

Biochemical and Haematological Effects of the Leaf Extract of *Newbouldia laevis* in Alloxan-Induced Diabetic Rats

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Abstract

Dichloromethane-methanol (1:1) extract (DME) of *N. laevis* leaves was prepared by cold maceration. The effects of the extract on the haematological and some biochemical parameters of alloxan-induced diabetic rats were investigated. The results showed that the oral administration of the extract (250, 500, 1000 mg/kg) caused a significant ($P < 0.5$) and dose-dependent increase in red blood cell count (RBC) and its indices, as well as a significant ($p < 0.05$) and dose-dependent reduction in the platelet count and the white blood cells (WBC). The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were significantly ($p < 0.05$) decreased. This effect was not dose related. The serum levels of total bilirubin, urea and creatinine were significantly ($p < 0.05$) decreased. The serum total protein and total antioxidant status (TAS) significantly ($p < 0.05$) increased dose dependently. Overall, administration of DME has significant ameliorative effect on alloxan-induced anaemia and other haematological alterations in diabetes and this may be of immense benefits in the management of diabetes and its associated haematological complications. Improved liver and kidney functions as well as improved antioxidant status are beneficial in the management of chronic diseases such as diabetes.

Keywords

Haematological, Biochemical, *Newbouldia laevis*, Diabetes, Anti-Oxidant

1. Introduction

Newbouldia laevis (family Bignoniaceae) is a non-leguminous, medium sized angiosperm, commonly called boundary tree (planted as hedgerows and as a life fence), chieftaincy tree (used in chieftaincy and traditional religious ceremonies)

[1], or tree of life (possibly because of its longevity). Locally, it is called Ogirisi (Igbo), Akoko (Yoruba), Aduruku (Hausa) [2]. It is a plant with diverse claims of effectiveness, widely utilized in traditional medicine by various cultures throughout the tropical Africa, including but not limited to Nigeria, Togo, Senegal, Ghana, Congo, Cote de Voire, and Cameroun. Some of the documented medical uses include in the folk treatment of fevers (including yellow fever), malaria, stomach ache, cough, sexually transmitted infections, skin infections, tooth ache, breast cancer, constipation, pain (pelvic pain in females, chest pain, ear ache), gonococcal orchitis, elephantiasis, sorefeet, ulcer, epilepsy, convulsion, migraine, sickle cell anaemia, as a febrifuge, as a vermifuge, in female reproductive healthcare (fibroids, infertility, hemorrhage), as aphrodisiacs, eye problems, snake bites, wound healing, diabetes, arthritis, rheumatism and other inflammatory conditions [3] [4] [5] [6] [7]. Some of the folkloric uses of this plant have been scientifically validated.

Pharmacological studies on extracts of different parts of *N. laevis* have revealed the antioxidant and free radical scavenging [8], antimicrobial and antimalarial [9], sedative and anticonvulsant [10], analgesic, antinociceptive and anti-inflammatory [11], hepatoprotective [12], anticancer [13], uterine contraction [14], wound healing and antiulcer [15], antisickling [16], hypoglycemic [17] activities among others.

Recently, the antihyperglycemic activity of the leaf extract and active fractions of the plant was reported [18] and apigenin was reported to be one of the active metabolites responsible for the antihyperglycemic activity [19].

The popular traditional use of *Newbouldia laevis* in the management of various ailments, including chronic ailments such as diabetes, necessitates the evaluation of its toxicological potential. Studies have reported the altered biochemical parameters of the liver and kidney functions, and haematological parameters following the administration of alloxan and / or extracts of medicinal plants [20] [21]. Mansi and Lehham [22] reported alteration in various haematological parameters and the immune system during the course of diabetes. Also toxicological studies have revealed that ingestion of medicinal plants or drugs can alter the normal haematological values [23] and as such could be an important tool in the assessment of deleterious effect of drugs [24] as well as medicinal plants and their extracts.

Therefore, the present study was undertaken to provide information on the restorative effects of dichloromethane/methanol (1:1) extract of *N. laevis* leaves on alloxan-induced diabetic rats using biochemical indices of the liver and kidney functions as well as haematological parameters.

2. Materials and Methods

2.1. Collection of Plant Material

Fresh leaves of *N. laevis* were collected from plants growing in Igbo-Ukwu, Aguata Local Government Area of Anambra State, South-Eastern Nigeria, in October. Botanical identity was kindly confirmed by Mr. J.M.C. Ekekwe, of the

Department of Botany, University of Nigeria Nsukka, where a voucher specimen is deposited.

2.2. Preparation of Extract

The leaves were washed, air-dried under shade for 7 days and pulverized into a coarse powder using locally fabricated hammer mill. The powdered leaf material (5 kg) was extracted with dichloromethane /methanol (1:1) by cold maceration for 48 h. The filtrate was concentrated *in vacuo* at 40°C to dryness with a rotary evaporator (yield 14.2%) and labeled DME extract of *N. laevis*. The DME extract was stored in a refrigerator until required.

2.3. Experimental Animals

White albino mice (15 - 30 g) and adult Wistar rats (150 - 250 g) of both sexes obtained from the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, University of Nigeria Nsukka were employed for these experiments. The animals were allowed 14 days acclimatization period on transfer to the research area with free access to food and water before starting the experiments. All animal experiments were in compliance with National Institute of Health Guide for the care and use of Laboratory Animals [25].

