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Effects of ethanol fruit rind extract of *Nephelium lappaceum* on hematological indices, blood glucose, body and organ weights of alloxan-induced diabetic albino Wistar rats.

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Abstract

The study investigated the effect of ethanolic fruit rind extract of *Nephelium lappaceum* on blood glucose, body and organ weights, and hematological indices in alloxan-induced diabetic albino Wistar rats. Sixty male albino Wistar rats weighing between 116 and 231 g were randomly assigned to six groups of ten rats in each group. Groups 1-5 rats were induced with 150 mg/kg body weight of alloxan monohydrate intraperitoneally. Fasting blood glucose was measured four days after induction; rats with blood glucose levels of 200 mg/kg and above were considered diabetic. Group 4 was treated with 50 mg/kg body weight of metformin. Group 5 was the diabetic untreated group that equally had alloxan, and group 6 was the normal control group. Group 1-3 were treated with 500, 1000, and 1,500 mg/kg body weight of extract, respectively, for 28days. Glucose levels were checked weekly using a glucometer. Rats treated with Nephelium kappacism peel extract showed a dose-dependent decrease in glucose levels when compared to the untreated diabetic group. The result of the study also revealed a significant reduction ($p<0.05$) in body weights of the untreated diabetic group compared to the normal group. However, treatment of the rats with the extract showed improved body weight when compared to untreated rats. The hematological parameters such as RBC, HGB, and HCT were significantly reduced ($p<0.05$) in the untreated group compared to the normal group. The study also showed a low level of MCH and MCHC in the untreated diabetic group. The administration of peel extracts improved the production of RBC and its indices in the diabetic rats. The WBC, however, showed a significant ($p<0.05$) increase in the diabetic untreated group compared to the normal group. Administration of peel extract reduced the production of WBC, indicating that the extract had an anti-inflammatory effect on the rats, while PLT level was significantly high in the experimental groups compared to the normal control. The result of this study suggests that the extract possesses hematinic and antidiabetic potentials, which could be used in the management of diabetes.

Keywords: Diabetes; Alloxan; Hematology; *Nephelium lappaceum*; Metformin

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by an increase in blood glucose levels that results from a defect in insulin secretion, insulin action, or both. The following clinical symptoms may occur: polyuria, increased thirst, blurred vision, and weight loss. It may also lead to coma and eventually death in a severe case [1]. Medicinal plants are globally used extensively for the treatment of various diseases. Based on World Health Organization comments, investigation of hypoglycemic agents from medicinal plants has gain much attention and importance in the field of medicine [2]. Medicinal plants are also used with the intention of maintaining good health and for financial benefits to people in rural areas who harvest, process, and distribute them for the treatment of various diseases [3]. Effective management of diabetes is crucial as its halts the aggravation of diabetes associated complications such oxidative stress, hypercholesterolemia, hyperlipidemia, retinopathy etc. [4]. In search for the treatment of diabetes, several plants have been screened and reported to possess antidiabetic effects. Among such plants is *Nephelium*

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lappaceum. *Nephelium lappaceum* is a tropical fruit that belongs to the sapindaceae family. It is a fruit that originates from Malaysia but is grown in other parts of the world. The name is derived from Malay word "Rambut", which means "Hair" [5]. Pharmacological studies of this plant reveal that it possesses antioxidant, antimicrobial, and analgesic properties. Despite the use of this plant in health management, only a few studies have been conducted on its anti-diabetic properties and protective effects. Therefore, the present study was designed to investigate the effects of ethanolic extract of *Nephelium lappaceum* fruit peels on some haematological indices, blood glucose, body and organs weights of alloxan-induced diabetic rats in order to evaluate the safety or toxicological potentials of the extract.

2. Materials and Methods

2.1. Collection and Preparation of Plant Extract

Fresh fruit peels of *Nephelium lappaceum* were collected at Ibok Ndiya in Ikono Local Government Area, Akwa Ibom State, Nigeria. The fruits were identified and authenticated by a botanist in the Department of Biological Sciences, Akwa Ibom State Polytechnic, Ikot Osurua. The fresh peels were cut off from the fruits, sorted, rinsed with distilled water, and then sliced and dried in the shade. The dried fruit peels were then ground into powder form using an electric blender, Kenwood model 309 Japan. 600 g of the powdered sample was extracted in 70% ethanol for 72 hours at room temperature. The macerated fruit peels were filtered using Whatman No. 1 Filter paper. The filtrate was concentrated for 3 days in a water bath at 40 0C and stored at 4 0C in the refrigerator for the various analyses.

