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Role of in-utero Neem leaf (*Azadirachta indica*) supplemented diet on some hematological parameters and glucose storage in offspring of Wistar rats

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Abstract

Azadirachta indica (AI) Neem leaf is native of India which grows in most of tropical and subtropical countries. It has adaptability to a wide range of climatic, topographic and edaphic factors. This study explored the impact of AI supplementation during gestation on some hematological parameters and glucose storage in male and female offspring of Wistar rats. 18 pregnant female and 12 male Sprague-Dawley rats with a weight range of 140-180g were employed for this study and they were exposed to either a standard diet or AI supplementation (AIS). The pregnant rats were exposed to AIS up to birth (gestational AI supplementation) which comprised of both treated males (TM) and treated females (TF). Control rats with control diet was administered in analogous comparatively and this comprised of control male (CM) and control females (CF). During postnatal day 49, the rats were sacrificed and blood sample was obtained for assay of white blood cells (WBC), platelets (PLT), red blood cell counts (RBC), hemoglobin (Hb) and packed cell volume (PCV). Liver and gastrocnemius tissues were obtained for skeletal and hepatic glycogen assayed and intestinal and pancreatic α-amylase and α-glucosidase were assayed from intestinal and pancreatic tissues. WBC, PLT, RBC, Hb, PCV, alpha amylase, hepatic and skeletal glycogen increased significantly ($p<0.05$) in TM and TF with a remarkable decrease and increase in alpha glucosidase ($p<0.05$) in TM and TF respectively compared with CM and CF. It can be inferred from the present study that perinatal AI supplementation provides a substantial justification to its use in folk medicine as a hematopoietic plant and the increased glucose storage observed may not be unconnected with its role as a hypoglycemic agent, though the effect were more marked in female offspring compared with their male counterparts.

KEYWORDS: *Azadirachta indica, amylase, glucose, gastrocnemius*

Introduction

Neem (*Azadirachta indica*), is native of India which is being cultivated in most of tropical and subtropical countries. It has adaptability to a wide range of climatic, topographic and edaphic factors. The tree survives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth. So that it requires little water and plenty of sunlight to survive in the environment [1]. It is widely distributed throughout the world providing a source of inspiration for novel drug compounds, as plant derived medicines which have made large contributions to human health and well-being. Presently it can be seen growing successfully in over 70 countries worldwide, in Africa, Australia, Asia, North, Central and South America. Evidence showed that the tree was introduced into West Africa at the beginning of the present century [2]. Every part of the tree has been employed as traditional medicine for household solution against various diseases [3]. It elaborates a vast array of biologically active compounds that are chemically diverse and structurally variable with different ingredients

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isolate from different parts of the tree [5]. The active ingredients include alkaloids, flavonoids, phenolic compounds, carotenoids, steroid and ketones, which have antihelminthic, antimicrobial, antiulcer, antifertility, antidiabetic, anti-inflammatory and antitumor properties and the plant is used in combination with oil for more effectiveness to reduce toxicity [5, 6,7]. The neem seed oil has toxicity effect against ectoparasites like ticks and mites which are common on cattle, equines, sheep, goats, wild ungulates and dogs [8]. Alcohol and aqueous extracts of flowers of the tree also effect against cattle filarial parasite [9].

All parts of the tree including leaves, bark, roots, seed and twigs contain active ingredients and used as medicine. Neem leaves are useful for increase immunity of the body, reduce fever, treating various foot fungi, useful against termites, used in curing neuromuscular pains and Anticlotting agent, Antihelminthic, Antituberculosis, antitumour, antiseptic, antiviral, Contraceptive, Cosmetics, Fertilizers, Insecticides and Insect repellents [12]. The processed Neem cake poses a good appetizer characteristic together with wormicidal activity which is used as poultry feed. Furthermore, Neem leaves has a significant amount of protein, minerals (except zinc) and digestible amounts of crude protein (CP) and total digestible proteins (TDP) which serves a better nutrition to the animals such as goat, sheep and cow [13]. Despite its bitter components, livestock consume diets containing varied percentage of neem cake. Alkali treatment of this by-product with caustic soda (10-20g sodium hydroxide) yields palatable products by removing the toxicant tritepenoids. After treatment it is incorporated into poultry feed [14].