2.4. Acute Toxicity (LD₅₀) Test and Phytochemical Studies

The oral and intraperitoneal median lethal doses (LD₅₀) of DME in mice were determined using the method described by Lorke [26], while the freshly prepared extract was chemically tested for the presence of chemical constituents using standard protocols [27].

2.5. Induction of Diabetes

Alloxan monohydrate (Sigma, St. Louis, MO, USA) was used to induce diabetes in adult Wistar rats of both sexes. A freshly prepared alloxan monohydrate (65 mg/kg in 0.9% saline) was administered intravenously to the animals [28]. Glucose solution (50%) was used to prevent the initial hypoglycemia caused by alloxan monohydrate [29]. After 3 days of alloxan treatment blood samples were collected from overnight fasted animals through the tail vein and blood glucose level was estimated using Accu-check Active® (Roche Diagnostics) Glucometer. Rats with blood glucose levels above 250 mg/dl [30] were considered diabetic and selected for the study.

2.6. Effect of DME on Alloxan-Induced Diabetic Rats

A total of twenty five (25) diabetic and five (5) normoglycemic rats of both sexes were used. The rats randomly selected were divided into six (6) groups of five (5) rats per group. The treatment schedules were as follows:

Group 1: normal control treated with 3% Tween 80 (5 ml/kg). Group 2: diabetic control treated with 3% Tween 80 (5 ml/kg). Group 3: diabetic rats treated with the standard drug glibenclamide (5 mg/kg). Group 4: diabetic rats treated

with 250 mg/kg of DME. Group 5: diabetic rats treated with 500 mg/kg of DME. Group 6: diabetic rats treated with 1000 mg/kg of DME.

The treatments were per os daily for 21 days. Fasting blood glucose was measured on day 0, 7, 14 and 21. The animals were weighed on those days to assess possible weight changes. On day 22, the animals were euthenized under chloroform anesthesia after overnight fast. Blood samples were collected through retro-orbital plexus into EDTA coated bottles and simple plain bottles for determination of haematological and biochemical parameters.

2.7. Determination of Haematological Parameters

Blood samples collected in EDTA bottles were analyzed for haematological parameters using a haematology analyzer (Mindray Auto Hematology Analyzer, BC-5200, USA) following the manufacturer's instructions. The parameters analyzed include white blood cell count (WBC) and the differentials, platelets, red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

2.8. Determination of Biochemical Parameters

Blood samples collected in plain bottles were centrifuged and the resultant serum was analyzed for urea [31], creatinine [32], and total bilirubin [33]. The liver function marker enzymes including alkaline phosphatase (ALP) [34], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [35], total protein [36] and total antioxidant status (TAS) [37] were analyzed using diagnostic kits from Randox laboratory UK.

2.9. Statistical Analysis

Data obtained were analysed using One Way Analysis of Variance (ANOVA) (SPSS Version 20.0 software USA) and expressed as mean \pm SEM. Differences between means were considered significant at $p < 0.05$ (LSD post hoc test).

3. Results

3.1. Acute Toxicity Test and Phytochemical Analysis

The oral LD₅₀ of DME in mice was greater than 5000 mg/kg, while the intraperitoneal LD₅₀ was estimated as 3807.9 mg/kg. The preliminary phytochemical tests on the extract gave positive results to alkaloids, flavonoids, glycosides, steroids, saponins, tanins, terpenoids, carbohydrates, proteins, oils, acidic compounds, reducing sugars and resins.

3.2. Effect of DME Extract on Mean Fasting Blood Glucose Levels of Diabetic Rats

There was no significant change in fasting blood glucose level of normal control rats throughout the study. All the DME treated groups showed significant ($p < 0.05$) and dose-related reductions in the mean fasting blood glucose levels at the

end of week one which was sustained till week 3. The effect of 1000 mg/kg (73.48% reduction) was similar to that produced by glibenclamide (74.19% reduction) at the end of week 3 (**Figure 1**).

3.3. Effect of DME Extract on Body Weight of Diabetic Rats

There was a weight increase of 9.64% in untreated normal rats at the end of week 3. A significant ($p < 0.05$) decrease of 13.02% occurred in the body weight of alloxan diabetic rats when compared to normal control rats (**Figure 2**). Treatment of diabetic rats with different doses of extract or glibenclamide did not result in significant weight change within the 1st week. A dose related increase in weight

