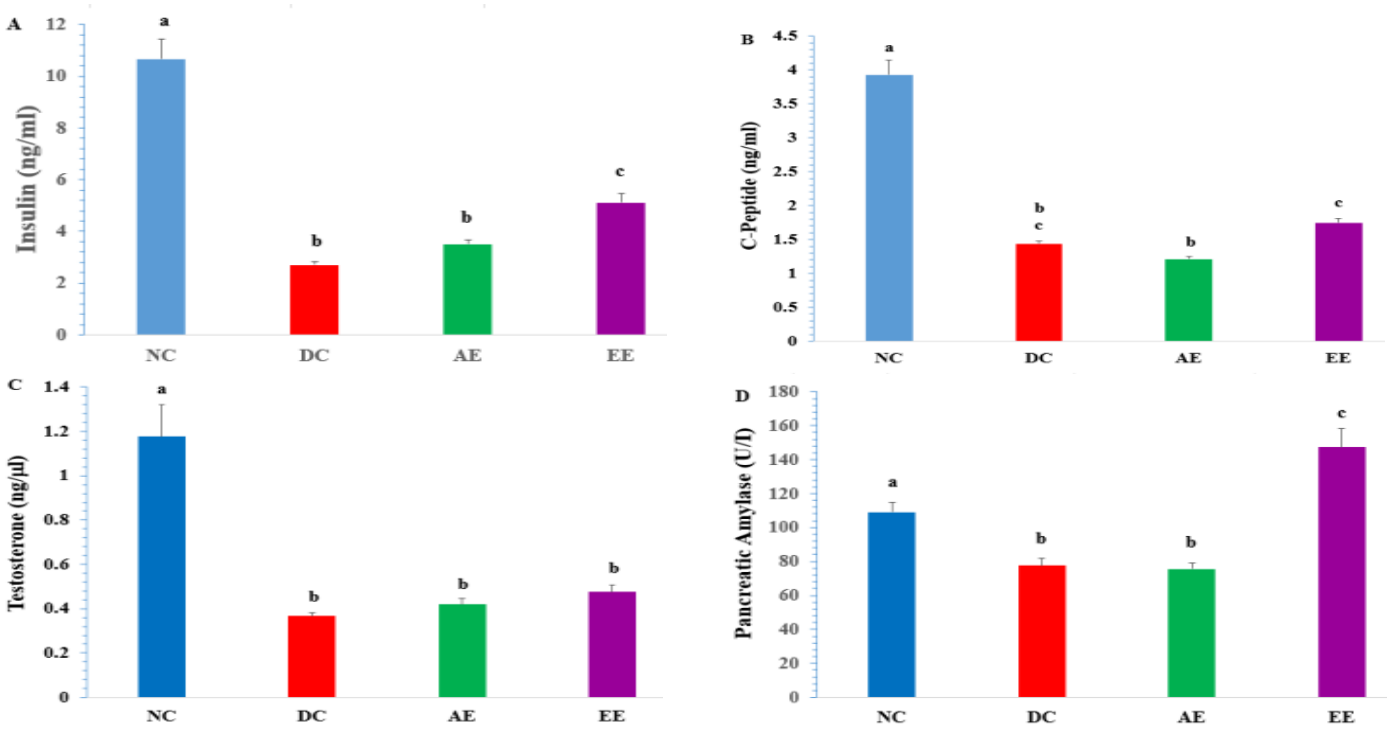

Figure 3: A. Insulin levels, **B.** C-peptide levels, **C**. Testosterone levels, and **D**. Pancreatic amylase activities in control and treatment rat groups administered water and *Terminalia macroptera* stem bark (TMSB) extracts, respectively. NC = normal control; DC = diabetic control; AE = aqueous extract-treated diabetic rats (200 mg/kg TMSB), and EE = ethanol extract-treated diabetic rats (200 mg/kg TMSB). Height of each bar represents mean ± SEM for *n =5* determinations. Statistical differences among groups were made significant at $p < 0.05$, and were indicated by different lower case alphabets.

