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ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF LEAVES OF *NEWBOULDIA LAEVIS* IN DIABETIC RATS

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ABSTRACT

This study was designed to evaluate anti-inflammatory and antioxidant potentials of ethanolic extract of leaves of *Newbouldia laevis* in diabetic rats. Diabetes was induced in rats by intravenous injection of freshly prepared solution of streptozotocin (60 mg/kg body weight). Diabetic rats were then treated with extract of the leaves of *N. laevis* (500 mg/kg body weight) for 28 days after which serum levels of tumor necrosis factor alpha (TNF-α) and interleukin -1 beta (IL-1β) were estimated using ELISA kit while serum concentration of nitric oxide (NO) was determined by Griess assay. The activities of catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were also estimated. Free radical scavenging activity of the extract was measured by decrease in the absorbance of methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The extract significantly reduced ($P < 0.05$) serum levels of nitric oxide, IL-1β as well as TNF-α in diabetic rats. The activities of CAT, GSH, GPx and SOD were significantly increased ($P < 0.05$) in treated diabetic rats compared to diabetic control. The extract also possesses free radical scavenging activity against DPPH with IC_{50} of 7.2 µg/ml. The study showed that ethanolic extract of *N. laevis* leaves possesses anti-inflammatory and antioxidant properties in streptozotocin-induced diabetic rats.

Keywords: *Newbouldia laevis*, inflammation, antioxidant, diabetes, cytokines.

INTRODUCTION

Under normal physiologic condition, inflammation is a protective response elicited by tissue injury. It is the mechanism through which the body destroys or neutralizes invading harmful agents and also restores homeostasis after stress (Lumeng and Saltiel, 2011). During inflammatory process, cytokines such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and Interleukin 1β (IL-1β) are released when monocytes and macrophages are activated. However, activation of macrophages must be tightly regulated in order to avoid unrestrained inflammatory process through inappropriate release of cytokines (Sakic *et al*., 2011). When there is distortion in the normal regulatory control of the inflammatory process, the innate and acute phase responses are sustained and disease progression ensues. The connection between inflammation and free radical generation has also been established (Yeh *et al*., 2005). Overproduction of radicals such as superoxide anions, hydroxyl radical, hydrogen peroxide and nitric oxide (NO) results in oxidative stress and chronic inflammation. The reaction products of these radicals trigger lipid peroxidation, oxidation of enzymes and proteins and modifications of nucleic acids (Barrera, 2012). This is the fundamental mechanism underlying many pathological conditions.

Reports from several clinical and experimental studies have linked increased oxidative stress and low grade chronic inflammation with the development of insulin-dependent diabetes as well as noninsulin-dependent diabetes (Ferreira *et al*., 2010; Parveen *et al*., 2012). In diabetes, chronic hyperglycemia leads to increased generation of superoxide by the mitochondrial electron transport chain (Tushuizen *et al*., 2005). An imbalance in the generation and scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) results in oxidative stress. This eventually leads to altered function of intracellular proteins, DNA damage, and activation of NF- kB which triggers abnormal changes in gene expression as well as increased generation of pro-inflammatory cytokines and inducible nitric oxide (Mohora *et al*., 2007). As ROS and RNS accumulate, more and more beta cells of the pancreas get destroyed and this contributes to the progression and complications of diabetes. Therefore, agents with antioxidant and anti-inflammatory activities are expected to be effective in the treatment of diabetes and its complications.

In spite of the health benefits of the current antidiabetic drugs, each drug has its own range of side effects which may compromise the disease status or even worsen the condition in some cases. Some of the side effects of antidiabetic drugs which may offset their benefits include weight gain, hyperinsulinemia, hypoglycemia, edema and

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volume expansion (Modi, 2007). Thus, the prevalence of the disease continues to rise worldwide and there is little that could be done to prevent its complications. Therefore, there is the need to search for better antidiabetic remedies with good antioxidant and anti-inflammatory properties.

