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EFFECT OF *VERNONIA AMYGDALINA* (BITTER LEAF) EXTRACT ON THE CARBOHYDRATE AND AMYLOSE CONTENTS OF THE FAECES OF ALBINO RATS

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ABSTRACT

The effect of *Vernonia amygdalina* leaf extract on the carbohydrate and amylose contents of the faeces of rats fed with rice were investigated in this study. Thirty-five (35) albino rats were divided into seven treatment groups: A= Negative control, B= animals fed with glucose (positive control), C= animals fed with extract and rice (0.1ml/body weight), D= animals fed with extract and rice (0.2ml/body weight), E= animals fed with extract and rice (0.3ml/body weight), F= animals fed with extract and rice (0.4ml/body weight) and Group F= animals fed with rice for this experiments. The experiment lasted for 12 days. Rat fecal Samples were collected at intervals and evaluated for its percentage carbohydrate and amylose contents. The result of the percentage carbohydrate and amylose in the rice and *vernonia amygdalina* samples used in the present study are (30.59±0.33, 8.6±0.72) and (3.86±0.29, 0.31±0.22) respectively. There was a dose dependent decrease in the carbohydrate content of the fecal samples in the treatment groups compared to the controls from days 3 to 12. Rats fed with 0.4ml of extract/kg body weight had the lowest percentage carbohydrate in their faeces. The percentage fecal amylose content was highest in the treatment group with 0.4ml of extract/kg body weight of rats closely followed by the treatment group with 0.3ml of extract/kg of rat's body weight compared to the controls and the other treatment groups from days 3 to 12.Overall, the *vernonia amygdalina* leaf extracts used

in the present study significantly enhanced carbohydrate metabolism in the rats and thus has great potential in the treatment and management of diabetes mellitus.

Keywords: ***Vernonia amygdalina,*** **carbohydrate and amylose contents**

INTRODUCTION

Rice is an important staple food in many countries of the world and is ranked as the worlds' number one human food crop [1]. Rice is popular in Nigeria and accounts for daily supply of 27% energy, 20% protein and 3% fat [2]. Minerals like calcium, magnesium, phosphorus are present along with some traces of iron, copper, zinc and manganese [3]. Over three billion people depend on it as a major source of their subsistence diet [4].

Rice starch, like other starches, consists of amylose and amylopectin which affect blood glucose response amongst other factors. Amylose and Amylopectin content of starch is inversely correlated to glycemic index (GI) [5]. Rice has given a wide range of results in GI studies around the world. Prevalence of diabetes mellitus, cardiovascular disease and metabolic syndrome are lower in subjects having diets with low glycemic response than in those with diets with a high glycemic index [6]. Amylose content of rice influences the GI value where rice that is high in amylose usually has lower GI value [7]. A low (GI) may results in a slower rate of digestion and absorption, hence reducing the rapid elevation of postprandial hyper-glycemia as well as insulin concentration which will then influence the management of diabetes [8]. Rice varieties with higher proportion of amylose have been shown tohave a slower rate of digestion and produce lower glycemic and insulin responses [9]. Much of the variation in the GI for rice is due to differences in the amylose to amylopectin ratio.

Vernonia amygdalina is a small shrub that grows predominantly in tropical Africa.It is medium in size with petiolate green leaf of about 6mm diameter and elliptic in shape [10]. In Nigeria the plant is commonly called bitter leaf in English, Oriwo in Benin, Origbo in Urhobo and Olubu in Igbo, [11]; [12]. The plant leaves have a bitter taste, hence the name bitter leaf. There are reports of *Vernonia amygdalina* curing cancer, liver damage, drug resistant microbial infections, constipation, fever and high blood pressure [13]. The use of *vernonia amygdalina* in the treatment of diabetes mellitus is very common among ethno medical practitioners [14]. In this study, the effect of *Vernonia amygdalina* leaves extract on the

amylose/amylopectin ratio of the faeces of rats fed with rice was investigated.

MATERIALS AND METHODS

Collection of Samples

The leaves of the plant, *V. amygdalina* and the riceused for this study were obtained from Abakaliki, Ebonyi State, Nigeria. The albino rats were purchased from the Animal House, College of Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

Preparation and Extraction of Leaf Extracts

The leaves collected were washed in clean water and air dried on a clean surface at room temperature. The dried leaves were pulverized into a homogenous powder using manual grinder (Corona, China). One hundred grams (100 g) of the powder was soaked in a litre of distilled water for 24 hours with intermittent stirring. The extracts were filtered using Whatman no.1 filter paper. Dark green filtrate of *V. amygdalina* was obtained. The filtered extracted was stored at (4 – 8) ^{o}C in clean sterile bottles.