Apart from their traditional uses, there are several reports on the biological activities and pharmacological actions of AI based on modern scientific investigations [15-21] blood glucose lowering activity of AI seed oil and leaf extracts have been reported in various models of diabetic animals [15-18] ethanol extracts of AI leaves have been shown to demonstrate antilipid peroxidative, antihyperglycemic and anti-hypercholesterolemic activities as well as reduced serum triglyceride level in diabetic rat model [18] Also, a significant decrease in some hematological parameters in chickens fed with AI leaves [19] and no significant difference in some hematological parameters following the extract use in diabetic rats [20] have been reported.

In Nigeria, AI supplements are popularly employed in the treatment of malaria. Some people have been observed consuming raw AI during pregnancy and lactation and anecdotal reports from them suggest that they consume it because of the myth that it is generally harmless, it is hematopoietic and protects them against malaria parasite. Despite these convincing evidences on its therapeutic use in folk medicine, however, there is paucity of information on the effects of AI supplemented diet during pregnancy and the subsequent effects on some hematological parameters and glucose storage in offspring. The present study therefore sought to unravel the role of AI supplementation on some hematological parameters and glucose storage and whether the effect is sex dependent.

MATERIAL AND METHODOLOGY

The Experimental animals

Eighteen (18) pregnant Wistar rats with a weight range of 140-180g were employed and were sheltered with cages with quality lighting conditions 12hours light and dark cycles, and had free access to tap water with quality food and acclimatization lasted for 1week. The mechanism undertaken were in line with the presentations of the Experimentation Ethics Committee on Animals Use of the College of Medicine, University of Lagos, Lagos State in accordance with the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals.

Neem leaf (*Azadirachta indica*) AI collection

Fresh and matured AI leaves were collected from a tree within a community located in Afowowa in Ewekoro local government of Ogun State, Nigeria and was identified at the Biology unit of D.S Adegbenro ICT polytechnic, Eruku-Itori, Ewekoro, Ogun state. The identified sample was deposited in the institution's herbarium. The collected samples were air dried and grinded with electric blender to get the powdered form.

Diet, mating and grouping

Five hundred grams of powdered AI leaves were grinded with 25kg of standard rat chow and this constitute AI supplemented diet with another 25kg normal rat chow and this formed the CONT diet. AI supplementation with these whole foods as a dried ground powder: maize (WN+M), sesame (WN+SO), soya bean meal (WN+SBM), palm kernel cake (WN+PKC), bone meal (WN+BM), industrial salt (WN+IS), grower premix (WN+GP), limestone (WN+LS), lysine (WN+L), methionine (WN+M), enzyme (WN+E), alphatox (WN+A) and threonine (WN+T). The diets gave a typical dietary intake of 1.5 servings of each food/d in a human diet, based on an energy content of a serving of food as set by the FDA for nutrition facts panels and a total daily diet intake of 2000kcal. Female rats were subjected to sexual act overnight with approved male breeders, 1 male per 2 females and they were maintained in their respective diet throughout pregnancy. The day on which spermatozoa was observed on a vaginal smear that was washed with normal saline NaCl 0.9% was assigned conception day

0. The rats were thereafter allocated to 1 of the 4 groups to be exposed to either a control diet or AI supplementation. Water and food were made available for all rats and grouped as follows (6 animals per group):

group I: Control male (CM) (exposed to control diet throughout the experiment).

group II: Control female (CF) (exposed to control diet throughout the experiment).

group III: Treated male (TM) (Male offspring exposed to AI supplementation only during gestation).

group IV: Treated male (TM) (Female offspring exposed to AI supplementation only during gestation).

All the pups were transferred to normal rat chow, except the control rats, until the end of the procedure which was PND 49. Offspring were reduced to 8-10 pups on postnatal PND 1 (birth, day 0) and were all weaned on PND 21 and housed in groups of 3 or 4, male and female offspring separately per cage.

Phytochemical analyses

Qualitative and quantitative phytochemical screening of *Azadirachta indica* were carried out according to the method described by [22] to identify the active components present.

Blood sample

Four (4ml) of blood was obtained through cardiac puncture into an EDTA bottles and was centrifuged at 3000 rpm for 15 min with the plasma carefully obtained with rubber pippete in a clean Eppendorf bottle and were stored at −20 °C until analyses [23].

Isolation of tissue

On day 49 the rats were sacrificed via dislocation the of cervical vertebrate after a light anesthesia. The rats were dissected and skeletal, liver, pancreatic and intestinal tissues were obtained, washed in an ice cold and washed with 1.15 % KCl which was blotted and weighed [24].