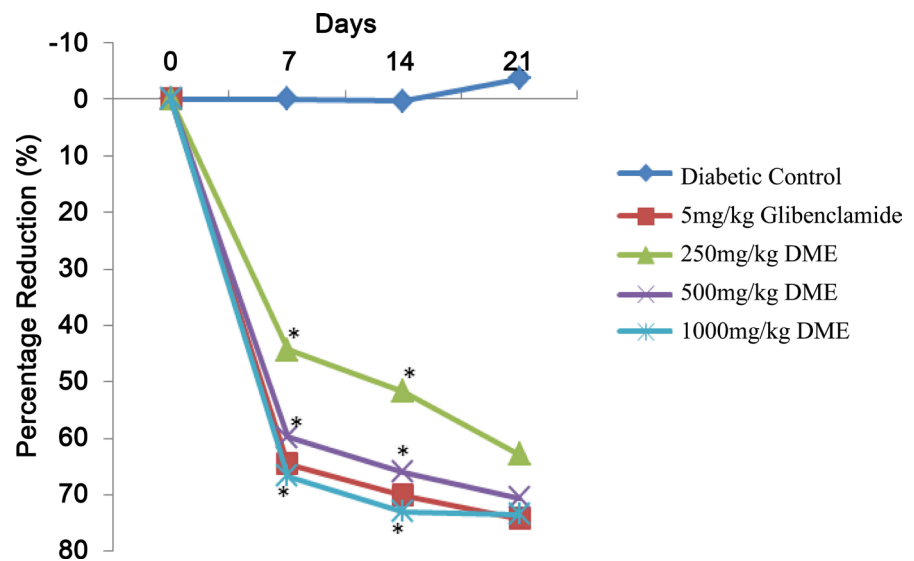


Figure 1. Effects of dichloromethane/methanol extract (DME) on mean fasting blood glucose levels of diabetic rats. * $p < 0.05$ compared to diabetic control. $n = 5$.

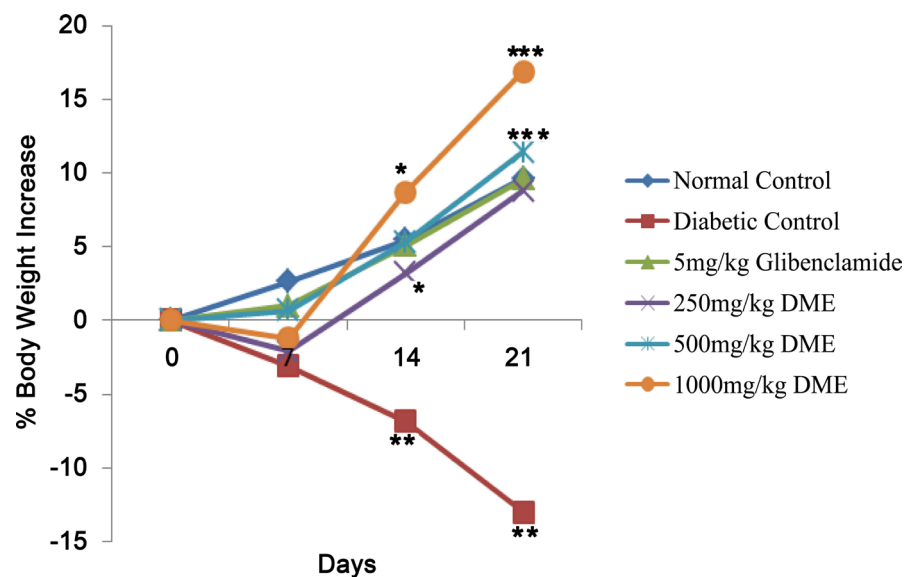


Figure 2. Effects of dichloromethane/methanol extract (DME) on average body weight changes of diabetic rats. * $p < 0.05$ compared to diabetic control; ** $p < 0.05$ compared to normal control. *** $p < 0.05$ compared to glibenclamide treated group.

was observed at 2nd and 3rd week of treatment ($p < 0.05$). The weight increase (11.41% and 16.87%) in the 500 mg/kg and 1000 mg/kg treated groups respectively was significantly ($p < 0.05$) higher than that of glibenclamide treated group (9.61%) at the end of the 3rd week (Figure 2).

3.4. Effect of DME on Haematological Parameters of Diabetic Rats

The red blood cells (RBC) and its indices were significantly ($p < 0.05$) reduced in diabetic rats. Treatment with DME significantly ($p < 0.05$) increased these parameters, while significant and dose related reduction in the white blood cells (WBC) and platelet count was evident ($p < 0.05$).

No significant changes occurred in the neutrophils and lymphocytes counts following DME treatment (Table 1).

3.5. Effect of DME on Liver Marker Enzymes of Diabetic Rats

The activities of liver function marker enzymes—alanine amino transferase

Table 1. Effects of dichloromethane/methanol extract (DME) on hematological parameters of diabetic rats.