Liquid-Liquid Fractionation of Crude Extract

The crude extract obtained as described above was evaporated to dryness at room temperature by employing air current. The concentrated extract was re-extracted with chloroform for 6 hours and washed severally with chloroform (five times). The extract was filtered with Whatman no.1 filter paper. The chloroform fraction and the residue were concentrated to dryness by air drying. The residue was again extracted with methanol for 6 hours and washed five times with methanol. The methanol extract was filtered with Whatman no.1 filtered paper. The methanol extract and the residue, termed 'water soluble fraction' were again concentrated to dryness by air drying. The concentrated methanol extract was washed 3 times with ethyl-acetate. The ethyl-acetate fraction (upper layer) was separated using separating funnel and concentrated to dryness by air drying. The residue (lower layer) was then fractionated with n-butanol saturated with water (50% n-butanol) and then the fractions were separated with separating funnel. The two fractions were concentrated to dryness by air drying [15].

Carbohydrate Estimation

1mg/ml of glucose was prepared and used as standard carbohydrate, 50µl was made up to 100µl. the sample was prepared into a solution of 5mg/ml and 50µl of the sample was made up to 100µl. 50µl of 80% phenol in distilled water was pipette into the sample and standard. Thereafter 2ml of concentrated H_2SO_4 was mixed well and heated in boiling water for 30mins. The Absorbance was

measured at 480nm using spectrophotometer and compared with the standard.

Extraction of Amylose from Rice

Purified amylose fraction was obtained using aqueous leaching. A 4% (w/v) of ground rice meal was prepared. The mixture was heated in water bath up to 65℃ and stirred intermittently and left to stand at 65 ℃ for an hour. The heated mixturewas centrifuged at 3000g for 10mins and the precipitate was discarded. N-butanol was then added to the supernatant at the ration of 1:3 (v/v)mixed and the solution was allowed to stay for 12 hrs. The pellet was then collected after centrifugation at 3000g and freeze-dries to obtain the amylose fraction. The potatoes amylose was obtained from potatoes starch in a similar fraction with the exception of heating at 55℃ for an hour.

Animal study

Thirty-five albino rats (0.1 to 0.2kg) were used for this experiment. The rats were allowed to acclimatize for a period of six days during which they werewith standard rodents feed (rat chow) and tap water. Then, the rats were equally divided (5 rats/group) into seven groups namely groups: A= Negative control, B= animals fed with glucose (positive control), C= animals fed with extract and rice (0.1ml/body weight), D= animals fed with extract and rice (0.2ml/body weight), E= animals fed with extract and rice (0.3ml/body weight), F= animals fed with extract and rice (0.4ml/body weight) and Group F= animals fed with rice.The weights of the rats in the test groups were measured and used to calculate the dosage of bitter leaf extract, 1 ml/kg/day, to be administered to each rat throughout the two weeks of treatment. The animals were given the extracts once every morning orally before meals. Administration was done via the oral route with the aid of oral cannula and syringe. After three hours of administration, fecal sample was collected from the rats into labeled. The fecal samples were then analyzed for various parameters.

Administration of aqueous extract to animals

The animals were carefully grouped according to similar body weights. There were a total of seven groups with five animals per group. One group was set as a control while the other six groups were administered different concentrations of the aqueous leaf extracts. The control groups were administered distilled water while other groups were administered, 100, 200, 300, and 400 mg/kg of the aqueous extracts, respectively, once daily of each sampling days (three days' interval) for three weeks. The aqueous extracts were administered in

distilled water orally. The animals were given the extracts once every morning orally before meal.

Amylose determination in fecal sample of rats fed with aqueous extract of *Vernonia amygdalina* and rice.

10% weight/volume of the sample was suspended in 1 molar aqueous conc. HCL for 30mins with constant stirring at 50 ^{o}c.The suspension was neutralized with NaOH and the pH adjusted to 7.0 ± 0.5. The acidified sample was centrifuged and freeze-dried overnight. 0.1g of a deposit was suspended in 1ml of 95% ethanol and 9mls of 2 molar NaOH and allows dissolve at 95 ^{o}c. one milliliter (1ml) of mixture was added to 4mls of distilled water which when completely dissolved, the solution was diluted with deionized water in100mls volumetric flask.

Five milliliter (5mls) aliquot was taken and mix with 0.4mls of 1 molar acetic acid and 2mls of freshly prepared iodine reagent and 91mlsof distilled water was added. The standard was prepared in similar manner but at different concentration using 0.25mg/ml, 0.5mg/ml, 1mg/ml, 1.5mg/ml, 2mg/ml and 4mg/ml. the absorbance was read using spectrophotometer at 610nm.

