Anti-diabetic Effect of Two Medicinal Plants: *Cataranthus roseus* and *Nauclea latifolium* on Some Biochemical Indices of Streptozotocin Induced Diabetic Albino Wistar Rats

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**Authors’ contributions**

This work was carried out in collaboration among all authors. Authors EEO and KA conceived the idea and designed the research work. Authors JE, TC and SU carried out the experiment. Author JE did the statistical analysis. Author EEO wrote the paper and finally author EU read and approved the final manuscript. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/JPRI/2019/v29i3330240

**Editors(s):**

(1) Dr. R. Deveswaran, Associate Professor & Head, Drug Design and Development Centre, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, India.

**Reviewers:**

(1) Keagile Bati, University of Botswana, Botswana.
(2) Javed Intekhab, SVPG College, India.
(3) Miikue-Yobe Togenu, Laboratory Technology Rivers State Polytechnic, Nigeria.

Complete Peer review History: [http://www.sdiarticle3.com/review-history/50594](http://www.sdiarticle3.com/review-history/50594)

**Received 02 June 2019**

**Accepted 13 August 2019**

**Published 28 August 2019**

**ABSTRACT**

The study was carried out to investigate the anti-diabetic effects of two medicinal plants *Cataranthus roseus* (C.R) and *Nauclea latifolium* (N.L) on some biochemical indices of streptozotocin induced diabetic albino wistar rats.

**Methods:** Ethanolic leaf extracts of C.R. and N.L. were given at daily doses of 500 mg/kg body weight in two divided doses each for 14 days. Thirty albino wistar rats were divided into five (5)

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groups, consisting of 6 rats each viz: Group 1(normal control), Group 2 (diabetic control), Group 3 (insulin treated), Group 4 (received N.L) and Group 5 (received C.R.).

**Results:** The results of the phytochemical screening contain flavonoids, polyphenols, and alkaloids were found to be present in appreciable amount in N.L while saponins and tannins were found in traceable concentration. Fasting blood glucose levels showed significant decrease (P<0.05) in all the test groups compared to diabetic control and closely related to the insulin treated groups. A significant increase (P<0.05) was observed in (TG) and (TC) concentration of all treated groups compared to the diabetic control group. The concentration of HDL was significantly increased while there was also a significant decreased (P<0.05) in VLDL and LDL in the diabetic control group and insulin group when compared to the normal control group, except for C.R treated group that shows a significant decrease compared with the diabetic control group. Enzymes activities were increased in insulin and diabetic groups. A significant reduction (P<0.05) was observed with the treated group of C.R. and N.L compared to the normal control group. Also, observed was a decrease in albumin level in groups treated with the extracts. Marked reduction in total protein level was observed in groups treated with extracts and insulin, compared to the normal control group. Serum concentrations of Na⁺, K⁺, Cl⁻ in diabetic control groups showed a significant increase (P<0.05) compared to the normal control group. K⁺ concentration was observed to be significantly decreased (P<0.05) in all groups treated with extract and insulin compared to the normal control group.

**Conclusions:** The results demonstrated that *C. roseus* and *N. latifolium* have anti-diabetic and antihepatotoxic properties and could be potential herbal remedy in treating and managing diabetic conditions.

**Keywords:** *Cataranthus roseus; Nuclea latifolium; triglycerides (TG); total cholesterol (TC) concentration.*

**1. INTRODUCTION**

Herbal medicines are popular remedies for disease used by a vast majority of the world’s population. Formulation from herbs, have attained widespread acceptability as therapeutic agent [1]. Report by World Health Organization (WHO), [2], estimates that more than 80% of the world population relies on traditional medicine for their primary health care needs. People living in small isolated villages and natural communities use folk medicine from treatment of common infectious diseases [3]. Medicinal plants have acquired increasing significance in development and trade over the last few years (http://www.traffic.org/about/priority medicinal trade html). Their use and conservation are cross-sectorial concerns that embrace not only health-care but also natural conservation, biodiversity, economic assistance, trade and legal aspects, including intellectual property. Even today, the majority of the world’s population is dependent upon traditional medicine and also on the use of plants and plant extracts. This is particularly true of poorer sections of the population in developing countries, because natural remedies are not only cheaper than modern medicines but are often the only medicines available in various rural regions. Beside serving medical and cultural functions, medicinal plants in developing countries have important economic role. The gathering of wild medicinal herbs provides economically disadvantaged groups such as small holders and handless herd’s people with their only form of cash income.