Medicinal plants have contributed greatly to the development of modern drugs, including those used in the management of diabetes mellitus. It is estimated that more than 400 plant species are used as anti-diabetic remedy, but only a limited number of them have been studied and validated for their antidiabetic properties using laboratory diabetic animal models and in clinical studies using human subjects (Sharma *et al*., 2011). *Galega officinalis*, *Momordica charantia*, *Gymnema sylvestre*, and *Opuntia streptacantha* are among plants that have been reported to be effective in the management of diabetes by virtue of their anti-inflammatory and antioxidant properties (Bnouham *et al*., 2006; Gupta and Sharma, 2006).

Newbouldia laevis (P. Beauv) is one of the plants employed in the management of diabetes in Nigeria, which have not been subjected to proper scientific investigations. Its common names are 'African border tree' and 'fertility tree'. The leaves of the plant are soaked in ethanol and the filtrate is taken orally to treat diabetes. The extract of the leaves has been reported to lower blood glucose level in diabetic rats (Owolabi *et al*., 2011). The anti-inflammatory, analgesic and antipyretic properties of the stem bark and flowers of the plant have been studied (Olajide *et al*., 1997). In this study, the leaf extract of *N. laevis* was evaluated for antioxidant and anti-inflammatory activities in diabetic rats.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Newbouldia laevis* were collected from the premises of College of Health Sciences, Ladoke Akintola University of Technology, Mercyland, Osogbo Campus, Nigeria. The plant sample was identified and authenticated by a taxonomist in Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen was deposited in the herbarium of the institute (voucher specimen no: FHI 107753).

Preparation of plant extract

The leaves were thoroughly washed with distilled water to remove soil and other debris that may contaminate the plant sample. The washed sample was then air-dried under shade in the laboratory for 5 days and the dry plant sample was pulverized using an electric grinding machine. The resultant powder sample weighing 500g was then extracted with 80% ethanol at 70^0C by continuous hot percolation using a Soxhlet apparatus. The extraction was carried out for 24h and the resulting ethanolic extract was concentrated at 40^oC in a rotary evaporator. The solid sample obtained weighed 47.5g (yield = 9.5%). The crude ethanolic extract (NLet) was kept in air-tight container and stored in a refrigerator at 4^0C until the time of use.

Experimental animals

Male Wistar rats weighing 180-200g were obtained from the Animal Holding Unit of the Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology (LAUTECH), Nigeria. The animals were housed in polypropylene cages inside a well-ventilated room. The animals were maintained under standard laboratory conditions of temperature (22 ± 2° C), relative humidity (55-65%) and 12 hour light/dark cycle. They were allowed to acclimatize for 2 weeks before the experiment. During the experimental period, animals were fed with a standard balanced commercial pellet diet (Ladokun Feeds Ltd. Ibadan, Nigeria) and potable tap water *ad libitum*.

Ethical consideration

All experimental procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Institute of Health, 1985) as well as Ethical Guidelines for the Use of Laboratory Animals in LAUTECH, Nigeria.

Induction of Diabetes mellitus

Experimental diabetes was induced in rats, which had fasted for 12hr by a single intravenous injection through the tail vein of a freshly prepared solution of streptozotocin (STZ) (60mg/kg b.wt) dissolved in 0.1M cold citrate buffer, pH 4.5 (Chen *et al*., 2005). The rats were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia. Estimation of fasting blood glucose (FBG) was done 72hours after injection of STZ to confirm induction of diabetes and then on the 7th day to investigate the stability of diabetic condition. Fasting blood glucose was estimated by One Touch® glucometer (Lifescan, Inc. 1995 Milpas, California, USA). Blood sample for the FBG determination was obtained from the tail vein of the rats and those with blood glucose value ≥ 200 mg/dl were selected for the study.

Biochemical assays

After treating diabetic rats with NLet (500mg/kg body weight) for 28 days, serum levels of TNF- α and IL-1β were estimated using Rat TNF-α and Rat IL-1β ELISA kits (RayBiotech Inc, USA). Measurement of NO was carried out as described by Zahedi *et al*. (2008) using Griess Reagent System procured from Sigma-Aldrich (St Louis, MO, USA). The activities of enzymatic antioxidants in rat liver were estimated. Catalase (CAT) activity was assayed as described by Sinha (1972). The activity of glutathione peroxidase (GPx) was determined as described by Rotruck *et al*. (1973). The level of

reduced glutathione (GSH) was estimated using the method of Jollow *et al*. (1974). Superoxide dismutase (SOD) activity was assayed by the method of Kakkar *et al*. (1984). Glibenclamide (5mg/kg body weight) was used as reference drug. Free radical scavenging activity of the extract was measured by decrease in the absorbance of methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Hantano *et al*., 1989) and ascorbic acid was used as reference drugs.