Parameter	Treatment Groups			Dose (mg/kg)		
	Normal Control	Diabetic Control	Glibenclamide	250 mg	500 mg	1000 mg
RBC ($\times 10^{12}/L$)	8.18 \pm 0.35	3.81 \pm 0.40** (53.42)	5.10 \pm 0.62* (33.86)	6.21 \pm 0.17* (62.99)	6.95 \pm 0.30* (82.41)	8.64 \pm 0.24* (126.77)
PCV (%)	47.16 \pm 2.20	23.8 \pm 1.68** (49.53)	38.02 \pm 1.10* (59.75)	42.52 \pm 0.65* (78.66)	46.98 \pm 1.39* (97.39)	50.88 \pm 0.93* (113.78)
HB (g/dL)	15.06 \pm 0.17	9.79 \pm 0.32** (34.99)	12.82 \pm 0.46* (30.95)	14.12 \pm 0.17* (44.23)	15.08 \pm 0.17* (54.03)	16.14 \pm 0.45* (64.86)
WBC ($\times 10^9/L$)	8.24 \pm 0.50	15.80 \pm 0.54** (91.75)	11.51 \pm 0.53* (-27.15)	10.28 \pm 0.38* (-34.94)	9.35 \pm 0.31* (-40.82)	7.35 \pm 0.32* (-53.48)
MCV (fl)	68.61 \pm 1.83	46.93 \pm 3.27** (30.94)	62.00 \pm 1.21* (32.11)	67.14 \pm 4.05* (43.06)	68.08 \pm 3.45* (45.07)	78.67 \pm 8.79* (67.63)
MCH (pg)	22.80 \pm 0.71	18.61 \pm 1.14** (18.38)	18.73 \pm 0.67 (0.64)	21.82 \pm 0.74* (17.25)	26.37 \pm 2.67* (41.70)	26.59 \pm 2.20* (42.88)
MCHC (g/dL)	34.21 \pm 1.37	21.54 \pm 1.71** (37.04)	33.25 \pm 0.81* (54.36)	31.77 \pm 0.97* (47.49)	33.84 \pm 1.58* (57.10)	39.55 \pm 2.07* (83.61)
Platelet ($\times 10^9/L$)	216.66 \pm 6.92	804.22 \pm 68.04** (271.19)	555.78 \pm 20.67* (-30.89)	544.00 \pm 31.51* (-32.36)	501.64 \pm 28.17* (-37.62)	465.52 \pm 19.24* (-42.12)
Lymphocytes (%)	56.54 \pm 0.58	48.69 \pm 1.08** (-13.88)	51.29 \pm 0.87 (5.34)	51.48 \pm 1.22 (5.73)	47.40 \pm 0.94 (-2.65)	45.14 \pm 1.29 (-7.29)
Monocytes (%)	13.93 \pm 0.27	1.71 \pm 0.14** (-91.60)	10.45 \pm 0.55* (511.11)	13.97 \pm 0.20* (716.96)	14.50 \pm 0.26* (747.95)	16.80 \pm 0.68* (882.46)
Neutrophils (%)	38.20 \pm 0.33	36.42 \pm 1.14 (-4.66)	37.76 \pm 1.23 (3.68)	34.90 \pm 0.62 (-4.17)	37.73 \pm 1.48 (3.60)	36.79 \pm 0.54 (1.02)
Eosinophils (%)	0.44 \pm 0.11	2.31 \pm 0.12** (425.00)	0.19 \pm 0.04* (91.77)	0.09 \pm 0.02* (96.10)	0.26 \pm 0.07* (88.74)	0.62 \pm 0.053* (73.16)
Basophils (%)	0.31 \pm 0.07	1.29 \pm 0.12** (316.13)	0.29 \pm 0.03* (77.52)	0.08 \pm 0.01* (93.8)	0.19 \pm 0.04* (85.27)	0.57 \pm 0.07* (55.81)

Values are mean \pm SEM (n = 5) * $p < 0.05$ compared to diabetic control; ** $p < 0.05$ compared to normal control. The numbers in parenthesis represent percentage increase or decrease of the parameter compared to the control group. Minus (-) represent a decrease.

(ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were significantly ($p < 0.05$) elevated by 96.06%, 66.38% and 74.92% respectively in alloxan-induced diabetic rats. Treatment with graded doses of DME for 21 days caused a significant ($p < 0.05$) decrease in the ALT, AST and ALP levels (**Figure 3**). Maximum reduction (59.04%) in ALT activity occurred in 500 mg/kg treated group while maximum reduction occurred in 1000 mg/kg treated group for AST. The reduction by 250 mg/kg (21.78%) and 500 mg/kg (21.78%) for AST was comparable to that of glibenclamide (23.36%) (**Figure 3**).

3.6. Effect of DME on Serum Total Bilirubin and Total Protein

Alloxan – induced diabetic rats exhibited significant ($p < 0.05$) increase (159.18%) in serum total bilirubin (**Figure 4**) and decrease (44.80%) in serum total protein

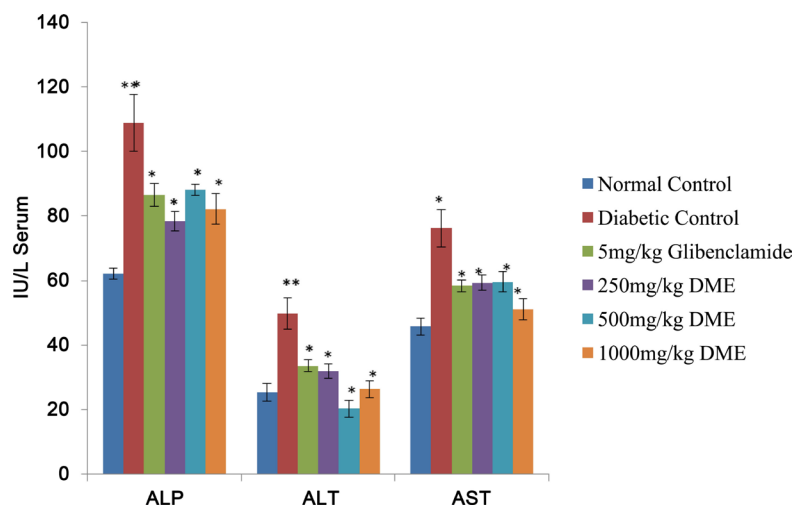


Figure 3. Effects of dichloromethane/methanol extract (DME) on serum liver marker enzymes of diabetic rats. Values are mean \pm SEM ($n = 5$) * $p < 0.05$ compared to diabetic control; ** $p < 0.05$ compared to normal control.