Worldwide, a total of at least 35,000 plant species are used for medicinal purposes. The most important industrial medicines nowadays are based on not more than about 90 species, whilst traditional remedies in developing countries are usually based on mixtures of herbs collected in the wild. In Indonesia, for example up to three quarters of all instance of sickness are treated with mixture of teas – known as Jamu – which contain plant extracts from up to 30 different kinds of dried plant species. Plants are not just the main component of traditional medicines, according to estimates by the World Health Organisation, they also form up to about 70 percent of the basis of modern pharmaceutical products. One example is acetylsalicylic acid, the main ingredient, in painkillers (headache tablets) which was first extracted from domestic willow as long as 150 years ago [4]. According to World Health Organization estimates of about 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million died from consequences of high blood sugar and more than 80% of diabetic related death occurring in low – and middle income countries. WHO projects that diabetic death will double in 2030? A report estimated...
that 25.8 million people in United State are affected with diabetes, 8.3% of the U.S population, (18.8 million people) are diagnosed while 7.0 million are undiagnosed. In Nigeria, WHO estimated diabetes mellitus prevalence to be 3.4% which may be under represented as more than half a million people in Lagos State alone are living with diabetes mellitus [4]. Vernonia amygdalina, Gongronema latifolium and Cataranthus roseus are three known plants used in traditional medicine for the treatment or management of disease conditions. Nauclea latifolium and Cataranthus roseus will be used in this research. Cataranthus roseus, common name Madagascar periwinkle, or rosy periwinkle is an attractive small shrub with graceful pink or white salveer form flowers. Native to South eastern and eastern Madagascar [5]. Nauclea latifolium commonly known as pin cushion tree, is a straggling shrub or small tree of about 10ft high and is a native of the tropic, Africa and Asia. The leaves are broadly elliptic to round ovate. It is found in areas like Abuja, Enugu, Akwa Ibom, Cross River, Kontangora, Shaki and some other parts of Nigeria [6]. However, this plant has been over time used in the management of some other metabolic diseases in Nigeria. Progressive metabolic disorder characterized by hyperglycaemia mainly due to absolute (Type1 DM) or relative (Type2 DM) deficiency of insulin hormone. DM virtually affects every system of the body mainly due to metabolic disturbances caused by hyperglycaemia, especially if diabetes control over a period of time proves to be suboptimal. Until recently it was believed to be a disease occurring mainly in developing countries, but recent findings reveal a rise in number of new cases of type 2 DM with onset and associated complications in developing countries [7]. Diabetes is associated with complications such as cardiovascular diseases, nephropathy, retinopathy and neuropathy, which can lead to chronic morbidities and mortality. World Health Organization [7] estimates that more than 346 million people worldwide have DM. This number is likely to be more than double by 2030 without any intervention. Recent report, India today heads the world with over 32 million diabetes patients and this number is projected to increase to 79.4 million by the year 2030.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Ethanol (90%) was obtained from James Burrogh Limited, 60 Montford place London 99.9%v/v min, one touch plus blood glucometer strips which were purchased from Globus Chemical, 55 Mayne Avenue, Calabar, Cross River State, Nigeria. A7413-106 Streptozotocin was obtained from sigma – Aldrich, Inc, St Louis, Mo63103, USA. All routine assay kits were from Agape Diagnostic Switzerland GmbH. Langackerstress 29-6330-Switzerland were obtained from spectrum Egyptian Company for Biotechnology (S.A.E) Obour City industrial area. Block 20009 8 pieces 19A Cairo, Egypt, human insulin injection was obtained from Atrapid Novo Nordisk A/s, DK-2880 Bagsvaerd, Denmark, Needles and other syringes used were purchased from Peace Land Pharmacy Limited, 476 Ndidem Isang Iso road, opposite Calabar Municipal Council Calabar.