Hematological parameters

Packed cell volume (PCV), hemoglobin Concentration (Hb), white blood cell count (WBC), red blood cell count (RBC) and platelet count (PLT); were analyzed using an Automated Analyzer (Sysmex, KX-21, Japan).

Skeletal and hepatic glycogen

This was measured in both skeletal and liver tissue samples (homogenate) [23].

Assay of pancreatic and intestinal α-amylase

Tissue intestinal and pancreatic α-amylase were assayed according to the method described [24].

Assay of pancreatic and intestinal α-glucosidase

Tissue intestinal and pancreatic α-glucosidase were assayed according to the method described by [24].

Statistical analysis

Results are provided as the mean and standard error of mean (SEM). GraphPad Prism 5 Software (GraphPad, Inc, La Jolla, CA, USA) was used for statistical analysis and one-way analysis of variance with post hoc Tukey's multiple comparison test was employed with significant level set at $p<0.05$.

RESULTS

Table 1 showed the qualitative presence of phytochemicals which include tannins, Saponin, alkaloids, Flavonoids, Steroids, glycosides, Terpenoids and phenol.

Table 1. Qualitative Phytochemicals Analysis of Powdered Neem Leaves

Phytochemicals	Result
Tannins	Positive
Saponin	Positive
Alkaloids	Positive
Flavonoids	Positive
Steroids	Positive
Glycosides	Positive
Terpenoids	Positive
Phenol	Positive

Table 2. Quantitative Phytochemicals Analysis of Powdered Neem Leaves

Minerals	Values
Tannins	1.382mg/100g
Saponin	2.914mg/100g
Alkaloids	1.282mg/100g
Flavonoids	1.073mg/100g
Steroids	1.014mg/100g
Glycosides	2.134mg/100g
Terpenoids	4.356mg/100g
Phenol	1.002mg/100g

Table 2 revealed the presence of quantitative phytochemicals which include tannins (1.382mg/100g), Saponin (2.914mg/100g), alkaloids (1.282mg/100g), Flavonoids (1.073mg/100g), Steroids (1.014mg/100g), glycosides (2.134mg/100g), Terpenoids (4.356mg/100g), phenol (1.002mg/100g).

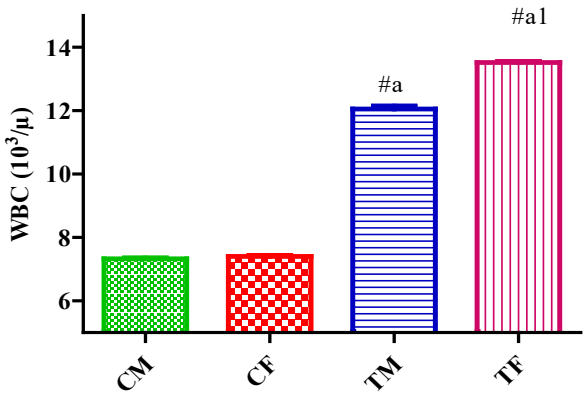

Figure 1. Outcome of AI supplementation on WBC in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

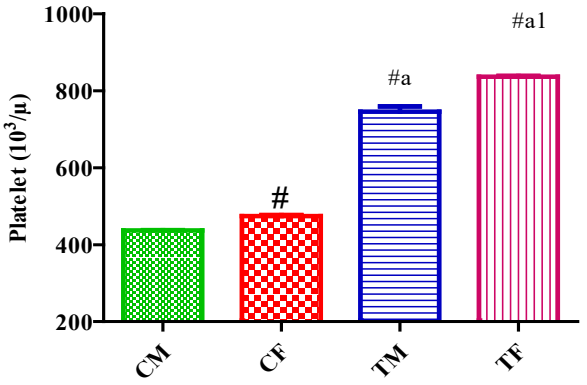

Figure 2. Outcome of AI supplementation on PLT in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

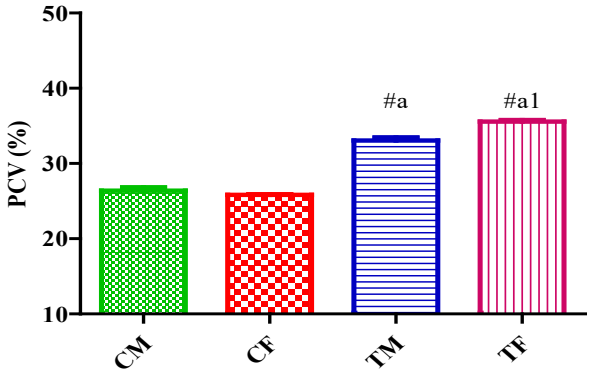

Figure 3. Outcome of AI supplementation on PCV in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