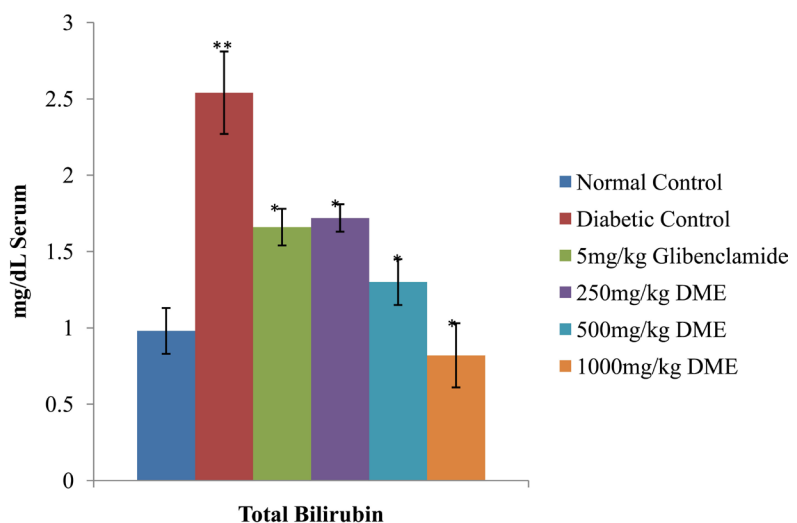


Figure 4. Effects of dichloromethane/methanol extract (DME) on serum total bilirubin. Values are mean \pm SEM ($n = 5$) * $p < 0.05$ compared to diabetic control; ** $p < 0.05$ compared to normal control.

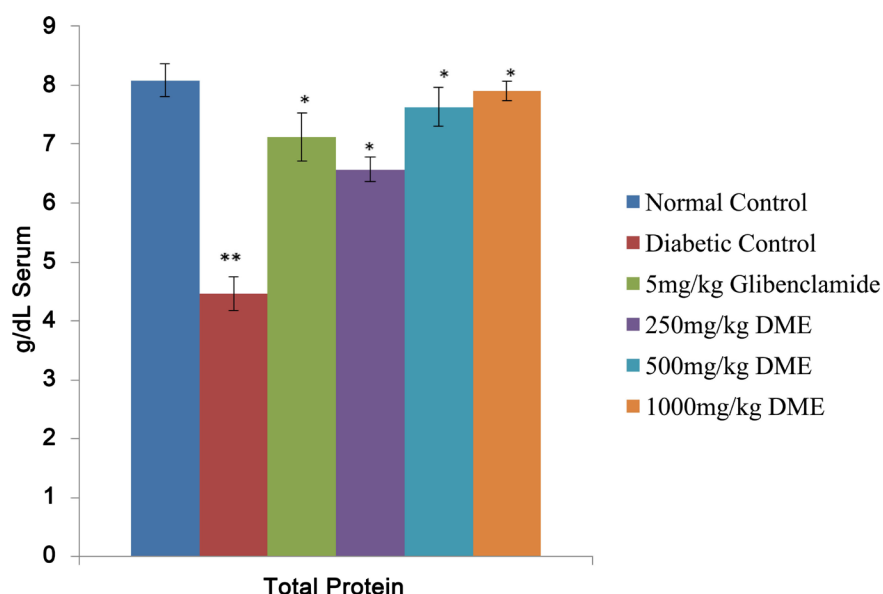


Figure 5. Effects of dichloromethane/methanol extract (DME) on serum total protein in diabetic rats. Values are mean \pm SEM (n = 5) * p < 0.05 compared to diabetic control; ** p < 0.05 compared to normal control.

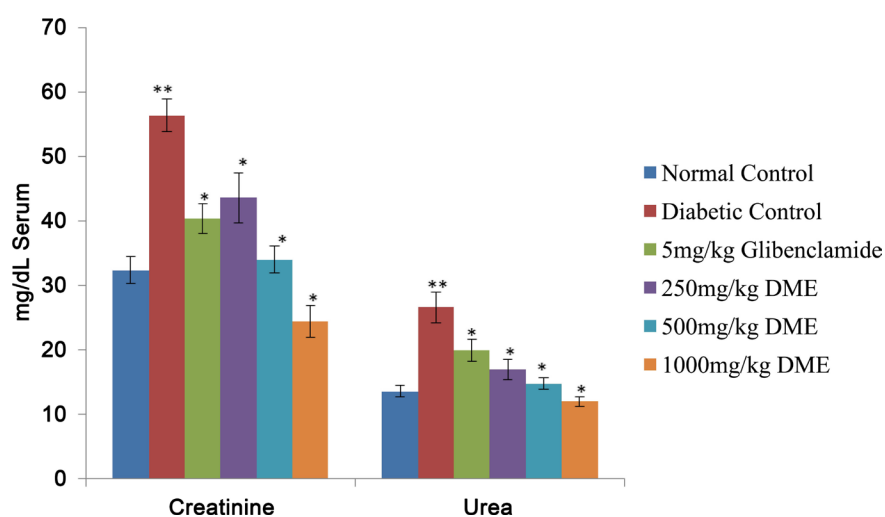


Figure 6. Effects of dichloromethane/methanol extract (DME) on serum total urea and creatinine levels of diabetic rats. Values are mean \pm SEM (n = 5) * p < 0.05 compared to diabetic control; ** p < 0.05 compared to normal control.