2.2. Experimental Design

Sixty albino Wistar rats were divided into 6 groups of 10 rats each

- Group 1 - Diabetic rats treated with 500 mg/kg body weight of extract
- Group 2 - Diabetic rats treated with 1000 mg/kg body weight of extract
- Group 3 Diabetic rats treated with 1500 mg/kg body weight of extract
- Group 4 Diabetic rats treated with 50 mg/kg body weight of metformin
- Group 5 - Diabetic untreated group
- Group 6 - Normal control group.

The experiment lasted for 28 days, and the treatment was administered orally once a day for 28 days.

3. Methods

3.1. Induction of Diabetes

Sixty (60) male albino Wistar rats weighing 116-231 g were obtained from an animal farm in Uruk Uso Village, Ikot Ekpene Local Government Area, Akwa Ibom State, Nigeria. The rats were divided into six (6) groups of ten rats in each cage and acclimatized for one week before the commencement of the experiment. Diabetes was induced in the rats through intraperitoneal administration of 150 mg/kg body weight of alloxan monohydrate, which was dissolved in 0.9% saline. All the rats were allowed free access to feed and clean water *ad libitum*. Four days after induction, a blood sample was obtained by puncturing the tip of the rats to confirm diabetes using a glucometer. Rats with fasting blood glucose concentration 200 mg/dl and above were considered diabetic, then selected for the experiment [6].

3.2. Determination of Blood Glucose Concentration

Fasting blood glucose was determined six (6) times during the period of the study. First, before the induction of diabetes with alloxan. Thereafter, 72 hours after induction, 7 days, 14 days, 21 days and 28 days of treatment, respectively. Blood glucose concentration in the rats was measured using a glucometer. A sterile lancet was used to prick the tip of the tail of each rat to obtain blood after an overnight fast. Blood was then introduced into the glucometer using a fine test strip.

3.3. Determination of Body Weights

The body weight of the rats was taken with the use of an electronic weighing balance. Each rat in each group was weighed six times during the course of the experiment.

3.4. Blood Sample Collection for Analysis

At the end of 28 days of treatment, the rats were made to fast overnight and then euthanized under chloroform vapour and sacrificed. Whole blood was obtained by cardiac puncture into non-heparinized tubes and was allowed to clot for 1 hour and 30 minutes. The sample was then centrifuged at 4000 rpm for 30 minutes to extract the serum for the various biochemical assays.

3.5. Determination of Organs Weight

Organs of interest were harvested from each rat and weighed with the use of analytical weighing balance.

3.6. Determination of Hematological Parameters

Hematological parameters were determined using an automated hematology analyzer (Mythic 18 machine). A blood sample was introduced into the machine through the probe, the instrument executed automatic analysis, and the result was displayed on the screen.

3.7. Statistical Analysis

The data obtained from the experiment were presented as mean ± standard error of the mean. The results were analyzed by one-way analysis of variance (ANOVA). Differences between means were considered significant at $p<0.05$ by Bonferroni multiple range test.

4. Results

Table 1 Effect of Ethanolic Extract of ***Nephelium lappaceum*** Fruit rind on Hematological Indices of Alloxan-Induced Diabetic Wistar Rats

