

FEEDING BEHAVIOUR, WEIGHT GAIN AND BLOOD SUGAR OF MALE WISTAR RATS FED ON A HIGH-CALORIE DIET AND VEGETABLES

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ABSTRACT

The transition in eating behaviour toward a diet rich in calories and low in vegetables is a major factor responsible for the rapid increase in the incidence of obesity and diabetes. The research aimed at investigating the effect of a high-calorie diet and vegetables on feeding behaviour, weight gain and blood sugar in male Wistar rats. The vegetables were dried, blended, and preserved in airtight containers. Thirty male Wistar rats weighing an average of 127.4 g were housed in 6 cages with 5 rats in each cage. There were six groups comprising the positive control which was fed standard rat feed and water, also the negative control which was given a high-calorie diet (high-fat feed and sugar water) and four treatment groups. The four treatment groups were fed on a high-calorie diet with a 5 % concentration of either *Corchorus olitorius*, *Crassocephalum crepidioides*, *Amaranthus hybridus* or *Solanecio bialafrae* respectively. Water (or sugar water) and feed intake of each group were measured and recorded daily. Weekly consumption of water and feed was computed for the entire 5 weeks of the experiment. The fasting blood sugar and weight of the test rats were recorded at baseline and weekly. Oral glucose tolerance test and serum insulin were determined at the end of the experiment using blood samples from the test rats. All results were analysed using ANOVA at $p \leq 0.05$ and means were separated with the use of Duncan's multiple range tests (SPSS 20.0). The high-fat feed was significantly different from the standard rat feed in the composition of fat (26.79 g) and calories (422.67 kcal). The negative control and the treatment groups got adapted to feeding on the high-calorie diet before the end of the experimental period. Water and feed intakes of the positive control were only significantly higher during the first three and four weeks, respectively. At the end of the experiment, the positive control had the highest weight gain of 22 g which was significantly different at $p \leq 0.05$. *C. crepidioides* and *S. bialafrae* significantly lowered the blood sugar (62.75 and 62.50 mg/dL) of the test rats. *A. hybridus* prevented insulin resistance by the attainment of peak level at 30 min alongside the positive control. There was a significant increase in the insulin level of the negative control while the vegetables prevented increased production of insulin.

Key words: Blood sugar, Diabetes, Feeding behaviour, High-calorie, Insulin, Obesity, Vegetable, Weight gain

INTRODUCTION

Eating (feeding) behaviour has considerable influence on health and it is the major risk factor for chronic diseases. People engage in eating behaviour as a matter of survival in contrast to seeking out food that would provide energy and nutrients [1]. Unhealthy dietary habits are becoming more frequent due to the nutritional transition that is affecting populations across developing countries where traditional healthy diets are being progressively replaced by more westernised dietary patterns [2]. The eating behaviour involving imbibing western dietary patterns is considered a significant contributor to the increases in body weight, adiposity and metabolic disease (such as type II diabetes) that have been taking place over the past years in Western and westernised societies [3].

Green leafy vegetables are known to offer various health benefits which include reduced risk of obesity, heart disease, high blood pressure and mental decline [4]. Vegetables contain a large amount of fiber as well as vitamins and are also known for their ability to prevent delayed colonic transit and increase bowel movement [5]. *Corchorus olitorius* L. (Jute mallow) is a traditional leafy vegetable that belongs to the family of *Malvaceae*. It can be found in most countries in tropical Africa where it is known by different names and its local name varies according to the different regions [6]. In terms of nutritional value, the leaves of *C. olitorius* are valued for their high dietary fiber, protein, vitamins, calcium, iron and folate. *Corchorus olitorius* contains more than 80 phenolic compounds and exhibits antioxidant properties. Consumption of *C. olitorius* helps to control blood pressure, cholesterol and lowers the risks of asthma, cancer, diabetes and heart disease [7].

Crassocephalum crepidioides is consumed and used for various purposes in many tropical and subtropical regions. The phytochemical screening of *C. crepidioides* showed the presence of pharmacologically important substances which possess antioxidant, anti-hyperlipidemic activity, chemopreventive and anti-inflammatory properties [8, 9]. It is used in several traditional therapeutics for liver dysfunction, stomach inflammation and breast cancer. A lotion of the leaves is used as a mild medicine that strengthens the stomach and excites its action [10]. *Solanecio biafrae* is another fresh succulent leafy vegetable that belongs to the family *Asteraceae*. It is an underutilized important African indigenous vegetable with nutraceutical properties. It is highly nutritive and medicinally important as a galactagogue and for the treatment of indigestion, diabetes, high blood pressure and infertility [11, 12].