Statistical analysis

Data obtained from the experiments are expressed as mean ± standard error of mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) followed by Student's t- test. A level of $P < 0.05$ was taken as significant. GraphPad Prism version 5.0 for windows was used for these statistical analyses (GraphPad software, San Diego California, USA).

RESULTS AND DISCUSSION

The results indicate that *N. leavis* extract has free radical scavenging activity against DPPH. The concentration of NLet that caused 50% inhibition (IC_{50}) was 7.2µg/ml while that of the standard drug, ascorbic acid was 4.4µg/ml. The result is presented in figure 1.

The extract stimulated the activity of enzymatic antioxidants in diabetic rats. In the rat liver, the cellular levels of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) decreased significantly ($P < 0.05$) in the diabetic control group relative to the non-diabetic control. Compared with the diabetic control group, there was a significant increase ($P < 0.05$) in the levels of the antioxidants in the groups treated with glibenclamide and NLet. NLet increased the level of GSH (53.1%), CAT (21.5%), GPx (20.2%) and SOD (33%) relative to diabetic control. There was no significant difference ($P > 0.05$) between NLet-treated group and the glibenclamide-treated group. The results are presented in figure 2.

As shown in figure 3, serum level of NO was significantly increased ($P < 0.05$) in diabetic control group compared to non-diabetic control group. NLet reduced serum level of NO in diabetic rats and the reduction was significantly different ($P < 0.05$) compared to diabetic control. Likewise, serum level of tumor necrosis factor alpha (TNF-α) and interleukin -1β (IL-1β) increased significantly in diabetic control when compared with non-diabetic control. This was reversed by NLet and the serum levels of both TNF-α (Fig. 4) and IL-1β (Fig. 5) in the NLet-treated group was significantly different from those of the diabetic controls.

Diabetes mellitus is usually accompanied by increased levels of free radicals and decreased concentration or activity of antioxidants. Excess production of free radicals triggers the process of oxidative stress that can seriously alter the cell membranes and other structures such as proteins, lipids and deoxyribonucleic acid (DNA). Oxidative stress sets in when cells cannot adequately mop up the excess free radicals formed. In other words, oxidative stress results when there is an imbalance between the formation and neutralization of free radicals (Genestra, 2007). Numerous studies have shown that enzymatic antioxidant defense system is compromised in diabetes (Martin *et al*., 2003; Atef and Ezz, 2012).

Superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione play significant role in protecting against tissue damage by free radicals. The functions of these antioxidants are interconnected and a decrease in their concentration leads to the accumulation of lipid peroxides and increased oxidative stress (Kaleem *et al*., 2006). In diabetes, hyperglycemia promotes glucose oxidation and this further leads to free radical accumulation. This is followed by protein glycation and oxidative degeneration. In this situation, the concentration and activity of the enzymatic antioxidants are significantly reduced. In uncontrolled or poorly controlled diabetes, advanced glycation endproducts (AGEs) are irreversibly formed. These are involved in the pathogenesis of many of the irreversible complications of diabetes, including hypertrophy, hyperplasia, expanded extracellular matrix and vascular complications (Martin *et al*., 2003).

In the present study, the ethanolic extract of the leaves of *Newbouldia laevis* was demonstrated to have free radical scavenging activity against DPPH with IC_{50} of 7.2µg/ml. The results also showed that the extract stimulated the activity of SOD, CAT, GPx and GSH in the liver of diabetic rats and there was no significant difference ($P > 0.05$) between the group treated with NLet and the glibenclamide-treated group. This indicates that NLet contains antioxidant principles that can protect the enzymatic antioxidant system against damage by excessive oxidative stress.

