Original Research Article

Haemoglobin and packed cell volume (PCV) of high-fat diet/streptozotocine-induced diabetic Wistar Rats treated with ethanol extract of a herbal mixture (Aju Mbaise).

ABSTRACT

Aim: This study was carried out to evaluate the effect of ethanol extract of Aju Mbaise herbal mixture on some haematological indices of diabetic Wistar albino rats.

Sample: Packed cell volume (PCV) and haemoglobin (Hb) concentration was estimated in diabetic rats treated with ethanol extract of Aju Mbaise herbal mixture.

Study design: In the course of the experiment, fifty-four (54) rats with initial weight range of 30 – 40g were grouped into 6 of 9 rats per group. The first group served as the normal control (NC) while the remaining five groups were induced with diabetes type 2 using high-fat diet for 8 weeks and streptozotocin at 35mg/kg body weight. Group II served as the diabetic control while the remaining groups (III, IV, V & VI) were treated with metformin and three different concentrations of the plant extract respectively.

Place and Duration of Study: The study was carried out in the Animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, between July 2018 and January 2019.

Methodology: The haemoglobin and packed cell volume were estimated after 4^{th}, 8^{th} and 12th week of treatment using MINDRAY Auto-Haematology analyzer.

Results: From the results obtained, it was observed that the diabetic control group has a PCV and haemoglobin concentration that is significantly ($P<.05$) lower when compared to that of the normal control group and the other treated groups.

Conclusion: The study has shown that Aju Mbaise herbal mixture is a haematopoietic agent as it had the tendency to synthesize blood cells.

Keywords:

Aju Mbaise, Haematopoietic, Streptozotocin, Metformin and Mindray.

Introduction

Diabetes mellitus is a chronic widely spread human autoimmune disease associated with abnormally high level of sugar (glucose) in the blood. According to [1], diabetes mellitus describes

a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. According to Nishimura [2], a critical evaluation of mortality patterns by race showed that blacks had a higher mortality rate than whites, and it also affect individuals of all ages [1]. According to [3], patients with diabetes mellitus show a significant derangement in various haematological parameters. A high prevalence of anaemia was identified in type-2 diabetic patients [4]. Thus, about 27% of diabetics' patients are anaemic [5]. Diabetes mellitus has been regulated with the use of some antidiabetic drugs, including insulin and other biochemical hypoglycemic agents such as tolbutamide, phenformin, troglitazone, rosiglitazone and repaglinide. According to [6], these therapies has shown to control the blood glucose level only when they are regularly administered, but also are tedious and have several undesirable side effects and fail to significantly alter the course of diabetic complications [7]. Thus, gave rise to the use of medicinal plant for the treatment and/or control of diabetes mellitus. The resource plant 'Aju Mbaise' is a traditional medicine, composed of combination of different leaves, roots and trunk of medicinal tree wrapped together and is taken in the form of concoctions. [8], reported that this plant decoction has good amount of quality proteins, minerals and vitamins, and also possesses antibacterial activity. The ability of this plant to demonstrate such quality is dependent on the accumulated natural products, biologically active materials and ingredients found in them. Due to the phytochemicals, minerals, vitamins, and nutrients present in the individual plants that make up Aju Mbaise, there is a likelihood that this plant possesses haematopoietic activity. This research work encompasses the induction and treatment of type-2 diabetes mellitus which is a chronic condition that affects the glucose metabolism in the body. To achieve the outlined objectives of this research work, high fat diet (HFD) and streptozotocin was used to induce type 2 diabetes mellitus, while the ethanol extract of Aju Mbaise plant samples was used for the treatment of the diabetes for a period of twelve (12) weeks, and the plant's effect on packed cell volume (PCV), and haemoglobin (Hb) concentration of female Wistar albino rats was measured with an auto-haematology analyser.

Materials and Methods

Procurement of Experimental Animals

A total of fifty-four (54) female Wistar albino rats was used for this experiment. They were acquired from the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State. They were housed in the animal house of the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria. The animals were left for 1 week to acclimatize to the

laboratory conditions during which they were administered normal feed (Top feeds- grower's mash) and clean water. The animals were later grouped into six groups of nine animals per group.

Collection of Plant Samples

Fresh samples of the plants that make up Aju Mbaise were collected at Obodo Ujichi, Ahiazu and Amuzi, Ahiara Towns, both in Aboh Mbaise L.G.A, of Imo State, Nigeria. The fresh plants after collection were air-dried, cut into small pieces and blended before the extraction process. The extraction was done with ethanol as the solvent.

Preparation of Extract

The whole plants parts (leaves and stem) were washed, air dried and blended to a powdered form. Powdered sample weighing 1,000g was soaked in 3,000ml of 95% ethanol for 48 hours after which it was sieved using a muslin cloth and afterwards filtered through a Whatmann filter paper No. 1. The filtrate was concentrated using a rotary evaporator at 45^{o} C and afterwards placed on a thermostatic water bath for further drying. The concentrate (paste) was collected, weighed, kept in sterile bottles and stored at 4^{o} C until usage.

Preparation of high fat diet

The high fat diet was compounded according to the method of [9], using standard laboratory chow (Top feed) growers mash, lard and sucrose in the ratio of 3:1:1 respectively. The diet was carefully homogenized, then fed to the animals (groups II to VI) with the exception of the normal control group.

Induction of Type 2 Diabetes mellitus

A single dose intraperitoneal injection of $35mgkg^{-1}$ body weight of streptozotocin was used to induce type 2 diabetes mellitus to the experimental animals in groups II to VI. Respective doses of the streptozotocin were dissolved in 0.2ml normal saline per rat before administration. The development of hyperglycaemia in the rats was allowed for 7 days after the streptozotocin injection and fasting blood glucose levels checked before the commencement of treatment. The treatment was done by oral administration of the drugs and extracts using intra-gastric gavage daily for 12 weeks. At every 4 weeks interval, 3 animals were fasted overnight, anaesthetized, sacrificed and blood samples collected using EDTA for haematological analyses.

Method of analysis

The PCV and haemoglobin concentration was estimated using a MINDRAY auto-haematology analyzer. The blood sample was mixed with a sample mixer for 2-5 minutes, after which it was introduced into the auto-analyzer and 250µl of the blood sample was aspirated by the analyzer. The haematological parameters to be analyzed were selected and the machine was allowed to run for 2-3 minutes after which the results were printed out from a printer.

Experimental Design

The experimental female Wistar albino rats were grouped into six groups of nine animals. Group I animals (normal control) are non-diabetic, Group II (diabetic control) animals are diabetic rats that remained untreated throughout the experimental period. Groups III to VI animals were made diabetic but were treated with a known antidiabetic drug (7.2mgkg^{-1} body weight metformin), and three different concentrations (500mg/kg, 250mg/kg & 100mg/kg) of the herbal mixture extract.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Statistical Analysis

Data were presented as Mean ± standard error of mean. The results were analyzed using one way ANOVA and Tukey HSD Post Hoc Test with significance at $P< 0.05$.

Results

The result of the PCV and haemoglobin level were presented below (Tables 1 & 2).

Table 1: Effect of Aju Mbaise herbal mixture extract on Packed Cells Volume (PCV) in HFD/STZ-induced female diabetic rats

	PCV (%)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	42.00±2.08^{b}	43.00±2.08^{b}	39.33±0.88
DC	35.33±2.03^{a}	36.00±1.53^{a}	36.33±1.45
Metformin	40.67±0.88^{b}	41.67±0.33^{b}	38.33±1.45
500mg Extract	39.33±0.88^{ab}	38.33±2.19^{ab}	38.67±0.67
250mg Extract	38.33±2.03^{ab}	40.33±1.20^{ab}	37.33±1.86
100mg Extract	39.67±0.67^{ab}	38.33±0.67^{ab}	38.00±1.00

Values represent Mean ± SEM, and n = 3. Groups with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500mg Extract** = Treated with 500mg/kg of the extract of the cocktail herbal mixture; **250mg Extract** = Treated with 250mg/kg of the extract of the cocktail herbal mixture; **100mg Extract** = Treated with 100mg/kg of the extract of the cocktail herbal mixture.

Table 2: Effect of Aju Mbaise herbal mixture extract on haemoglobin (Hb) level in HFD/STZ-induced female diabetic rats

	Hb (g/dl)		
Groups	**Week 4**	**Week 8**	**Week 12**
NC	12.67±0.88	13.33±0.33^{b}	12.97±0.09
DC	10.67±1.20	9.67±1.76^{a}	11.30±0.98
Metformin	11.43±0.98	11.00±0.58^{ab}	11.77±0.96
500mg Extract	11.30±0.74	11.63±0.74^{ab}	11.87±0.83
250mg Extract	11.47±0.45	10.57±0.66^{ab}	11.17±0.69
100mg Extract	10.93±1.03	9.60±0.31^{a}	11.53±1.47

Values represent Mean ± SEM, and n = 3. Groups with different Superscript(s) are significantly different at $p<0.05$, while groups with same superscript(s) are not.

Key: NC= Normal control; **DC=** Diabetic control; **Metformin**= Treated with metformin; **500mg Extract** = Treated with 500mg/kg of the extract of the cocktail herbal mixture; **250mg Extract** = Treated with 250mg/kg of the extract of the cocktail herbal mixture; **100mg Extract** = Treated with 100mg/kg of the extract of the cocktail herbal mixture.

Discussion

The present study showed that diabetes mellitus has effect on some haematological parameters such as packed cell volume (PCV) and haemoglobin. According to [10], a wide range of haematology laboratory values change significantly in patients with diabetes. In this study, PCV which is an index of anaemia was significantly lower among diabetic subjects when compared to the non-diabetic controls and the treated animals. Anaemia is known to be prevalent among diabetic's patients and may also be significant in determining the outcome of heart failure and hypoxia-induced organ damage in patients with diabetes [11]. Due to the incomplete life span of

erythrocytes in diabetic condition, Hb concenteration which depends on plasma volume and erythrocyte mass is lowered [12]. Also according to [13], anaemia has been seen to occur in diabetic mellitus condition and this may be due to the increase in non-enzymatic glycosylation of erythrocyte membrane proteins which correlates with hyperglycemia. Oxidation of these glycosylated membrane proteins can cause an increase in the production of lipid peroxides causing haemolysis of the red blood cells [14]. [15], reported that diabetes related anaemia has been observed in diabetic nephropathy, and about 20% of those with type 2 diabetes may eventually develop kidney damage and later kidney failure. It was also suggested that diabetic anaemia may be because of insufficient androgen releasing function of adrenal glands or less erythropoietin concentration [16]. In other study of [17], it was also suggested that anaemia has a high prevalence in type 2 diabetic patients and it has high correlation with kidney disorders. [18], also reported that diabetes incorporation with kidney nephropathy can cause anaemia. Anaemia may also be significant in determining the outcome of heart failure and hypoxia-induced organ damage in diabetes. While several factors contribute to the increased prevalence of anaemia in diabetes, the failure of the kidney to increase erythropoietin in response to falling haemoglobin appears to be the dominant factor. In this study, Wistar albino rats were induced diabetes mellitus type-2 with administration of high fat diet and a low dose (35 mg/kg) injection of streptozotocin. The experimental animals were treated with a standard antidiabetic drug (metformin) and three (3) different concentrations of extract of cocktail of Aju Mbaise herbal mixture. From the result of the experiment presented in Table 1, it was observed that there was a significant decrease in PCV of the diabetic control animals when compared to that of the normal control and diabetic animals treated with metformin and the three different concentrations of the herbal mixture extract, throughout the experimental period. This was also noted in the Haemoglobin result presented in Table 2; where there was a significant decrease in haemoglobin concentration of the diabetic control animals when compared to that of the normal control and diabetic animals treated with metformin and the three different concentrations of the herbal mixture extract. The results obtained from this study is in agreement with other studies by [19, 20], that found decreased hematological parameters (PCV, Hb, and RBC) in type 2 diabetic patients. Similarly, a previous study observed a lower mean values of RBC, Hb, PCV and MCHC in diabetic patients when compared to the control group, indicating the presence of anaemia in the former group [21]. There may also be decreased PCV in patients with type-1 diabetes because of the higher levels of blood glucose which can potentially cause intracellular dehydration. This finding does indicates that PVC may not be a good marker for the monitoring of anaemia among type-1 diabetic patients. Thus, it may be better to use haemoglobin level in the monitoring of anaemia among patients with type-1 diabetes. The ability of the Aju

Mbaise herbal mixture extract to improve/increase the PCV and haemoglobin levels in diabetic animals could be attributed to the phytochemicals as well as the minerals and vitamins content of the herbal mixture.

Conclusion

This study has shown that the mean values of PCV and haemoglobin are lower among diabetic Wistar albino rats when compared to the non-diabetic controls and the diabetic rats treated with metformin and ethanolic extract of Aju Mbaise herbal mixture respectively. It was observed that the administration of the cocktail herbal mixture of Aju Mbaise increased PCV and haemoglobin level in diabetic rats. Thus, this herbal mixture is a potential erythropoietic agent.

Competing Interests

Authors have declared that no competing interests exist.

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