Amaranthus hybridus popularly known as “Amaranth or pigweed” is highly nutritious, cheap to produce and easily adapts to the environment in which it grows [13]. Tea made from the leaves is used in the treatment of intestinal bleeding and diarrhea. It can be used as an antioxidant and to promote high-density lipoprotein (HDL) cholesterol. It also eases digestion and promotes satiety due to the amount of fiber it contains [14]. Thus, the study intended to determine the feeding behaviour, weight gain and blood sugar of Wistar rats fed on a high-calorie diet and the possible ameliorating effect of the vegetables.

MATERIALS AND METHODS

A. hybridus was obtained from Agboju market in Lagos State, while *C. crepidioides* and *C. olitorius* were from Isara-Remo market, Ogun State and *S. bialfrae* was bought from Oje market in Oyo State, Nigeria. The Wistar rats were purchased from the Animal Facility at Babcock University where the experiment was carried out. Babcock University is situated in Ilishan – Remo which is located within Latitude: 6.8932 East and Longitude: 3.7105 North in the Rainforest climatic region of Nigeria.

Processing of the vegetables

The vegetables were picked, washed, cut into smaller sizes and subsequently dried in a hot air oven at 40 °C. They were then ground into powder and packed in polythene bags until they were required. The vegetables were added to the feed individually at a concentration of 5 % for the different treatment groups.

High-calorie diet

The high-calorie diet was comprised of high-fat feed and sugar water. High-fat feed was made by the addition of vegetable oil to the rat standard feed at the ratio of 30:70 while sugar was added to the rats' drinking water at the concentration of 30 % to produce sugar water [15]. The vegetable oil is a common cooking oil that contains an appreciable amount of monounsaturated fatty acids (38 g), omega-9-fatty acids (38 g) and antioxidants with no cholesterol and less than 1 g of trans fatty acids.

Animal studies

The reference number for the experimental protocol is BUHREC803/19 which was approved by Babcock University Health Research Ethics Committee. A total of 30 male Wistar rats with an average weight of 127.4 g were housed in cages (45 × 90 × 35 cm) at a temperature of about 28 °C and 12 hours of light for acclimatization

for 10 days before the experiment started. During this period, the rats were fed on standard feed and water.

The rats were then divided into 6 groups comprising 2 control groups and 4 treatment groups. The positive control group was fed standard rat feed and water while the negative control group was given a high-calorie diet. The treatment groups were fed a high-calorie diet with vegetables and each group received only one of the vegetables. Experimental diets and drinking water were supplied in a feeding trial that lasted for 5 weeks.

The rats in each group were marked for identification before being weighed and the fasting blood sugar was taken using an Accu-check glucometer (CE 0088) before the experiment began, after which both parameters were measured and recorded weekly. Daily feed and water intake were also recorded and the weekly consumption of rats in each group was computed. The rats were deprived of feed and water for 12 h after the last day of the experiment for the oral glucose tolerance test. The blood samples of the rats in the different groups were collected by an ocular puncture for the determination of serum insulin. The blood was centrifuged for 10 minutes at $2,000 \times g$ to obtain blood serum which was preserved in the refrigerator until it was analysed.

Analysis

Proximate composition of standard rat feed and high-fat feed

The proximate composition of the standard rat feed and the high-fat feed (the main component of the high-calorie diet) used in this study was determined using the method described by the Association of Official Analytical Chemists [16] while the energy content was determined using the Atwater system. The Atwater general factor system includes energy values of 4 kcal per gram (kcal/g) (17 kJ/g) for protein, 4 kcal/g for carbohydrates and 9 kcal/g (37 kJ/g) for fat [17].

Oral Glucose Tolerance Test

This test measures the body's response to sugar or resistance to insulin. The test was determined according to the method described by Adeoye *et al.* [18]. Rats were deprived of food and water overnight for 12 h followed by placing in fresh cages without food. Cages and the rats were identified and the basal glucose concentration ($T = 0$) of each rat was measured by removing one rat at a time from its cage and making a small incision over the lateral tail with sterilized scissors. A small blood sample (3 μ L) was placed directly on the test stripe and inserted into the blood glucose monitor (CE 0088). Direct pressure was applied to the incision until the blood clot and the rat was returned to its cage. After all the rats had been

measured for basal glucose concentrations, glucose solution (45 %) was administered by oral gavage to each animal at a 30-sec interval between rats and the timer started with the first rat gavage. All administrations were finished within 15 min to perform the next blood glucose measurement at T = 30 min. This was repeated at T = 60 and T = 120 min.