Inflammatory cytokines are important mediators of β-cell destruction in animal models of diabetes as well as in human islets. A combination of interleukin-1(IL-1), γ-interferon (INF-γ) and tumor necrosis factor (TNF) stimulates inducible nitric oxide synthase (iNOS) expression in the islet and this leads to increased production of nitric oxide (NO) that causes the destruction of the islet cells (Thomas *et al*., 2002).

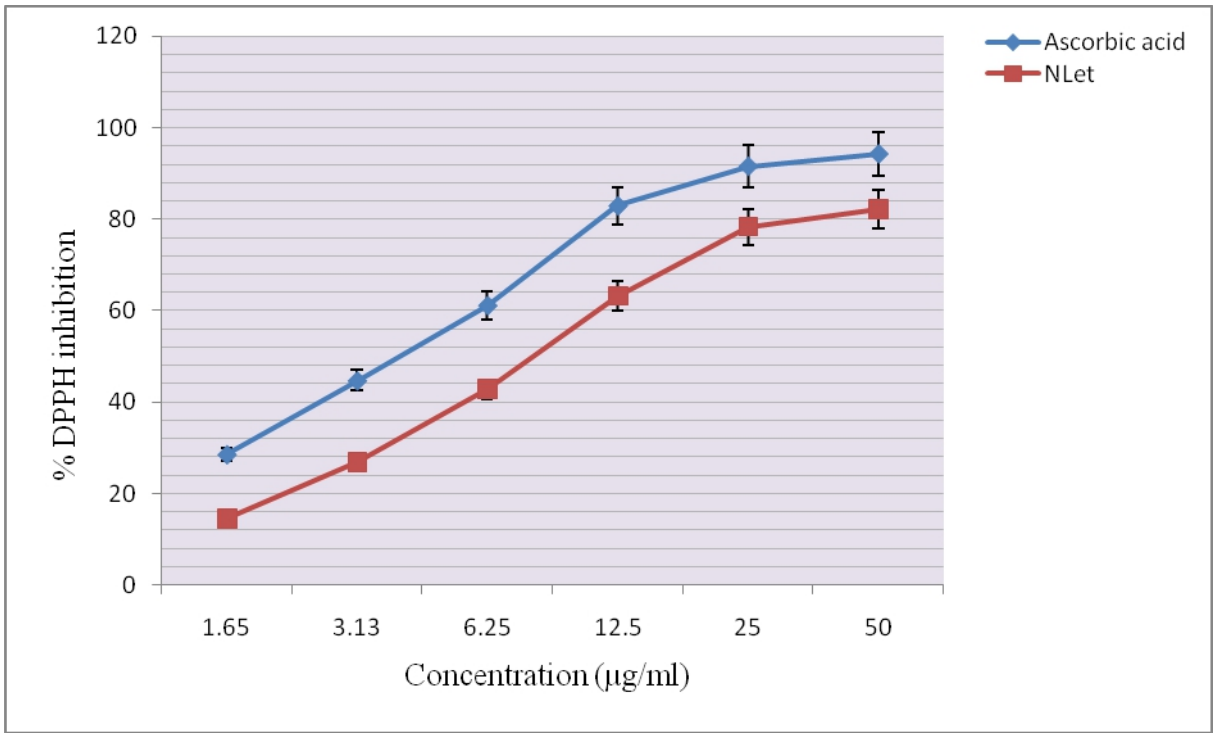

Fig. 1. DPPH free radical scavenging activity of *N. laevis* extract.
Values are means ± SEM of three replicates.