2.2 Methods

2.2.1 Collection of plant materials

Fresh and matured leaves of Nauclea latifolium was harvested from the Endocrine Research Farm while Cataranthus roseus were harvested from the staff village environment, University of Calabar, in August 2018. They were authenticated by a botanist Dr Mike Eko, Department of Botany, University of Calabar, Calabar and voucher specimens deposited in an herbarium in the Department of Botany. The leaves were rinsed severally with clean tap water to remove dust particles and debris followed with distilled water thereafter allowed to completely drain. The dry Nauclea latifolium and Cataranthus roseus leaves were blended with the use of Cornono (EL legitima) VC.I.A. S.A manual hand blended, (Medellin-Colombia) into powder and 3 kg of the Nauclea latifolium, was weighed out and soaked in 2000 ml of 80% ethanol while 800 g of Cataranthus roseus was weighed out and soaked in 1000 ml of 80% ethanol. The mixture was allowed for 48 hours in the refrigerator at 40°C for thorough extraction of the plant’s active components. These were then filtered with a cheese cloth and later with Whatman No.1 filter paper to obtain a homogenous filtrate. These filtrates were then concentrated in vacuo at low temperature (37 – 40°C) to about one tenth the original volume using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness yielding 34.9 g (3.49%) and 29 g (3.62%) respectively. The extracts were then refrigerated at 2 – 80°C until when used.
2.3 Animals

Thirty albino rats (males only) of Wistar strain weighing about 140-180 g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Calabar. The animals were divided into five (5) groups containing six (6) animals were allowed to acclimatize for three weeks in the animals' house of the Department of Biochemistry. The animals were housed in well ventilated cages (wooden bottom and wire mesh top) and kept under controlled environmental conditions of temperature (25±50°C), relative humidity (29±2%) and 12 hours’ light/dark cycle.

2.4 Method of Acute Toxicity Test LD 50

The acute toxicity study of *Cataranthus roseus* and *Nauclea latifolium* was carried out using the [8]. The LD50 value was determined, Confirmatory test was carried out and the LD50 was calculated from the graph of percentage (%) of mortality (converted to probit) against Log-dose of the extract.

2.5 Induction of Experimental Diabetes

2.5.1 Principle

Streptozotocin is approved by the U.S. Food and Drug Administration (FDA) for treating metastatic cancer of the pancreatic islets cells. Since it carries a substantial risk of toxicity and rarely curing the cancer, its use is generally limited to patients whose cancer cannot be removed by surgery. In these patients streptozotocin can reduce the tumour size and reduce symptoms (especially hypoglycaemia due to excessive insulin secretion by insulinomas).

2.6 Anti-diabetic Activity

Fasting blood glucose was determined after deprivation of food for 16 hours with free access of drinking water. Hyperglycaemia was induced by a single intraperitoneal injection of 100mg/kg body weight streptozotocin (STZ), a-Aldrich, Inc, St. Louis, Mo63103, U.S.A) in sterile saline. After 5 days of streptozotocin (STZ) injection, the hyperglycaemic rats (glucose level >8.3mmol/l) were separated and divided into different groups comprising of 6 rats each for the anti-diabetic study.

2.7 Extract and Drug Administration

Before administration, the extracts were reconstituted in normal saline (vehicle) and administered orally twice daily at a dose of 500 mg/kg body weight for the single dose of 250 mg/kg of the extracts of C. R and N. L, Insulin was administered at 5IU/kg b.wt, And the control animal received 0.2 mlq of normal saline (Placebo) Respectively.

2.8 Experimental Design

Diabetic animals were grouped as shown in Table 1 below.

<table>
<thead>
<tr>
<th>Table 1. Animal grouping and treatment scheme</th>
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<td>Groups</td>
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<td>4</td>
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<td>5</td>
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Animals were accordingly treated with extracts and insulin. The plant extracts were also determined from preliminary work in our laboratory whereas insulin dose, NPH (5IU/kg b.w) was adopted as previously used. The plant extracts were administered via oral gastric intubations, twice per day (10.00am; 4:00pm) in a 6 hours cycle and insulin was administered once per day postprandial (10.00am), subcutaneously (S.C.). Treatment lasted for 14 days and throughout this duration periodic changes in glucose and body weight were measured with the use of a glucometer and animal weighing balance respectively. The animals were maintained on rat pellets prepared by Vital Feeds, Jos, Plateau State, Nigeria and tap water, both the feed and water were provided ad. libitum.
2.9 Collection of Samples for Analysis

At the end of the 14 days' food was withdrawn from the rats and they were fasted overnight but had free access to water. They were then euthanized under chloroform vapour and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles. The blood was transferred into plain tubes and allowed to clot for about two hours, the clotted blood was thereafter centrifuged at 3,000rpm for 10 minutes to recover serum from clotted cells. Serum was separated with sterile syringes and needles and stored frozen until used for biochemical analysis.