Serum insulin

The serum insulin of the test rats was determined using Mercodia insulin ELISA assay kit (10-1113-01). All reagents and samples were at room temperature before use. Enzyme conjugate 1X solution, wash buffer 1X solution, insulin control solutions and calibrators were prepared according to the direction of the kit. The samples along with sufficient microplate wells to accommodate calibrators and samples in duplicate were also prepared and a plate plan was done.

Calibrators and samples (25 μ L each) were pipetted into different wells. Enzyme conjugate 1X solution (100 μ L) was added to each well and incubation was done on a plate shaker (700-900 rpm) for 2 hours at room temperature (18-25°C). Each well was washed 6 times with wash buffer 1X solution. The reaction volume was discarded by inverting the microplate over a sink. Wash solution (350 μ L) was added to each well which was later discarded and the microplate was tapped firmly several times against absorbent paper to remove excess liquid. The process was repeated 5 times and prolonged soaking during the washing procedure was avoided. Substrate tetramethylbenzidine with hydrogen peroxide (200 μ L) was added to each well which was incubated for 15 minutes at room temperature (18-25 °C) after which stop solution (50 μ L) was added to each well and the plate was placed on the shaker for approximately 5 seconds to ensure mixing. Optical density at 450nm was read within 30 minutes using spectrophotometric microplate reader (A51119700C) and the results were obtained from the calibration curve [19].

Statistical analysis

All analyses were carried out in triplicate and T-test was used to obtain the difference between the feeds at $p < 0.05$. While one-way analysis of variance was used to determine the significant difference among the groups followed by separation of the means by Duncan's multiple range tests using SPSS version 20.0. Standard error in the graphs indicates the difference among the groups.

RESULTS AND DISCUSSION

Unhealthy eating behaviour characterized by the consumption of a high-calorie diet is one of the factors associated with metabolic changes which result in an

increased risk of obesity and high blood sugar. [20]. Thus, the feeding behaviour, weight gain and blood sugar levels of rats fed on a high-calorie diet and the possible ameliorating effect of the commonly eaten vegetables were determined in this study.

Nutrient composition of standard and high-fat feed

Table 1 shows the results of the nutrient composition of the standard feed (Feed A) and high-fat feed (Feed B). Feed A was significantly ($p < 0.05$) higher in protein (17.78 %), carbohydrate (50.03 %), moisture content (7.68%) and ash content (9.71 %) while feed B was significantly higher in fat content (26.79%), fiber (14.83 %) and energy content (422.67 kcal/kg). After five weeks of the experiment, it was observed that the rats in the treatment groups and the negative control group had rough and deep yellow hair compared to rats in the positive control group. The feeds were different in composition and this observation is likely to be due to the yellow pigment (the carotene) in the oil. The observation corresponds with the report of Majeed *et al.* [21] who reported the development of rough and greasy hair in adult male rats after administration of vegetable oil.

Feeding behaviour of the test rats

The feeding behaviour of the rats was determined as mean water intake and mean feed consumption. Figures 1 and 2 present the mean water intake and feed consumption of the test rats per week, respectively. The water intake and feed consumption of the positive control were significantly higher than that of the other groups in the first three and four weeks of the experiment. This is because the rats in these other groups were not familiar with the high-calorie diet which corroborates the report of Duca *et al.* [22] and the behavioural studies by Sclafani [23] who demonstrated innate impairment of sweet detection but a well-developed taste for starch-derived polysaccharides (for example, polycose) in rats.

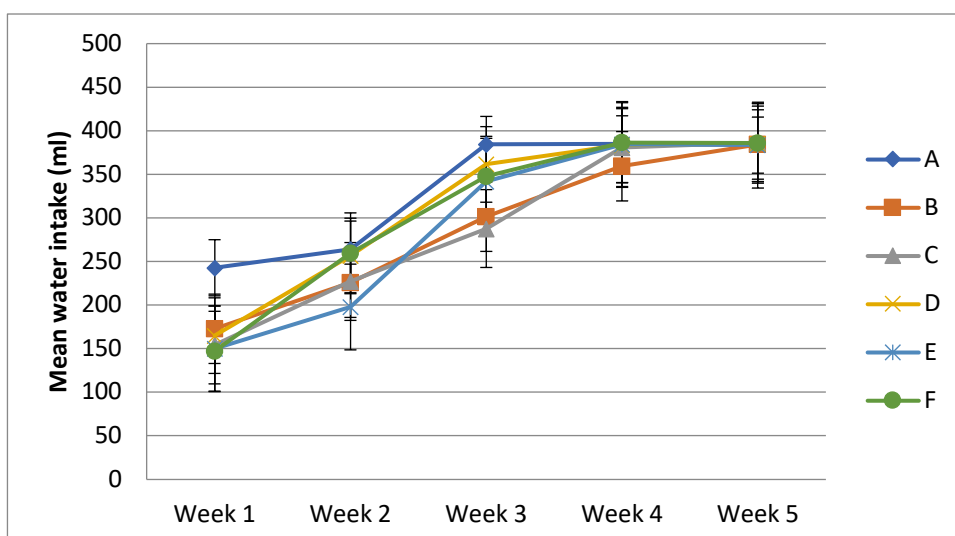


Figure 1: Mean weekly water (or sugar water) intake (ml)