RESULTS AND DISCUSSION

The result of the percentage carbohydrate and amylose in the rice and *vernonia amygdalina* samples used in the present study are (30.59±0.33, 8.6±0.72) and (3.86±0.29, 0.31±0.22) respectively as shown in **Table 1**. Rice being a starchy food had a relatively higher percentage carbohydrate and amylose compared to the *vernonia amygdalina* leaves. Carbohydrates are very good source of easily available energy in the body and as such makes rice an important energy food. The rice and the *vernonia amygdalina* leaf have relatively low percentage amylose content. This implies that the carbohydrate (starch) in the rice sample has high digestibility. The amylose content of starch is one of the very important factors affecting starch digestibility and thus metabolic activities in vivo [16]. Amylose unlike amylopectin is not highly branched and thus has less surface area and more intra-molecular hydrogen bonds [17].

There was a dose dependent decrease in the carbohydrate content of the fecal samples in the treatment groups compared to the controls from days 3 to 12. The rats fed with rice only had the highest percentage carbohydrate in their faeces compared to the other treatment groups across the duration of the experiment. Interestingly, rats fed with 0.4ml of extract/kg body weight had the lowest percentage carbohydrate in their faeces as shown in **Figure 1**. The observed

dose dependent effect of the *vernonia amygdalina* leaf extract on the percentage carbohydrate of the rat's fecal samples suggests that *vernonia amygdalina* increased carbohydrate metabolism in the rats. This observation may support the claim by local herbal medicine practitioners on the anti-diabetic effects of *vernonia amygdalina* [7]. In other reports, *vernonia amygdalina* has been shown to enhance glucose uptake by the cells by targeting of insulin productionfrom the beta-cells of the islet of Langerhans [11]. *Vernonia amygdalina* is reported to possess hypoglycemic properties [4]. [16] opined that bitter leaf has a beneficial effect in enhancing the gastro intestinal enzymes thereby improving digestion and assimilation of nutrients.

The result in **Figure 2** shows that the percentage fecal amylose content was highest in the treatment group with 0.4ml of extract/kg body weight of rats closely followed by the treatment group with 0.3ml of extract/kg of rats' body weight compared to the controls and the other treatment groups from days 3 to 12. This observation indicates a possible interference or hindrance in the breakdown of the amylose chain of the rice starch at high dosage of the *vernonia amygdalina* leaf extract. The inhibition of amylose breakdown at high dosage of the extracts could be one of the factors that contribute to the hypoglycemic properties of *vernonia amygdalina*. However, it is important to note that the greater hydrogen bonding between glucose units in amylose molecule than amylopectin hinders its exposure to enzymatic digestion [5]. Rice varieties with higher proportion of amylose have been shown tohave a slower rate of digestion and produce lower glycemic response [9]. The observed effect of the *vernonia amygdalina* extracts in inhibiting amylose breakdown and improving carbohydrate metabolism is in this study lend credence to the use of *vernonia amygdalina* in the treatment and management of diabetes mellitus especially in the tropics where this plant is in abundance.

Table 1: Amylose and Carbohydrate content in Rice and *Vernonia amygdalina*

Sample	% Carbohydrate	% Amylose
Rice	**30.59±0.33**	**8.6±0.72**
Vernoniaamygdalina	**3.86±0.29**	**0.31±0.22**