2.10 Data and Statistical Analysis

Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons with the control group according to Punnett's method using SPSS software version 17. The value of P<0.05 were taken as significant.

3. RESULTS

The result in Table 2 shows that the phytochemicals present in *Cataranthus roseus* and *Nauclea latifolium*. Contains flavonoids, polyphenols and Alkaloids were found to be present in appreciable amount in *Cataranthus roseus* with saponins, tannins are found to be in traceable concentration. Also, saponins and hydrocyanide were detected at higher levels in *Nauclea latifolium* with flavonoids, polyphenols all in traceable amount.

3.1 Change in Fasting Blood Glucose (FBG) Level of Experimental Rat Model

The results in Fig. 1 illustrate the initial and final blood glucose level of diabetic rats. The results showed that at the initial period of treatment there was a significant increase (P<0.05) in fasting blood glucose of the insulin group. However, treatment with the extract of N.L and C.R, resulted in a significant (P<0.05) reduction in Fasting Blood Glucose (FBG) relative to diabetic control and insulin treated group. The reduction in serum glucose may be due to the regeneration of beta cells of the pancreas, which were destroyed by STZ [9]. Fig. 1 shows a presentation of the result of FBG that was observed in experimental period compared to the normal control. Upon treatment with both extracts and insulin respectively, a marked reduction in FBG was observed at the final day of the experimental period, which was significant (P<0.05) compared with the diabetic control and normal control. Therefore, this research reflected the beneficial effect of plant extracts on the glucose level of diabetes albino wistar rats. The reduction in glucose level in extract treated group may be due to the insulin-like effects of the extracts, as insulin increase glucose uptake by the cells. Reduction in glucose level of diabetic extract-treated group may also be due to the renewal of cell following extract administration. The renewal of cells in diabetics has been studied in several animal models. It has been suggested that regeneration of islet cells after the use of extract may be the primary cause of the recovery of STZ induced albino wistar rat.

3.2 Effect on Electrolyte Concentration

The effect of the two anti-diabetic plants *N. latifolium* and *C. roseus* indicated in Fig. 3 and Fig. 4, showed a representation of sodium, chloride and potassium ion concentration in diabetic rats. From the result, a significant decrease in sodium and chloride was observed in all treated groups with the extract and insulin at (P<0.05) compared to the normal control and related to the diabetic control. Also observed from Fig. 4 was a significant decrease in potassium concentration in all treated groups compared to both diabetic and normal control groups.

<table>
<thead>
<tr>
<th>Table 2. Phytochemical components of Ethanolic extracts of <em>Cataranthus roseus</em> and <em>Nauclea latifolium</em></th>
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<tr>
<td>Components</td>
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<tr>
<td>-------------------------------------</td>
</tr>
<tr>
<td>1. Flavonoids</td>
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<tr>
<td>2. Saponins</td>
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<tr>
<td>3. Polyphenols</td>
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<tr>
<td>4. Alkaloids</td>
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<tr>
<td>5. Tannins</td>
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<tr>
<td>6. Hydrocyanide (HCH)</td>
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Key + = Present, ++ = Highly present, +++ = Very highly present, N.D = Not Detected
Fig. 1. Initial and final blood glucose level of diabetic rats  
Values are expressed as mean ± SEM, n = 6, *Significantly different from NC at p<0.05  
a = p<0.05 vs DC, b = p<0.05 vs Insuline, c = p<0.05 vs NL

Fig. 2. Percentage change in blood glucose level of diabetic rats  
Values are expressed as mean ± SEM, n = 6, Also from Fig.2, percentage (%) change in the blood glucose  
showed a relative decrease in all the experimental groups
Fig. 3. Sodium and chloride ion concentrations of experimental rats
Values are expressed as mean + SEM, n = 6, *significantly different from NC at p<0.05
a = p<0.05 vs DC, b = p<0.05 vs Insuline, c = p<0.05 vs NL