Treated male (TM) and treated female (TF) investigated significantly increased (p<0.05) all blood parameters which include WBC (figure 1), PLT (figure 2), PCV (figure 3), RBC (figure 4), Hb (figure 5) assayed and also significantly decreased (p<0.05) for α-amylase (figure 6). However, alpha glucosidase significantly reduced (p<0.05) in TM compared with CM, CF and TF while it significantly increased (p<0.05) for TF compared with CM, CF and TM (figure 7) There was a significant increase (p<0.05) in skeletal glycogen (figure 8) and hepatic glycogen (figure 9) contents compared with CM and CF.

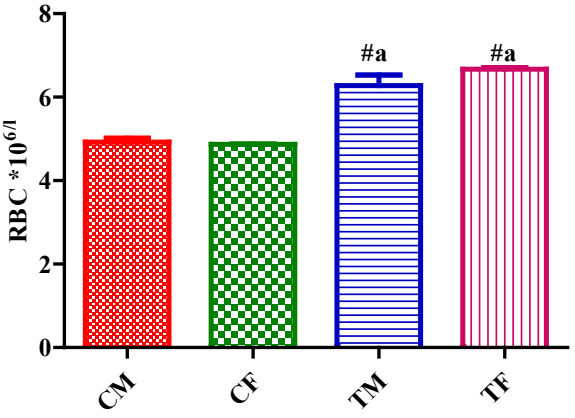

Figure 4. Outcome of AI supplementation on RBC in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

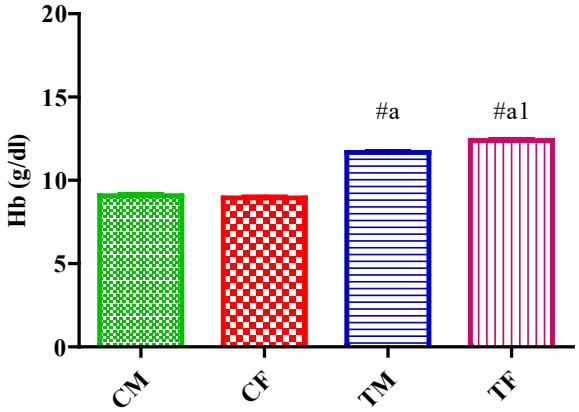

Figure 5. Outcome of AI supplementation on Hb in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

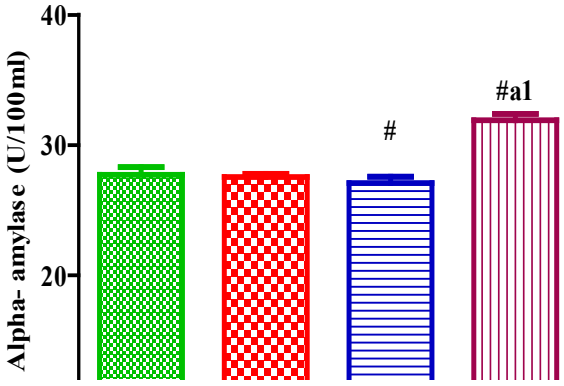

Figure 6. Outcome of AI supplementation on α-amylase in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

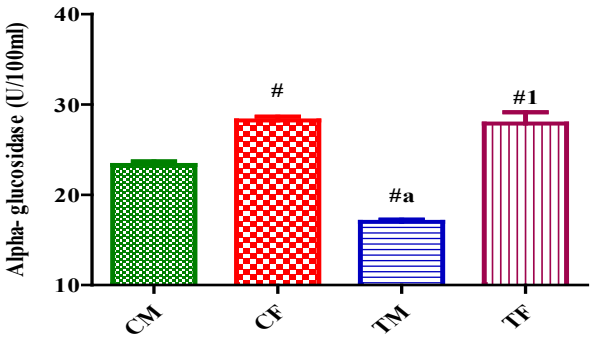

Figure 7. Outcome of AI supplementation on α-glucosidase in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