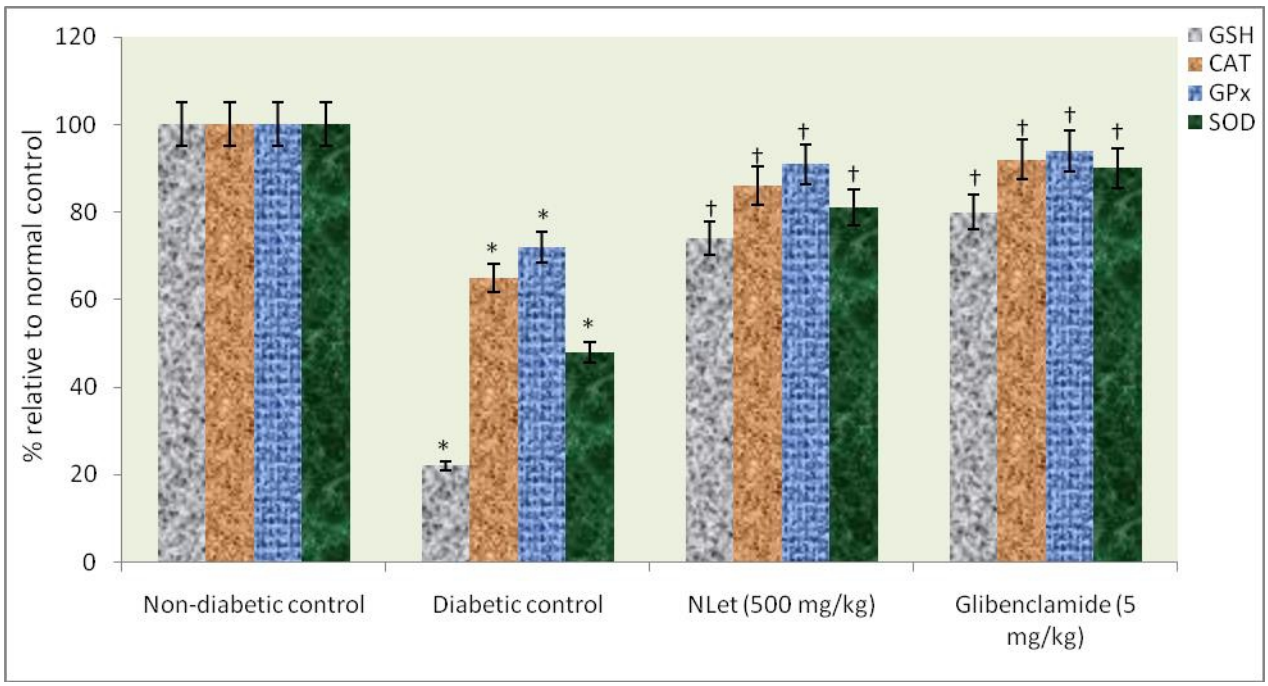

Fig. 2. Effect of *N. laevis* leaf extract on the activity of enzymatic antioxidants in the liver of diabetic rats. Values represent mean ± SEM (n = 6). $^{*}P < 0.05$ compared with normal control; $^{\dagger}P < 0.05$ compared with diabetic control.