GPS	WBC ($10\times^{9}$/l)	NEU (%)	LYM (%)	MON (%)	EOS (%)	BAS (%)	RBC ($10\times^{6}$Ul)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (Pg)	MCHC(g/dl)	PLT ($10\times^{3}$Ul)
1	8.70±0.61	3.62±0.22	4.37±0.53	0.80±0.27	0.26±0.04	0.02±0.00	8.84±0.14	15.91±0.38	60.33±2.69	68.70±1.82	18.28±0.09	26.50±0.71	775.25±24.12
2	10.28±0.75	4.39±0.15	5.85±0.27	0.89±0.03	0.30±0.13	0.01±0.00	8.72±0.14	15.65±0.06	56.43 ± 1.24	63.58 ± 0.50	18.18 ± 0.26	28.20±0.53	764.25±11.76
3	7.07 ± 0.30	2.10±0.32	5.09±0.57	0.05±0.01	0.08±0.03	0.02±0.00	8.95±0.04	15.45 ± 0.18	53.28 ± 1.37	60.10 ± 1.57	17.70 ± 0.42	28.66±0.44	516.50±61.51
4	10.59±0.12	4.39±0.07	5.50±0.08	0.10±0.28	0.19±0.02	0.02±0.00	9.45±0.12	16.78±0.38	60.30±0.40	62.20±0.31	17.85±0.56	24.18±3.63	551.50±10.57
5	8.10 ± 0.04	2.66 ± 0.78	4.68 ± 0.13	0.13± 0.02	0.13 ± 0.01	0.08 ± 0.02	7.44 ± 0.19	14.08 ± 0.33	49.60 ± 0.33	71.90 ± 2.20	16.58 ± 0.91	25.100.34	869.75 ± 9.76
6	6.81 ± 0.44	3.14 ± 0.04	4.72 ± 0.16	0.16 ± 0.01	0.16 ± 0.01	0.03 ± 0.00	9.84 ± 0.32	17.32 ± 0.22	57.58 ± 1.04	65.25 ± 2.04	18.05 ± 0.44	28.75±0.40	414.25 ± 6.75

Result presented as mean ± standard Error of Mean (SEM). Values are considered statistically significance at $p<0.05$ KEY: WBC – white blood cells, NEU – neutrophile, LYM – Lymphocytes, MON - Monocytes, EOS - Eosinophils, BAS - Basophil. RBC - Red Blood Cell, HGB - Hemoglobin, HCT - Hemoglobin concentration, MCV - Mean Corpuscular Volume, MCH - Mean Corpuscular Hemoglobin, MCHC - Mean Corpuscular Hemoglobin Concentration, PLT - Platelets

Table 2 Effect of Ethanolic Extract of ***Nephelium lappaceum*** Fruit rind on Glucose Level of Alloxan-Induced Diabetic Wistar Rats

Groups	Blood Glucose(mg/dl) before Induction	Blood Glucose (mg/dl) 4 days after Induction	Blood Glucose (mg/dl) after 1 Week treatment	Blood Glucose (mg/dl) After 2 Weeks Treatment	Blood Glucose (mg/dl) After 3 Weeks Treatment	Blood Glucose (mg/dl) After 28 Days Treatment
1	79.75 ± 2.46	215.00 ± 8.22	225.04 ± 5.81	219.01 ± 8.42	217.00 ± 8.69	211.50 ± 6.89
2	74.00 ± 1.08	226.25 ± 10.41	229.11 ± 6.72	218.04 ± 7.34	206.41 ± 3.68	200.50 ± 11.32
3	90.00 ± 5.64	230.00 ± 8.86	226.92 ± 11.48	226.61 ± 8.22	199.85 ± 8.52	197.25 ± 7.66
4	77.75 ± 1.93	222.75 ± 10.05	201.61 ± 7.68	184.46 ± 10.41	167.76 ± 10.55	149.25 ± 22.47
5	78.25 ± 7.19	230.00 ± 7.76	236.01 ± 8.41	231.11 ± 4.76	248.92 ± 7.69	249.25 ± 7.42
6	70.75 ± 1.80	72.50 ± 1.76	71.48 ± 2.06	76.59 + 1.66	75.91 ± 3.41	75.00 ± 2.86

Result presented as mean ± standard Error of Mean (SEM). Values are considered statistically significance at $p<0.05$

Table 3 Effect of Ethanolic Extract of ***Nephelium lappaceum*** Fruit rind on Body Weight of Alloxan-Induced Diabetic Wistar Rats

Groups	Initial Body Weight (g)	Body Weight (g) After Induction	Body Weight (g) After 28 Days Treatment
1	116.75 ± 6.57	101.50 ± 6.34	114.25 ± 6.26
2	152.00 ± 4.69	133.25 ± 3.04	149.75 ± 4.48
3	169.75 ± 2.14	144.75 ± 8.98	165.25 ± 2.50
4	190.00 ± 5.12	163.00 ± 7.38	181.75 ± 4.55
5	213.00 ± 2.74	207.50 ± 8.59	205.00 ± 4.71
6	231 .00± 5.21	252.25 ± 8.49	232.50 ± 5.92

Result presented as mean ± standard Error of Mean (SEM). Values are considered statistically significance at $p<0.05$.