(**Figure 5**). Groups of diabetic rats treated with different doses of the extract had significant (p < 0.05) decrease in serum total bilirubin and increase in serum total protein. The effects of 250 mg/kg were similar to the effects produced by glibenclamide

3.7. Effects of DME on Kidney Function Markers

The serum levels of urea and creatinine were significantly (p < 0.05) increased by 95.59% and 74.07% respectively in alloxan-induced diabetic rats (**Figure 6**). Administrations of DME for 21 days caused significant (p < 0.05) and dose-related decrease in the serum level of these kidney function markers. The reductions

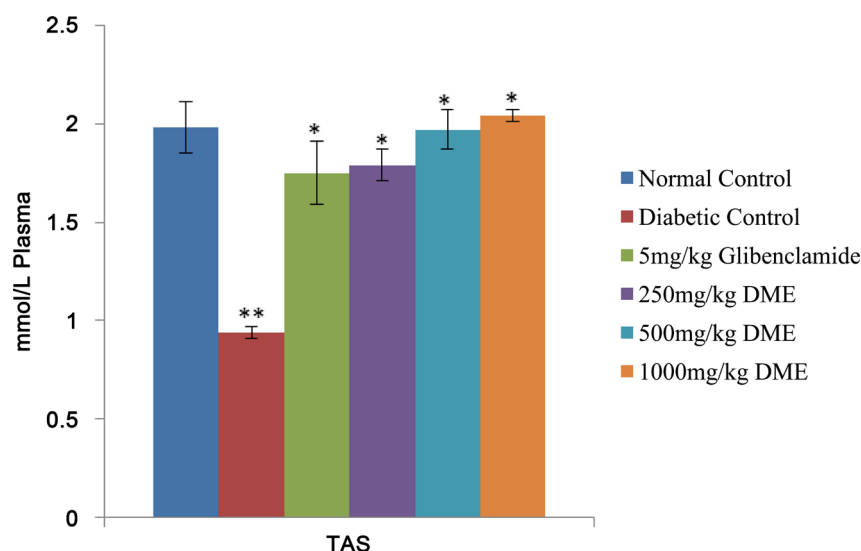


Figure 7. Effects of dichloromethane/methanol extract (DME) on serum total antioxidant status (TAS) of diabetic rats. Values are mean \pm SEM ($n = 5$) * $p < 0.05$ compared to diabetic control; ** $p < 0.05$ compared to normal control.

produced by 500 mg/kg and 1000 mg/kg were more than that produced by glibenclamide

3.8. Effects of DME Extract on Total Antioxidant Status (TAS) of Diabetic Rats

A significant ($p < 0.05$) decrease (52.53%) in total antioxidant status (TAS) occurred in diabetic untreated rats when compared with the normal control rats (Figure 7). Treatment with DME evoked a significant dose-related increase in TAS level in diabetic treated rats (90.43%, 109.57% and 117.02% respectively) ($p < 0.05$) when compared to diabetic untreated control. This effect was better than that of the reference drug (86.17%).

4. Discussion

Alteration in the various haematological parameters and the immune system during the course of diabetes have been reported [22]. Also toxicological studies revealed that ingestion of medicinal plants or drugs can alter the normal haematological values [23]. Therefore, haematological parameters could be an important tool in the assessment of deleterious effect of drugs [24] as well as medicinal plants and their extracts.

Anaemia has been noted to be a common pathophysiological feature and a complication of DM [38]. Diabetes associated anaemia is reported to be due to the increased non-enzymatic glycosylation of RBC membrane proteins which correlates with hyperglycemia [39]. Oxidation of these membrane proteins in the presence of chronic hyperglycemia in uncontrolled DM increases the production of lipid peroxides that leads to haemolysis of RBC [40]. One of the pathological consequences of this membrane lipid peroxidation is reduced erythrocyte survival [41]. Although the RBC membrane lipid peroxide level in diabetic rats was