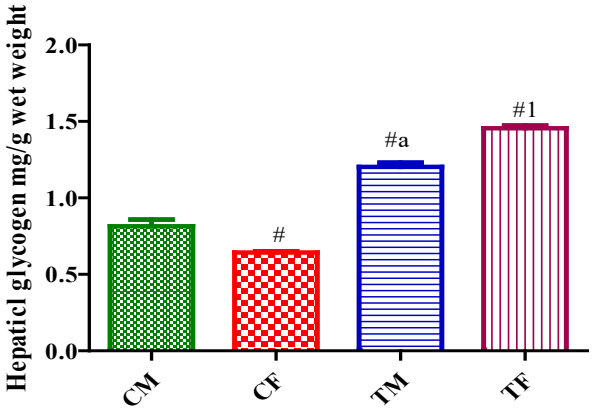

Figure 8: Outcome of AI supplementation on hepatic glycogen in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

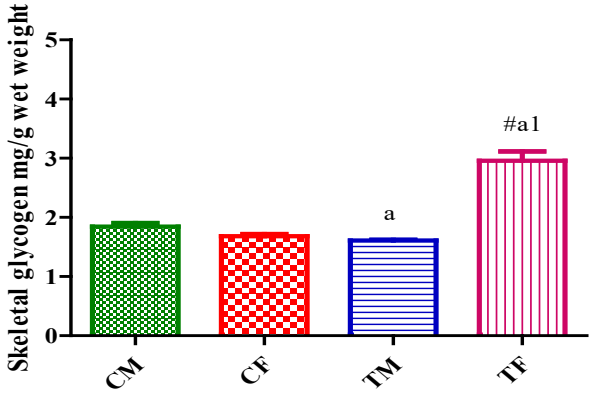

Figure 9: Outcome of AI supplementation on skeletal glycogen in CONT and treated rats. Values represent mean±SEM; n=6. Remarkable levels (#p<0.05 vs CM, ap<0.05 vs CF, 1p<0.05 vs TM).

Discussion

The offspring treated with AI supplemented diet at in-utero showed an increase in the WBC, PLT, RBC, Hb and PCV. This observation is in agreement with the report of [25]. Also [26], a report from HIV/ AIDS patients, also observed that an acetone/water leaf extract of AI (IRAB) exhibited a remarkable increase in some hematological parameters. Pregnancy is a potentially anemic state [27,28] and since is often complicated by malaria in Nigeria and this might have effect on offspring health during postnatal life, this observed increase in hematological parameters in the present study suggests that the AI supplementation may have potentials at boosting erythropoiesis in offspring. Indeed, this may justify its use in folk medicine during pregnancy. The significance of the observed increased hematological parameters in the present study cannot be ruled out as anemia has been reported to affect over 500 million women [29] and in pregnancy it is associated with impaired maternal and infant consequences.

The heightened blood parameters could be related to the established constituents of the extract such as flavonoids and it is evident from the present study qualitative and quantitative presence of flavonoids that have been shown to have hematopoietic properties [28]. Also, AI has been established to boost the body's macrophage response, which stimulates the lymphatic system of the body and also boosts the body's leukopoiesis [30,31]

The effect of AI as a hypoglycemic agent in normal and experimentally induced diabetic animals is well established and reported [32-34]. The results of the present study also showed that the AI supplementation significantly increased the glucose storage in these rats which is evident form increased glucose deposition in both hepatic and skeletal tissues as well as increased activities of alpha amylase activity. This suggests that AI supplementation in all the treated rats, also possess hypoglycemic effect. Several mechanisms through which AI decreases the blood glucose level have been investigated by several authors [35,36]. Jelodar, et al. [37], has earlier suggested that the hypoglycemic properties of the AI extract may not be unconnected with its ability to stimulate sufficient production of insulin by the pancreas, that aided in the peripheral utilization of glucose in the cells or the possible ability to regenerate the β-cells. Chattopadhyay, et al. [38], on the other hand suggested that AI's possible mechanism is by inhibiting the action of epinephrine on glucose metabolism resulting in increased utilization of peripheral glucose.

Alpha glucosidase is found in the mucosal brush border of small intestine where it catalyzes the final step of the digestion of starch and disaccharides which are present in abundance in human diet. Inhibitors of these enzymes delay the breakdown of carbohydrate in the small intestine and decreased the postprandial blood glucose movement levels in diabetic patients. Hence, these enzymes may be useful as effectiveness strategies to reduce the levels of post meal hyperglycemia [39].

Conclusion

In conclusion, the data described in the present study seem to provide a substantial justification, the folkloric use of AI as a hematopoietic agent with the potential of elevating glucose storage in offspring though the effects were more marked in female offspring compared with their male counterparts.

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