Fig. 4. Potassium ion concentration of experimental rats
Values are expressed as mean + SEM, n = 6, *significantly different from NC at p<0.05
a = p<0.05 vs DC, b = p<0.05 vs Insuline
3.3 Effect of Treatment on Serum Lipid Profile of Experimental Rats

Triacylglycerol

The effect of the two anti-diabetic plants N. latifolium and C. roseus indicated in Fig. 5, triacylglycerol concentration shows a significant (P<0.05) decrease in all the treated groups compared to the diabetic control groups. Also the result in Fig. 6, total cholesterol concentration shows a significant decrease (P<0.05) in the treated groups compared to the diabetic control groups and normal control groups. However, from the Fig. 7 there is a significant increase (P<0.05) was observed in the HDL-C level in all the treated groups when compared to the diabetic control and the normal control groups. In Fig. 8, it was observed that there was a significant increase in LDL concentration (P<0.05) in the insulin group compared to the diabetic control group and a significant decrease in all the extract treated groups compared to the normal control. The result for VLDL shown a significant (<0.05) decrease in the extracts treated groups compared to the insulin group and the diabetic control group respectively in (Fig. 9). The presence of these substances may be responsible for their antihyperglycemic action.

10] had earlier in his report indicated that plants endowed with flavonoids, glycosides and polyphenols are likely to possess both hypoglycaemic and anti-hyperglycaemic properties. However, it is not known how the ethanolic extract of the leaves of N. latifolium exert its hypoglycaemic effect. Moreover, the hypoglycaemic activity of the leaves of the plant may be due to this secondary metabolite involved in the stimulation of the β-cells and subsequent secretion of insulin [11]. The significant decrease in electrolyte in the extracts treated groups may be attributed to the actions of the bioactive components, and suggests that these extracts may be nephrotoxic. Diabetes mellitus is associated with disturbance in electrolytes metabolism. Electrolytes are dissolved mineral used by the body to conduct electricity. Potassium, sodium and calcium are all important for proper electrolyte balance. Electrolytes are vital for proper electric signals in the heart. Electrolytes are salts that conduct electricity and are found in the body fluid, tissues and blood. Examples are chloride (C), calcium, Magnesium, sodium and potassium. Sodium (Na+) is concentrated in the (ICF) proper balance is essential for muscles co-ordination heart fluid absorption and excretion, nerve function and concentration [12]. The kidney regulates fluid absorption and excretion and maintain a narrow range of electrolyte function. Sodium and potassium are filtered and its secretion into the bile by the liver [13]. Too much or too little sodium (hyponatraemia) or potassium hyper or hyperkalaemia) caused by poor diet, hydration, medication and disease, results in an imbalance. Hyponatraemia is the most common electrolytes imbalance [14]. It is associated with kidney disease such as nephrotic syndrome and acute renal failure (ARF). Sodium is the major positive ion (cation) in fluid outside of cells too much or too little sodium therefore can cause cells to malfunction, and extremes of it in the blood can be fatal. Potassium is the major positive ion (cation) found inside of cells. The proper level of potassium is essential for normal cell function. An abnormal increase in potassium or decrease in potassium (hyperkalemia) can profoundly affect the nervous system and increase the chance of irregular heart beat (arhythmias), which when extreme can be fatal. An abnormally low level of potassium (K⁺) is called hypokalemia. The adrenal gland makes a hormone (aldosterone) that signals the kidneys to excrete or conserve potassium based on the body needs. Electrolytes play a vital role in maintaining homeostasis within the body. They help to