not measured in this study, other RBC parameters such as Hb, PCV, MCV, MCH, and MCHC were measured so as to investigate the effect of DME on the anaemic status of alloxan-induced diabetic rats. The decrease in the RBC and its indices following treatment with diabetogenic agent in experimental diabetes is an indication of reduced and abnormal erythropoiesis. This observation is consistent with earlier reports [42] [43] but differ from the reports of some others [44] [45]. Administration of DME to alloxan-induced diabetic rats appreciably improved the levels of RBC and its indices ($p < 0.05$). This suggests that some phytoconstituents present in the extract can stimulate the formation or secretion of erythropoietin which stimulates the stem cells in the bone marrow to produce RBC [46] which is evidenced by the improved levels of MCH and MCHC [47]. Similar report of increased RBC and its indices in normal rats supplemented with *N. laevis* root extract is available [48]. Another study [49] however, reported slight increase in RBC and its indices in *N. laevis* leaf-supplemented normal rats and suggested that the increase may be due to stimulatory effect of the extract on the production of haematopoietic regulatory elements such as erythropoietin and colony-stimulating factors by the stromal cells and macrophages in the bone marrow. Phytoconstituent such as flavonoids [50] and specifically apigenin [19] present in the leaf of *N. laevis* may be responsible for these effects. This is evidenced by the improved level of MCH and MCHC. Similarly Mahmoud *et al.*, [51] reported that grape fruit flavonoids improved RBC and its indices in diabetic rats via its ability to lower lipid peroxidation level that causes haemolysis of erythrocytes. The attenuation of pro-inflammatory cytokines production and stimulation of adiponectin expression by hesperidin and naringin (grapefruit flavonoids) is reported to be directly related to the improvement in RBC and its indices and correction of the anaemic status in type 2 diabetic rats [52]. This may be true for apigenin present in *N. laevis* leaf.

White blood cell (WBC) serves as a scavenger that removes foreign substances. The number of WBC is known to rise as a body defense mechanism in response to toxic environment [53]. Changes in WBC have been associated with insulin resistance and cardiovascular complications [54]. Leukocytosis is reported to be associated with insulin resistance, Type 2 DM, coronary artery disease, stroke and diabetes induced macro and microangiopathy [52]. Leucocytes are reported to be activated by AGEs, oxidative stress, angiotensin II and pro-inflammatory cytokines [52]. The result of this study showed a significant ($p < 0.05$) increase in WBC of diabetic control rats which became reduced significantly ($p < 0.05$) on DME treatment in a dose related manner. This may have been as a result of the ability of the extract to restore insulin sensitivity, ameliorate AGEs production and reduce oxidative stress within the blood cells. This finding is in agreement with earlier reports [45] [52].

A report that platelet count was significantly higher among diabetics compared to non-diabetics and a positive correlation between platelet count and poor glycemic control exists [55]. Previous report suggests that higher platelet count may contribute to vascular events in patient with insulin resistance [56].

Platelet counts correlated positively with WBC count which may suggest a shared mechanism [57]. Raised platelet values are commonly seen in inflammatory and infectious diseases [58] and are considered as an acute phase reaction to infection or inflammation as is the case with alloxan-induced diabetogenesis caused by free radicals [59]. In the present study, thrombocytosis was evident in the diabetic untreated control rats. However, treatment with DME significantly ($p < 0.05$) reduced the platelet count dose dependently. This observation may suggest the ability of the DME to achieve glycemic control and protect against vascular events.

The results of the study on haematological parameters are consistent with the reports of other workers [52] [54] [55] and suggest the safety and erythropoietic property of *N. laevis* leaf extract.

A marked elevation in the levels of serum liver marker enzymes was evident in the diabetic control. Several studies have reported similar elevation in the activities of serum liver marker enzymes during alloxan administration [60] [61]. The elevated liver marker enzymes in diabetic control rats may be due to destructive changes in the hepatocytes due to alloxan toxicity since hepatic injury is often associated with alterations in the serum levels of these enzymes. Also increase in ALP activity may be indicative of peroxidation of cell membrane and loss of membrane integrity. It has been reported that ALT participates in gluconeogenesis and its transcription is suppressed by insulin and that increased activity is therefore suggestive of impairment of insulin signaling and not hepatocyte injury [62]. Treatment with DME or glibenclamide caused a significant ($p < 0.05$) decrease in the transaminases and ALP activities. This effect was not dose related. Plant extracts have been reported to inhibit transaminase activity [63]. The decrease may be attributed to hepatoprotective and antioxidant activity of DME [64]. Antioxidants are known to reduce the development of chemically induced liver damage [65]. The improved antioxidant status of DME treated diabetic rats may have contributed to these effects. The increase in the levels of serum proteins is also suggestive of hepatoprotective activity as the induction of protein synthesis speed up the regeneration and production of liver cells [64]. The result of this study supports an earlier report [66] of the modulation of serum liver marker enzymes and antioxidant activities by the ethanol extracts of stem and leaves of *N. laevis* in diabetic rats.

Serum total protein is a marker of the synthetic function of the liver and a valuable guide to assess the severity of liver damage [67]. A reduction in the serum protein levels in diabetic control rats may be as a result of possible damage to the hepatocytes induced by alloxan and chronic hyperglycemia. It may also be due to increased rate of amino acid conversion to glucose and reduced ribosomal protein synthesis as a result of insulin deficiency. Protein may also be damaged directly by specific interaction of oxidants or free radicals with particularly susceptible amino acids [68]. A good correlation between protein synthesis and insulin levels has been reported [60]. Although insulin levels were not measured in this study, a significant reduction in the blood glucose level may be taken as a

direct evidence of increased insulin levels of treated diabetic rats. Treatment with DME for 21 days caused a significant and dose related improvement of serum total protein content ($p < 0.05$). This effect was comparable to that of glibenclamide treated rats. Increase in serum total protein of alloxan-induced diabetic rats treated with glibenclamide for 21 days has been reported [69]. This may occur as the result of increased protein synthesis as a result of improved insulin levels, and could be linked to the protective effect of the extract against oxidative damage to the liver [64]. An increase in total protein level has been reported to have hepatoprotective effect [70]. The increase in serum total protein observed in this study may also be as a result of intrinsic nutritive property of *N. laevis*. Ogbonnia *et al.* [48] reported a similar increase in serum total protein of normal rats fed with *N. laevis* stem bark aqueous extracts and suggested a link between increased protein levels and protective effect of the extract against oxidative damage to the liver.