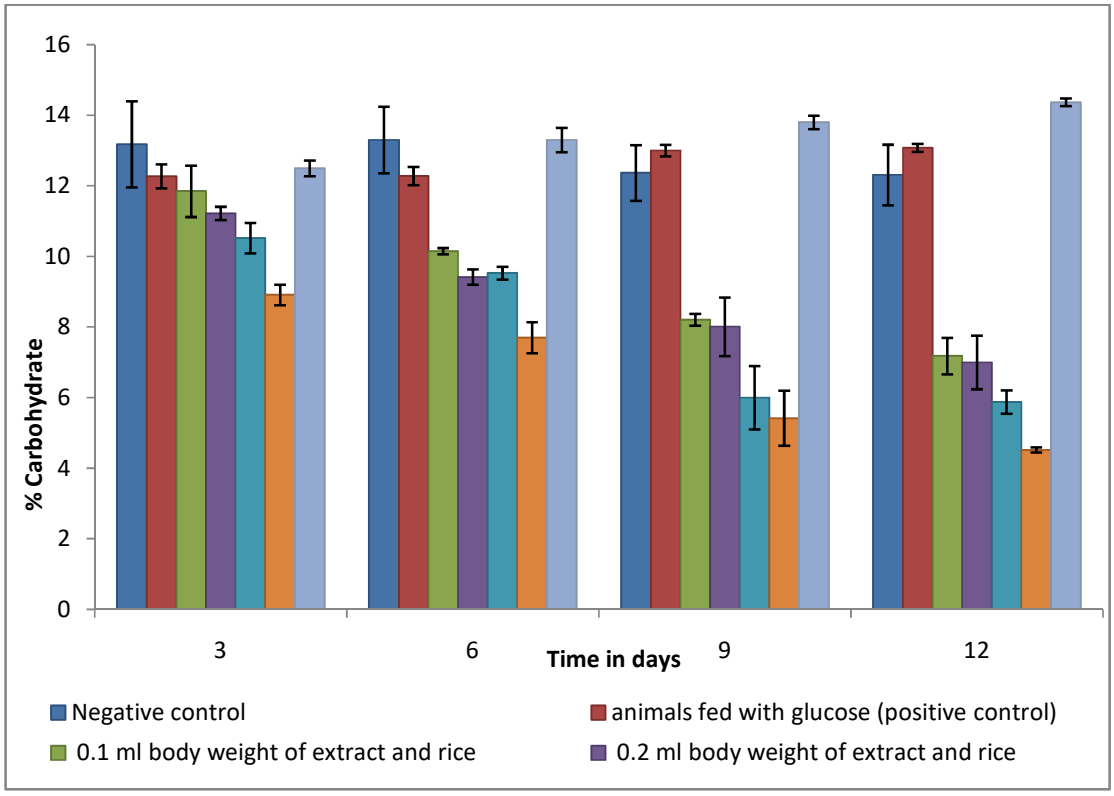

Figure 1: Carbohydrate content of the faeces of rats fed rice and different levels of *Vernonia amygdalina* extract

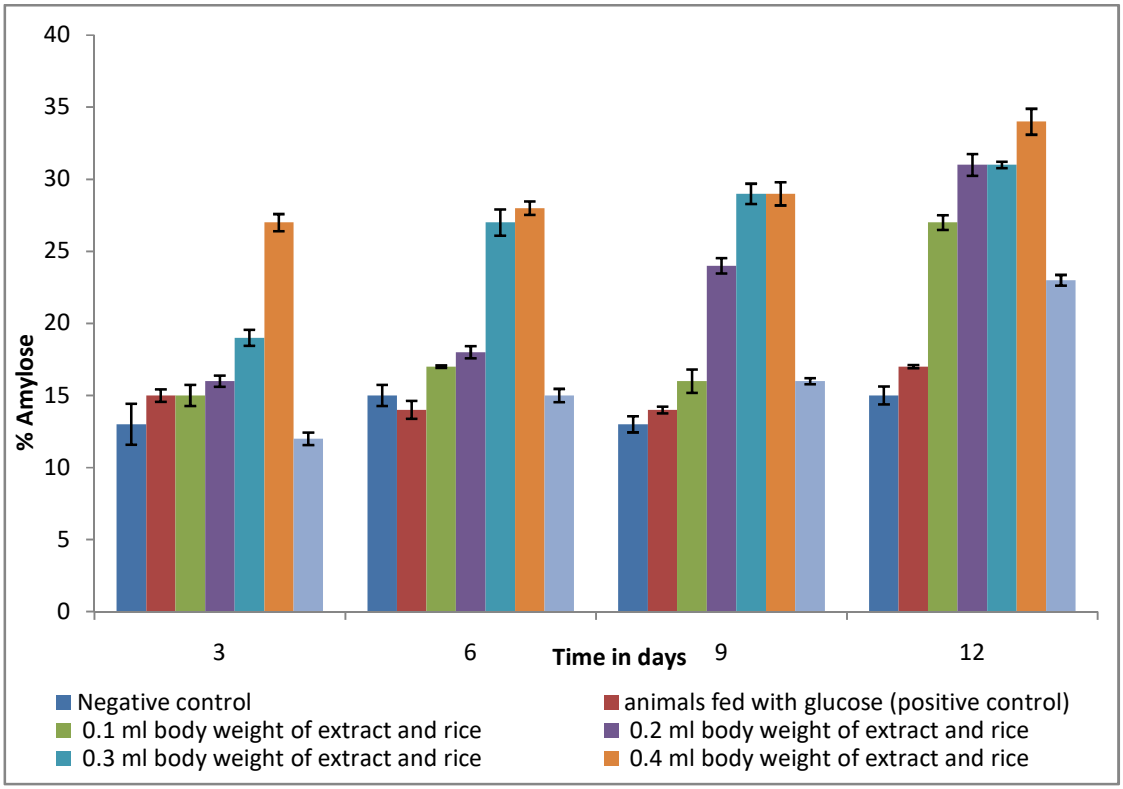

Figure 2: Amylose content of the faeces of rats fed rice and different levels of *Vernonia amygdalina* extract

CONCLUSION

The *vernonia amygdalina* leaf extracts used in the present study significantly enhanced carbohydrate metabolism in the rats. This suggests the huge potential of *vernonia amygdalina* in the treatment and management of sugar related metabolic disorders in man.

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