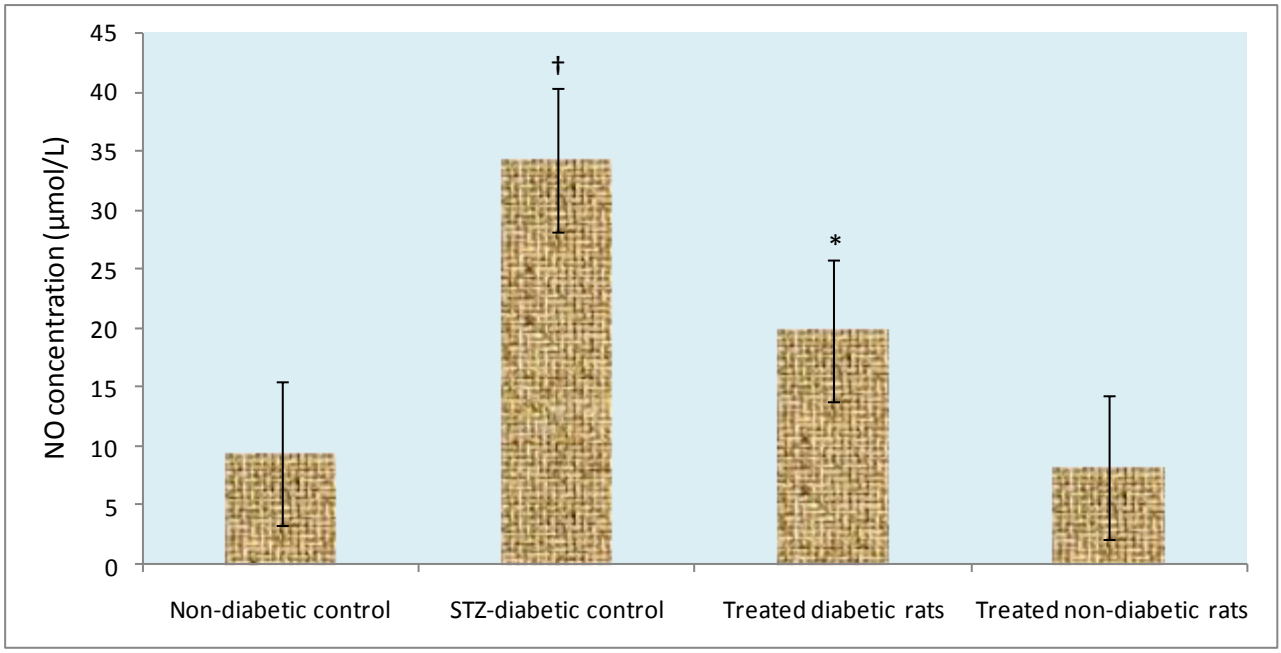

Fig. 3. Effects of *N. laevis* leaf extract on serum level of nitric oxide in diabetic rats. †P < 0.05 compared with the non-diabetic control; *P < 0.05 compared with diabetic control. Values represent mean ± SEM (n = 6).

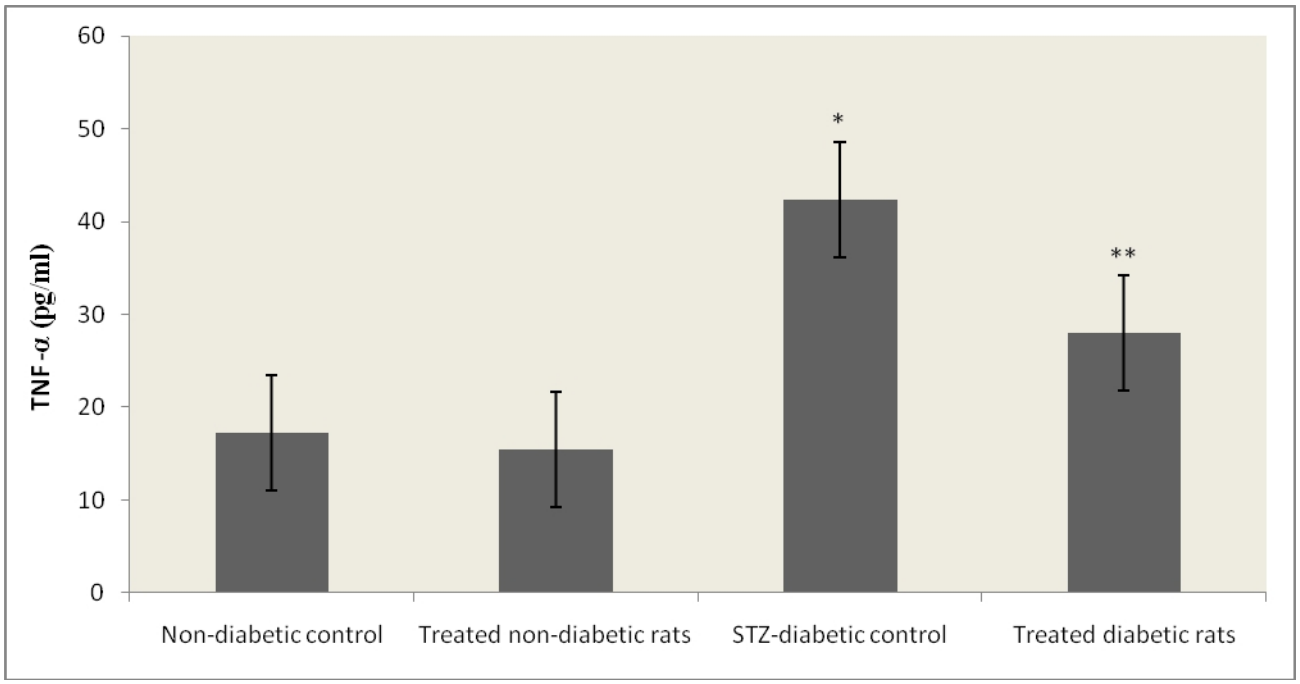

Fig. 4. Effect of *N. laevis* extract on the serum level of TNF -α in diabetic and non-diabetic rats. Values represent mean ± SEM (n = 3). *P < 0.05 compared with non-diabetic control; **P < 0.05 compared with STZ-diabetic group.