Total bilirubin level is also an important marker of liver function since it is conjugated in the liver for possible excretion via the kidneys or via bile. An increased total bilirubin level in diabetic control rats was evident, indicating a compromised liver function. This observation is consistent with an earlier report [71]. The increase in plasma bilirubin may occur as a result of decreased liver uptake, conjugation, or increase in bilirubin formation [72] following liver damage. Administration of DME to diabetic rats for 21 days significantly ($p < 0.05$) lowered the total bilirubin in a dose related manner suggesting an improvement in liver function. Similar observation was reported by Shah and Khan [71] using *Sida cordata* extract in alloxan-induced diabetic rats for 15 days. Sunmonu and Afolayan [73] also reported a significant reduction in the bilirubin level of STZ-induced diabetic rats treated with *Artemisia afra* aqueous extract for 15 days.

Kidneys are the major excretory organs and renal function tests are devised to detect possible renal damage. Increased serum levels of urea and creatinine are among the most sensitive indicators of kidney injury. In this study, urea and creatinine levels were significantly increased in diabetic rats ($p < 0.05$) indicating renal impairment. Hyperglycemia is known to induce these elevations. Alarcon *et al.* [74] reported that alloxan-induced hyperglycemia resulted to increased levels of plasma urea and creatinine. The high serum urea in diabetic control rats has been suggested to be due to the stimulation of gluconeogenesis as alternative glucose source as a result of insulin deficiency [75]. Gluconeogenesis is sustained by increased proteolysis which releases glucogenic amino acids that are subsequently deaminated in the liver resulting in high urea levels [75]. Administration of DME for 21 days significantly and dose dependently lowered these indices ($p < 0.05$). The stabilization of these parameters indicates improvement in renal function which could be attributed to the antihyperglycemic effect of the extract and thus increased insulin effect causing a decline in proteolysis. Similar observation has been reported [71].

Diabetes is characterized by low plasma levels of both enzymatic and non-

enzymatic antioxidant defenses which make the cells of diabetics vulnerable to oxidative stress. Depletion of antioxidant level has been demonstrated in DM and administration of antioxidants to compensate the depletion has been shown to improve diabetes and prevent its complications [76]. It is hypothesized that under severe oxidative stress (such as in DM), there is heavy production of reactive species which may result in the depletion of protective physiological moieties [68]. Increase in lipid peroxidation during diabetes is as a result of inefficient or overwhelmed antioxidant system due to free radical generation. Decreased activities of antioxidant enzymes and non-enzymatic antioxidants during experimental diabetes which correlates with hyperglycemia has been reported [66] [75]. Antioxidant activity or the inhibition of free radical generation is of paramount importance in diabetes management. Supplementation with antioxidants is believed to be effective in increasing the activities of antioxidant defense enzymes, scavenging free radicals, preventing oxidative damage and as such protecting lipid components of the cells against peroxidation [49] [77]. Total antioxidant status (TAS) (enzymatic and non-enzymatic) plays a pivotal role in cell defense against ROS. The decreased TAS level in diabetic control rats may be associated with ROS generation with subsequent oxidative stress in chronic hyperglycemia. The reduced level of the TAS of diabetic control rats is taken as direct evidence for oxidative stress which may have overwhelmed the antioxidant system due to free radical generation [78]. Administration of DME for 21 days caused a significant two-fold increase in TAS level in diabetic treated rats ($p < 0.05$). This effect was significantly ($p < 0.05$) more than that of glibenclamide. This suggests improved functionality of the antioxidant system of the diabetic treated rats probably due to the effect of the phytochemical antioxidants present in the DME. This may have significant implication on the antidiabetic efficiency of *N. laevis* leaf by offering a protective back-up against alloxan induced toxicity in the pancreas and the sustained oxidative stress due to chronic hyperglycemia.

Weight loss is one of the key features of DM, usually linked to degradation of structural proteins and muscle wasting [39]. In this study, induction of diabetes with alloxan is demonstrated to be associated with a characteristic decrease in body weight. The ability of DME to protect against massive weight loss in alloxan diabetic rats seems to be due to its ability to improve glucose utilization, reduce hepato-renal dysfunction, [18] [79], improve the TAS and restore haematopoietic function. Earlier report noted a significant increase in the body weight of normal rats supplemented with *N. laevis* extract [49].

5. Conclusion

Thus *N. laevis* leaf possesses the ability of managing hyperglycemia, improve haematological and biochemical derangements in alloxan induced-diabetic rats. It can also control muscle wasting and induce adipogenesis.

Conflict of Interest

The authors have no conflict of interest to declare.

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