4. DISCUSSION

Diabetes mellitus (DM) is an endocrine disorder characterized by chronic hyperglycaemia with many disturbances of carbohydrate, fat, and protein metabolism due to decrease in insulin secretion. The result of this research reflected the beneficial effect of plant extracts on the glucose level of diabetes albino wistar rats. The reduction in glucose level in extract treated group may be due to the insulin-like effects of the extracts, as insulin increase glucose uptake by the cells. Reduction in glucose level of diabetic extract-treated group may also be due to the renewal of cell following extract administration. The renewal of cells in diabetics has been studied in several animal models. It has been suggested that regeneration of islet cells after the use of extract may be the primary cause of the recovery of STZ induced albino wistar rat. The presence of these substances may be responsible for their antihyperglycemic action.
regulate myocardial and neurological function, fluid balance, oxygen delivery, acid-base balance and much more, the most serious electrolyte disturbance involved abnormalities in the level of sodium and potassium. The result of the lipid profile from the study shows a significant decrease in TG, TC, LDL with a significant increase in HDL and VLDL. These increase shows that HDL serve as acceptor of cholesterol from various tissues [15]. They promote the removal of cholesterol from cells and its secretion into the bile by the liver [16]. This result further confirmed the use of these two traditionally used medicinal plants for the management of diabetic and related cardiovascular implications. The best single indicator of the likelihood of developing atherosclerotic heart disease is not total plasma cholesterol but rather the ratio of plasma LDL cholesterol to plasma HDL-cholesterol. High levels of HDL are negatively associated with the risk of coronary heart disease, high level of TG, which in the fasting condition are found mainly in VLDL, are positively related to the risk for coronary heart disease [17]. As LDL carries most of the plasma cholesterol, the total plasma cholesterol may also be a good index for the risk of coronary heart disease, when the high cholesterol level is not due to a high HDL level. However, the total cholesterol of HDL ratio may be the most potent or efficient predictor for the risk of coronary heart disease [18]. The extract may cause increase in HDL level by inducing APOA-1 production [19]. Suggested that increase in HDL levels after treated may be due to the induction of APOA-1 production. In the present study the anti-diabetic effect of the extracts Cataranthus roseus and Nauclea latifolium indicates that there were increase in AST and ALT levels thus suggesting that these extracts are hepatoprotective on the liver where these enzymes are synthesized [20]. However, the result of Albumin and total protein shows a remarkable decrease when compared to the normal control treated with the extracts of Nauclea latifolium and Cataranthus roseus. The findings suggest that the extracts may have the potentials to reverse the potential risk of hepatotoxicity but probably requires long durations for total restoration of the liver synthetic function. The results are consistent with the report by [21] on the effect of Nauclea latifolium leaves aqueous extracts on blood glucose levels of normal alloxan induced diabetic rats. Our finding on Cataranthus roseus and Nauclea latifolium was consistent with earlier reports on the beneficiary importance of the two medicinal plants.

Fig. 5. Triglyceride concentration of experimental rats
Values are expressed as mean + SEM, n = 6, *significantly different from NC at p<0.05. a = p<0.05 vs DC, b = p<0.05 vs Insuline, c = p<0.05 vs NL
Fig. 6. Total cholesterol concentration of experimental rats
Values are expressed as mean + SEM, n = 6, *significantly different from NC at p<0.05
a = p<0.05 vs DC, b = p<0.05 vs Insulin, c = p<0.05 vs NL

Fig. 7. High density lipoprotein concentration of experimental rats
Values are expressed as mean + SEM, n = 6, *significantly different from NC at p<0.05
Fig. 8. Low density lipoprotein concentration of experimental rats
Values are expressed as mean ± SEM, n = 6, *significantly different from NC at p<0.05
a = p<0.05 vs DC, b = p<0.05 vs Insulin, c = p<0.05 vs NL

Fig. 9. Very low density lipoprotein concentration of experimental rats
Values are expressed as mean ± SEM, n = 6, *significantly different from NC at p<0.05
a = p<0.05 vs DC, b = p<0.05 vs Insulin, c = p<0.05 vs NL
Fig. 10. Total protein concentration of experimental rats
Values are expressed as mean + SEM, n = 6 *significantly different from NC at p<0.05
a = significantly different from DC at p<0.05, b = p<0.05 vs Insuline, c = p<0.05 vs NL

Fig. 11. Aspartate aminotransferase concentration of experimental rats
Values are expressed as mean + SEM, n = 6, *significantly different from NC at p<0.05
a = significantly different from DC at p<0.05, b = p<0.05 vs Insulin
Fig. 12. Alanine aminotransferase concentration of experimental rats
Values are expressed as mean ± SEM, n= 6, *Significantly different from NC at p<0.05
a = significantly different from DC at p<0.05, b = p<0.05 vs Insulin

5. CONCLUSION

The findings of the present research were concluded that the ethanolic extracts of *N. latifolium* and *C. roseus* have a beneficial effect on serum level of glucose, lipid profile, serum enzymes and electrolyte. This study also exposes the therapeutic value of this medicinal plants and its efficacy in the management of diabetes and it related complication.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES