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RESEARCH ARTICLE

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Hypoglycaemic effects of melatonin and ethanol extract of *Azadirachta indica* administration on blood glucose levels in streptozotocin-induced diabetic Wistar rats

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ABSTRACTS

The study was aimed at evaluating the hypoglycaemic effects of melatonin and Azadirachta indica administration on blood glucose levels in streptozotocin (STZ) induced diabetic Wistar rats. To achieve this, thirty Wistar rats were used in the study. The animals were assigned into the following groups: Group 1: Normal control + normal saline orally Group 2: Diabetic control + normal saline orally; Group 3: Diabetic + A. Indica (200 mg/kg b w) orally; Group 4: Diabetic + melatonin (10 mg/kg b w) IP; Group 5: Diabetic + A. Indica (200 mg/kg b w) orally + melatonin (10 mg/kg b w) IP; Group 6: Diabetic + metformin (500 mg/kg b w) orally. The extract and drug were administered once daily for a period of three (3) weeks respectively. The fasting Blood glucose level was taken at an interval of 7 days. The results showed that there was significant reduction ($p \leq 0.05$) in blood glucose levels in Azadirachta indica leaves extract treated diabetic group (DAI), melatonin treated diabetic group (DML) and the extract and melatonin combined treated diabetic group (DAI+DML) as compared with the metformin treated diabetic group and Diabetic control group. The results showed that melatonin and Azadiachta indic ethanol leaves extract possesses hypoglycaemic properties and synergistically reduced blood glucose levels in streptozotocin-induced diabetic Whistar rats.

Key words: Melatonin, *Azadirachta indica*, Hypoglycaemia, Streptozotocin, Whistar rats

INTRODUCTION

Diabetes mellitus (DM) represents one of the most important health problems worldwide and according to recent estimations, it is likely to worsen to critical levels in the next decades, it is of great concern that it is rising rapidly in younger population groups, especially children and adolescents [1]. Diabetes has long been viewed as a disorder of carbohydrate metabolism due to its hallmark feature of hyperglycemia, indeed hyperglycemia is not only the cause of the acute symptoms such as polydypsia, polyuria, and polyphagia [2], but also the long-term complications including retinopathy, nephropathy, and neuropathy. In addition, hyperglycemia may contribute to the development of macro-vascular disease, which is associated with the development of coronary artery disease, the leading cause of death in individuals with diabetes [3]. Thus, a primary goal in the prevention and the management of diabetes is the regulation of blood glucose to achieve near-normal levels. Hyperglycemia affects many tissues including vascular

endothelium and pancreatic β-cells which leads to their dysfunction, [4]-[5]. In this progression, the initial stage is a relatively long period of chronically elevated blood glucose; hyperglycemia persists and compromises metabolic activity leading to endothelial dysfunction (ED), β-cell dysfunction and disrupted vascular smooth muscle relaxation [5].

A study to assess the prevalent rate of type 2 diabetes and to determine the associated risk factors of the disease in Port-Harcourt, Nigeria was done using 502 subjects above 40 years. The study result revealed that 34 subjects had diabetes giving a prevalence rate of 6.8% with a male to female rate of 7.7% and 5.7% respectively. In another study in Dakace village in the outskirt of Zaria, Nigeria; of 199 subjects 94 was males and 105 females had their fasting blood glucose and body mass indices determined. The overall prevalence of diabetes was 2.0%. The study concluded that the prevalence of diabetes mellitus (Type 2 DM) in the semi urban community is rising as seen in other parts of Nigeria [6].

Various hypoglycemic drugs, such as sulfonylurea, metformin are being used for the treatment of diabetes but their use is restricted by their limited action and accompanying side effects such as hypoglycaemic shock and weight gain. Insulin treatment also fails to prevent the long term complication [7].

Plants and plant products continue to play a dominant role in traditional remedies against ailments from antiquity [8]. Conventional measures used in the management of diabetes usually aim at improving glucose homeostasis and delay the onset of complications but, these measures are not curative [8].While undue weight gain, drug resistance to Insulin and hypoglycaemia are some side effects associated with conventional measures. Many complications of diabetes result due to an increased free radical load [9]. The search for natural antioxidative agents that will ameliorate the harmful effects associated with hyperglycaemia still continues in spite of considerable progress in the management of diabetes mellitus by synthetic drugs.

Azadirachta indica is one of the most versatile medicinal plants having a wide spectrum of biological activities. Studies have reported the beneficial effect of *Azadirachta indica* leaves in the management of diabetes mellitus and the amelioration of the oxidative stress associated with the disease [10].

Melatonin has been shown to be a major scavenger of both oxygen and nitrogen based radicals [11-13], including peroxynitrite anion ($ONOO^-$) [14]-[15]. Melatonin may influence diabetes and associated metabolic disturbances not only by regulating insulin secretion, but also by providing protection against reactive oxygen species, since pancreatic β-cells are very susceptible to oxidative stress because they possess only low-antioxidative capacity [16].

In developing countries adequate treatment measures for diabetes mellitus are often unavailable or too expensive hence the need to test for the viability of *Azadirachta indica* ethanolic leaves extract and melatonin a known potent antioxidant as alternatives to conventional antidiabetic drugs. The result of this study can be applied in the management of diabetes by using natural, easily and readily available and cheaper products with negligible side effects thus, serving as a preferred alternative to known diabetic drugs especially in developing countries.

However the combined effect melatonin and Azadirachta indica administration on the blood glucose levels is yet to be determined. Thus this study was conducted to examine their hypoglycaemic effects on blood glucose levels in streptozotocin-induced diabetic Whistar rats.

MATERIALS AND METHODS

Experimental Animals

Thirty young adult male Wistar rats, weighing approximately 140g each were obtained from the Faculty of Pharmaceutical Sciences of Ahmadu Bello University, Zaria. They were kept in plastic cages and allowed to acclimatize for 2 weeks in the Faculty of Pharmaceutical Sciences Animal house before the experiment, and maintained under laboratory conditions of temperature, humidity and light. They were allowed free access to water and standard pellet diet obtained from Grand Cereals Ltd, Jos Plateau State. The animals were divided into six groups of five animals each.

Plant material

Leaves of fresh *Azadirachta indica* were harvested from Ahmadu Bello University Faculty of Medicine Zaria in the month of April 2012 and authenticated at the Department of Biological Sciences, Ahmadu Bello University Zaria Herbarium by Mr. U.S. Gallah with a voucher specimen number 900151.

Preparation of extract

The Fresh leaves of A. indica were air dried, minced and powdered using laboratory mortar. 1 kg of the leaves was extracted in 1.5 litres of 80% ethanol using a soxhlet extractor. This was filtered using a whatman filter paper (24 mm). The filtrate was dried in a laboratory water bath set at 67°C and total yield of 46.8g was obtained per 1000kg of the leaves.

EXPERIMENTAL DESIGN

Diabetes induction

A baseline blood glucose levels was taken for all the control and test animals before grouping them. This was done to ensure that the animals were all normoglycaemic. Thirty three Wistar rats were randomly selected and were given a single dose of intra peritoneal injection of streptozotocin, (STZ) (Sigma, Aldrich, USA), at 55mg/kg body weight in citrate buffer (0.1M, pH 4.5). The solution (STZ in citrate buffer) was used within 5 minutes to induce chemical diabetes in the wistar rats after overnight fasting of twelve hours Blood samples were collected at 72 hours after STZ treatment from the dorsal vein of the tail and the blood glucose levels detected using a One Touch Ultra 2 Glucometer, (Lifescan, CA, USA). Streptozotocin treated adult Wistar rats with fasting blood glucose level at 11mmol/L and above was considered diabetic. Twenty eight Wistar rats in this group were found to be chemically diabetic giving 84 % diabetic induction. These animals were further grouped into five groups of five Wistar rats each (Group 1, 2, 3, 4, and 5) called the diabetic group while group 6 is normal control groups with five rats per group.

Administration of extract and melatonin

The extract (200mg/kgbw/d) (Atangwo, *et al*., 2010) was administered by orogastric intubation once daily for three weeks while melatonin (10mg/kgbw/d) was administered intraperitoneally once daily for three weeks in all treated groups while metformin was the standard drug (500mg/kgbw/d).

Blood glucose studies

Blood was obtained from the tail vein of the rats after 7 days of treatment every week for 21 days and read with one touch ultra Glucometer.

Statistical analysis

Data generated were presented as Mean±SD and analysis performed using student's T-test and a prevalence less than or equal to 0.005 ($p \leq 0.05$) was considered to be significant. One way analysis of variance (ANOVA) was used to compare the level of difference between and within the groups, while Duncan Multiple Range Test was used as a Post hock Test. The analysis was done using Statistical Packages for Social Sciences (SPSS) 19 statistical packages.

RESULTS

Table 1: Hypolgycaemic effects of melatonin and ethanol leave extract of *Azadirachta indica* administration on blood glucose levels in streptozotocin-induced diabetic wistar rats

Treated Group	DAY 1	DAY 8	DAY 15	DAY 22
NC	**71.40 ±2.015^{b}**	**70.0±2.345^{b}**	**82.40 ± 8.078^{c}**	**96.20 ±3.734^{b}**
DC	**272.60 ±20.488^{a}**	**279.80 ±23.731^{a}**	**298.00 ±27.120^{a}**	**320.80 ±33.466^{a}**
DAI	**345.80 ±60.728^{a}**	**265.80 ±39.434**	**219.40 ±30.615^{b}**	**153.20 ± 8.680^{c}**
DML	**336.20 ±54.369^{a}**	**259.60 ±44.715^{a}**	**189.80 ±18.359^{c}**	**141.60 ± 4.331^{c}**
DAI/DML	**337.20 ±42.677^{a}**	**278.60 ±38.281^{a}**	**187.20 ±17.758^{c}**	**138.80 ± 5.544^{c}**
DMF	**353.40 ±42.646^{a}**	**337.20 ±43.718^{a}**	**321.40 ±44.667^{a}**	**299.40 ± 48.264^{a}**

Values are expressed as mean ±SD; Values with different superscripts in a column are significantly different ($p < 0.05$)

NC = Normal control + normal saline orally; DC = Diabetic control+normal saline; D+AI=Diabetic+Azadirachta indica (200 mg/kg b w); D+ML=Diabetic +Melatonin (10 mg/kg b w); DAI+ML=Diabetic+Azadirachta indica (200 mg/kg b w) +Melatonin (10 mg/kg b w); D+MF=Diabetic + Metformin (500 mg/kg b w)

Blood Glucose Level

Table 1 showed results obtained for blood glucose levels in STZ induced diabetic Wistar rats in control groups and diabetic groups. The table showed that there was significant reduction ($p \leq 0.05$) in blood glucose levels in *Azadirachta indica* leaves extract (DAI), melatonin treatment (DML) and the combined treatment groups (DAI/DML) in both the diabetic and control groups while diabetic control group (DC) and diabetic metformin (DMF) treated groups showed no significant decrease in blood glucose levels during the treatment period.

DISCUSSION

Blood Glucose Studies

In this study, decrease in blood glucose levels were observed in diabetic extract treated group, melatonin treated group and melatonin and extract combined treated group in the STZ induced diabetic. *Azadiracta indica* leaves extract treated group only, showed significant reduction in blood glucose levels at the end of the 21 days of treatment which is similar to the work of Das *et al* (2010)(17).

Chronic hyperglycaemia is the cause of many debilitating changes associated with diabetes mellitus [7], the pathophysiological changes of which can be alleviated by blood glucose control.

Melatonin treatment alone also reduced blood glucose level significantly. When the extract and melatonin were used in treating the animals, a highly potent and significant value was observed, showing that melatonin potentiates the efficacy of the extract in reducing the blood glucose levels and that melatonin on its own has hypoglycaemic effects. According to Meshchyshen *et al* (2010) [18], melatonin caused a decrease and normalization in blood glucose levels in alloxan induced diabetic rats, probably due to its inhibitory effects on catecholamines by inhibiting ACTH-stimulating cortisol production. The metformin treated diabetic group showed reduction in blood glucose but was not significant as compared with the extract and melatonin treated groups; which is similar to observations by [17].

CONCLUSION

The extract of *Azadirachta indica* leaves and melatonin showed hypoglycaemic effect on STZ induced diabetic Wistar rats, therapeutically ameliorating the effects of hyperglycaemic pathophysiological changes in diabetes mellitus. The ethanol extract and melatonin when combined gave better results in all parameters studied. This study supports the claims by traditional herbal healers of the efficacy of the extract and combining it with melatonin potentiates its efficacy in the study.

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