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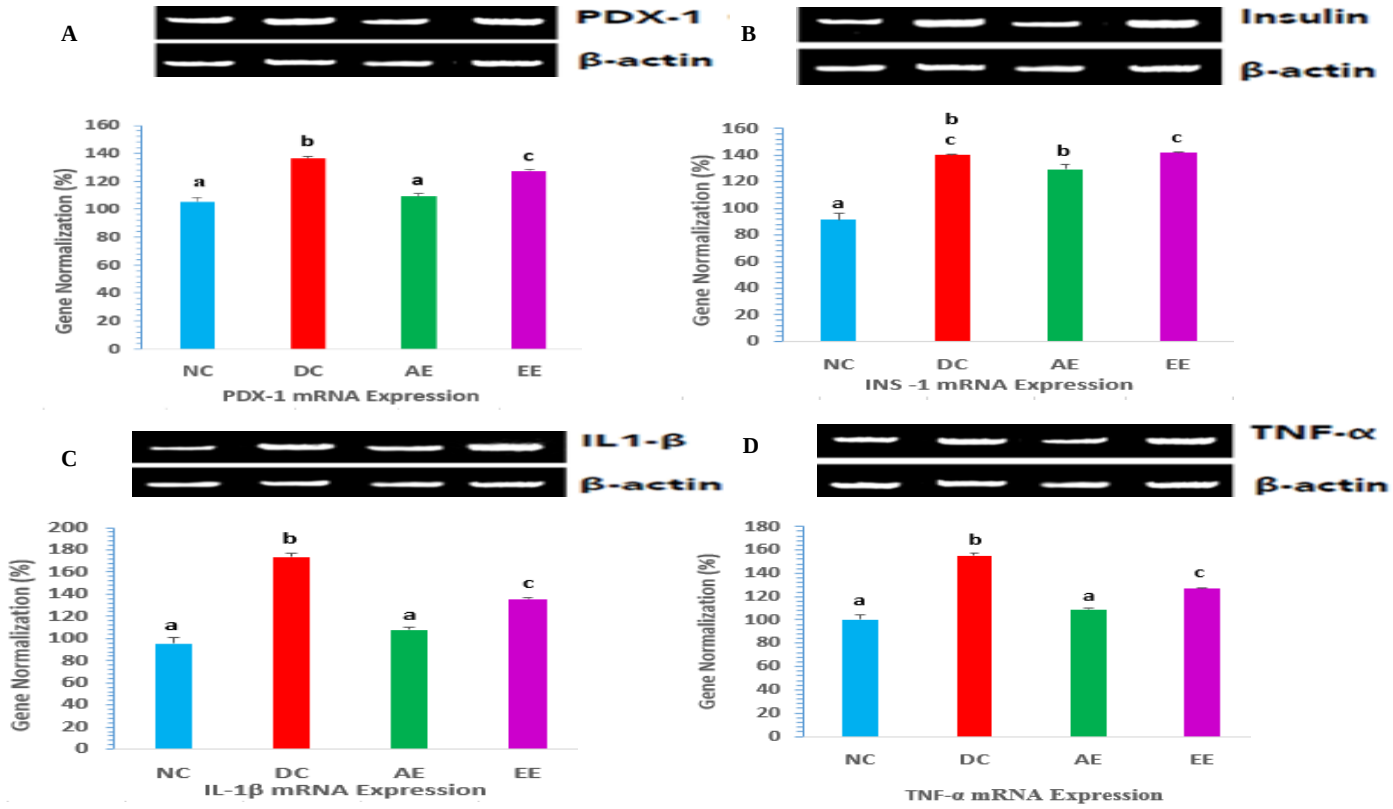

Figure 4: A. Homeodomain transcription factor-1 (PDX-1), **B.** Insulin-1 (INS-1), **C.** Interleukin-1 beta (IL-1β), and **D.** Tumor necrosis factor-1 alpha (TNF-1α) gene expressions in control and treatment rat groups administered water and *Terminalia macroptera* stem bark (TMSB) extracts, respectively. Bars represent expressions normalized with β-actin gene while bands captured in agarose gel are shown above each chart. NC = normal control; DC = diabetic control; AE = aqueous extract-treated diabetic rats (200 mg/kg TMSB) and EE = ethanol extract-treated diabetic rats (200 mg/kg TMSB). Significant differences between NC and other groups were indicated by different small case alphabets at $p < 0.05$ (n = 3).

It was also believed that during vacuolation of the β-cells, polypyrimidine tract binding proteins 1 were partly destroyed by firstly, STZ-induced ER chronic stress, and thereafter, by reactive oxidants generated from glucotoxicity. Consequently, these were not available to bind to the untranslated regions of the mRNA to initiate translation. In support, some researchers have reported notable increase in β-cell gene expressions, while metabolic enzymes related to insulin secretion were depleted under excessive production of glucose.[41]

The above partly gave an explanation for the PDX-1 and insulin mRNAs expressions that were increased in the diabetic group, whereas insulin protein and c-peptide remained decreased in concentrations. It is pertinent to note, therefore, that up-regulated nuclear factor genes may not necessarily cause restoration of β-cell functions if the cytoplasmic proteins needed for translation are not regenerated. PDX-1 gene expressions that were normalized in the AE-treated group did not relate to the decreased insulin molecules. Speculatively, any compound in AE with a strong ligand property could have bonded to the PDX gene-enhancer molecules and prevented their shuttling into the nucleus, leading to the downregulation of PDX-1 gene transcription. On the other hand, the gene expression as downregulated in the EE-treated rats may be suggestive of a feedback signal that insulin secretion has been initiated. The up-regulated expression of insulin mRNA coupled with the significantly increased production of insulin molecules altogether depicted the gradual recovery of the β-cell functionality. It is opined that these high expressions will down-regulate to control levels when the insulin secretory rate normalizes upon EE continuous administration beyond the experimental time.

Furthermore, very high blood glucose concentrations found in diabetic state have been noted to induce proinflammatory cytokines (TNF-α and IL-1β) production in β-cells, which are significant factors responsible for tissue damage.[42] The expressions of the proinflammatory factor genes were downregulated by AE and EE. A good proportion of the inflammatory response might have been attenuated by the suppression of ROS. Again, the case of AE-treated rats revealed that antioxidative power may not necessarily restore insulin secretory property where necrosis is involved. Consequent to the findings, it could be stated that the restorative attributes of EE were independently confined to the intrinsic components of the extract in restoring damaged β-cells.

It has also been reported that as long as IL-1β is overexpressed, PDX-1 nucleocytoplasmic shuttling would continue, and its gene expression would remain upregulated.[43] Since the extracts caused the down regulation of the proinflammatory genes, they consequently reversed the shuttling of PDX-1 and encouraged its binding to the promoter region of the insulin gene for onward transcription. In effect, the reversal most likely reduced the production rate of the insulin transcription factor. This partly explained the downregulation observed with the PDX-1 gene expression in the treated rats. Although AE failed to translate the changes to insulin production for reasons yet unknown.

Conclusion

The study has revealed that EE of TMSB possesses antidiabetic properties better than its AE counterpart. These properties were likely based on the reduction of glucose by insulin secreted from β-cell that regained functionality.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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