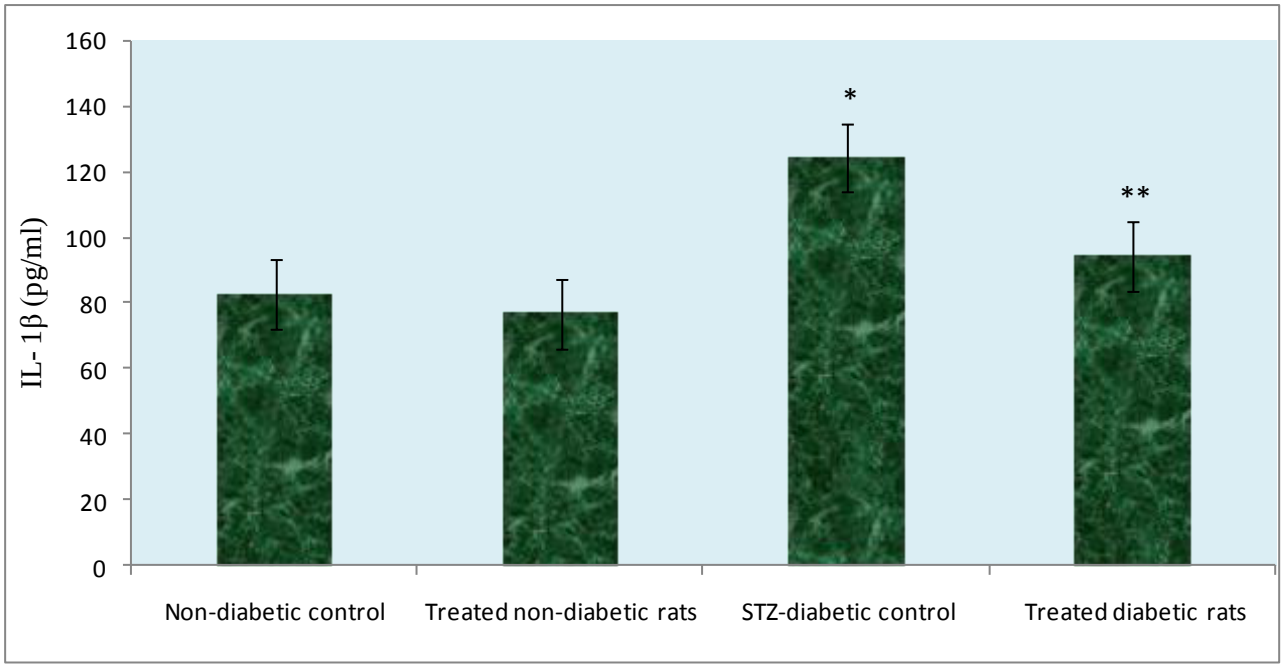

Fig. 5. Effect of *N. laevis* extract on the serum level of IL- 1β in diabetic and non-diabetic rats.
Values represent mean ± SEM (n = 3). *P < 0.05 compared with non-diabetic control; **P < 0.05 compared with STZ-diabetic control.

A number of studies have also shown that NO produced by macrophage and /or endothelial cells can mediate β-cell damage independent of local cytokine release. Thus, macrophage-generated NO, cytokine-induced NO and other radicals contribute to β-cell destruction. In this study, serum levels of NO, interleukin -1β (IL-1β), and tumor necrosis factor –alpha (TNF-α) were significantly increased in diabetic control rats. This is an indication of excess production of NO and cytokines in the diabetic rats. Treatment with NLet significantly reduced the levels of these parameters.

CONCLUSION

The results of this study suggest that ethanolic extract of the leaves of *N. laevis* possesses anti-inflammatory and antioxidant activities that could enhance amelioration of β-cell destruction and other oxidative stress-induced complications in diabetes mellitus.

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