Table 4 Effect of Ethanolic Extract of ***Nephelium lappaceum*** Fruit rind on Organs Weight of Alloxan-Induced Diabetic Wistar Rats

Groups	Liver (g)	Pancreas (g)	Tastes (g)	Lungs (g)	Spleen (g)	Kidney (g)
1	6.47 ± 0.48	0.75 ± 0. 10	2.20 ± 0.07	1.19 ± 0.15	0.44 ± 0.03	0.83 ± 0.04
2	6.67 ± 0.55	0. 66 ± 0.07	1.99 ± 0.17	1.26 ± 0.13	0.56 ± 0. 04	0.92 ± 0.06
3	6.21 ± 0.09	0.72 ± 0.02	2.30 ± 0.10	1.16 ± 0.07	0.73 ± 0.04	1.07 ± 0.13
4	6.26 ± 0.08	8.38 ± 0.02	2.13 ± 0.10	1.05 ± 0.02	0.91± 0.03	0.86 ± 0.07
5	7.33 ± 0.21	0.79 ± 0.04	2.13 ± 0.05	1.01 ± 0.03	0.91 ± 0.04	1.03 + 0.02
6	7.78 ± 0.10	0.93 ± 0.03	2.37 ± 0.10	1.11 ± 0.06	0.94 ± 0.02	1.03 ± 0.04

Result presented as mean ± standard Error of Mean (SEM). Values are considered statistically significance at $p<0.05$.

5. Discussion

Diabetes is a metabolic disease characterized by high blood glucose levels due to a total or relative deficiency of circulating insulin levels. The maintenance of glucose levels at homeostatic pace is crucial, as abnormal glucose levels could underscore the development of some clinical complications. Phototherapeutic approach or phytomedicines have gained great attention in recent times for ethnobotanical potential in disease management, particularly in resource-limited regions where cost and access to modern medications are not common. The present study documents the effects of ethanolic peel extract of *Nephelium lappaceum* on the blood glucose, body and organ weights, and some hematological indices of alloxan-induced diabetic rats. Alloxan is a chemical that induces diabetes by destroying β-cells of the islets of Langerhans and exerts a reduction in insulin release, therefore inducing hyperglycemia [7]. The present study confirmed that intraperitoneal administration of alloxan significantly increased the blood glucose levels in experimental rats when compared to the normal control rats. This is in line with the report of [8]. The upsurged glucose levels are an indication of the impaired insulin release due to the destruction of the β-cells of the pancreas. The mechanism of destruction has been reported to be via the generation of reactive oxygen species by alloxan.

However, oral administration of ethanolic peel extract of *Nephelium lappaceum* revealed a significant dose-dependent decrease in glucose levels when compared to the untreated diabetic group. The current results lend credence to the report of [9] that *Nephelium lappaceum* exhibited anti-hyperglycemic activity in vivo through the inhibition of α-glucosidase and α-amylase. The study also aligned with another study by [10], who reported that geranin, the bioactive compound in *Nephelium lappaceum* rind extract, had more profound inhibiting effects on both enzymes (α-glycosidase and α-amylase). Treatment of the diabetic rats with metformin showed a greater reduction in glucose level than the extract, although still significant. Thus, the decrease in glucose levels following the administration of Nephelium kappacism in treatment may be due to the presence of this bioactive compound (geranin) and other phytochemicals. The destruction of the pancreas by alloxan through oxidative stress may have been ameliorated by the extract, indicating the presence of antioxidant molecules. According to [10], geranin, the main bioactive compound in rambutan peels, possesses potent antioxidant activity. This suggests that rambutan peels may act by improving peripheral utilization of glucose, inhibition of hepatic glucose synthesis/ renal glucose reabsorption, and up-regulation of insulin sensitivity.

Body weight evaluation is one of the general indicators employed in assessing the metabolic regulation for diabetes. The study indicated a significant reduction in body weight in the untreated diabetic group when compared to the normal group. The decrease in body weight may lend credence to the fact that diabetes is associated with increased glycogenolysis, lipolysis, and gluconeogenesis, which result in muscle wasting and excessive breakdown of tissue proteins [11].

Histological findings [9], also support that diabetic rats showed reduced islet size and islet cell chamber and hepatic domain. Treatment of the rats with the peel extract resulted in improved body weight when compared to the untreated. It may be that the extract caused the system to shift to carbohydrate as an energy source, with the preservation of proteins and fat, which resulted in the prevention of body weight decrease. Also, the increased body weight of the treated groups may be attributed to the shielding property in monitoring muscle wasting by reversing lipolysis, glycogenolysis, gluconeogenesis, and increased lipogenesis [12].

Hematological evaluation is another important approach in assessing the health status of an individual, as an elevation or reduction in one or more of the hematological indices could be an indication of a disease condition or an injury to the blood-producing organs [13]. The present study suggests that hematological parameters are altered in diabetes, as RBC, HGB, and HCT were significantly reduced in the untreated diabetic groups when compared to the normal group. This is similar to the report of [14] (2018). The occurrence of anemia in diabetic mellitus has been reported to be due to the increased non-enzymatic glycosylation of RBC membrane proteins [3]. The oxidation of these proteins increases the production of lipid peroxides, which leads to hemolysis of RBC [15].

Glucose accumulation in diabetes also results in the depletion of NADPH through the polyol pathway that eventually leads to sorbitol production [16]. Blood cells, especially the RBCs, are destroyed by a concert of events from the superoxide radical produced in ETC in the mitochondria, with the declined availability of reduced glutathione and NADPH, which are consumed in the polyol pathway, results in anemia. Nephropathy is reported to be one of the clinical complications in diabetes that further leads to low RBC as a result of impairment in the production of erythropoietin that signals the production of red blood cells [17]. The decreased hemoglobin and PCV in the diabetic group may be due to microcytic anemia, which is reported to occur when the RBCs are too small and fragile to accommodate sufficient hemoglobin [13]. The present study also recorded a low level of MCH and MCHC in the untreated diabetic group. MCHC, as a diagnostic test, helps to identify the cause of anemia. The low level of MCHC may suggest insufficiency of hemoglobin

content, which is a reflection of the low level of hemoglobin in this report. Administration of rambutan peel extract ameliorated the red blood cells and their indices in diabetic rats. The improved blood levels may be due to improved or enhanced erythropoietin activity, repair of renal injury occasioned by the ROS generated antioxidant effect of the extract, making the plant possess hematinic potential. White blood cells (WBC) showed a significant increase in the diabetic group when compared to the normal control, which aligns with [18]. Increased WBC count is a marker of inflammation, and several reports have linked WBC count abnormality with diabetic risk [19]. Also, advanced glycation and products, oxidative stress, and cytokines activate leukocytes in a hyperglycemic state, thereby increasing the inflammatory state and the development of vascular complications in diabetes [20]. The WBC was improved following administration of peel extract, indicating the anti-inflammatory potential of the extract. PLT level was significantly high in the experimental groups when compared to the control, indicating that the thrombolytic domain was affected by the alloxan even upon administration of the exact dose.

6. Conclusion

Several plants possess potent therapeutic molecules that are useful for the treatment or management of an array of clinical challenges. *Nephelium lappaceum* is one of such plants that exerted potent antidiabetic and hematinic effects on alloxan-induced diabetic animals, with a significant improvement in the body and organ weights.

Compliance with ethical standards

Acknowledgment

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Statement of ethical approval

The use of animals for the study was carried out in accordance with guidelines set by the Institute for Laboratory Animal Research (ILAR) (2000). Hence, no ethical conflicts exist with the use of animals in this work.

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