A: Positive control group fed standard rat feed and water

B: Negative control group fed a high-calorie diet

C: Treatment group fed high-calorie diet and *Corchorus olitorius* leaves

D: Treatment group fed high-calorie diet and *Crassocephalum crepidioides* leaves

E: Treatment group fed high-calorie diet and *Amaranthus hybridus* leaves

F: Treatment group fed high-calorie diet and *Solanecio biafrae* leaves

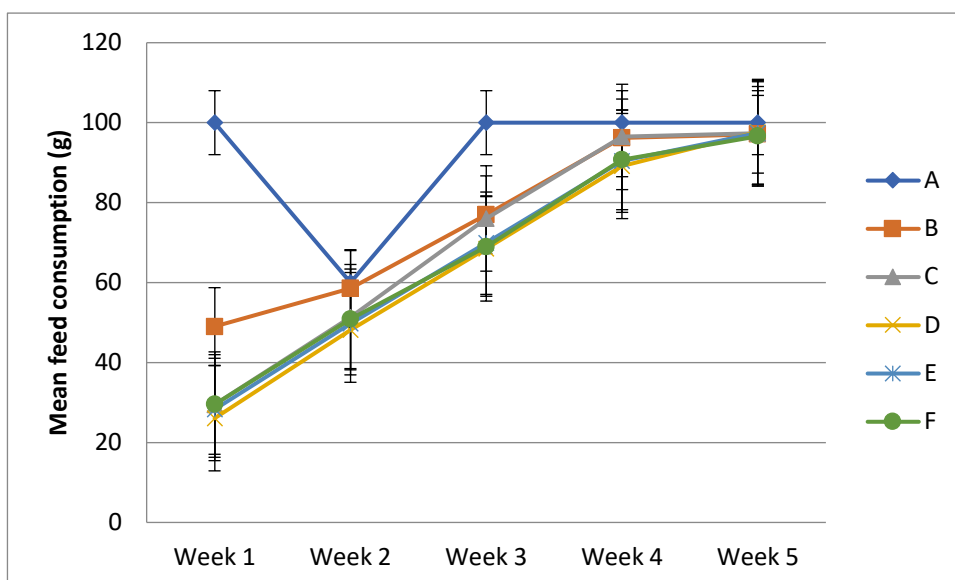


Figure 2: Mean weekly feed consumption (g)

A: Positive control group fed standard rat feed and water

B: Negative control group fed a high-calorie diet

C: Treatment group fed high-calorie diet and *Corchorus olitorius* leaves

D: Treatment group fed high-calorie diet and *Crassocephalum crepidioides* leaves

E: Treatment group fed high-calorie diet and *Amaranthus hybridus* leaves

F: Treatment group fed high-calorie diet and *Solanecio biafrae* leaves

The report of Zukerman *et al.* [24] that rats exhibit an experience-induced preference for sucrose corresponds with what was observed towards the end of the experimental period where the water intake and feed consumption of the rats in the different groups were not significantly different. This observation could be attributed to the impairment of the conditioned satiety response of the rats by the sucrose and the vegetable oil as reported by Aoyama and Nagano [25]. This observation towards the end of the experiment also supports the stimulus-response theories that behaviour is formed as a result of what we are repeatedly exposed to and this is an indication that eating habits are learned and developed over time when there is consistent exposure [26, 27]. Contrary to what was observed with the rats, there is a strong desire on the part of humans to seek and ingest sweet foods and drinks [28]. However, repeated experiences with initially disliked food can influence food preferences and eating habits [29].

Mean weight gain of the test rats (g)

The mean weight gain of rats in the positive control (22.54g) after five weeks was significantly higher than that of the other groups which could partly be attributed to a significant reduction in the protein content of the high-calorie feed fed to the negative control and the treatment groups. This finding supports the report of Li and Freeman [30] who reported a 10g loss in the weight of rats fed a high-fat, low-protein diet. However, this is contrary to the report of Buettner *et al.* [31] who reported significant weight gain in rats fed on lard, olive oil, fish oil and coconut oil.

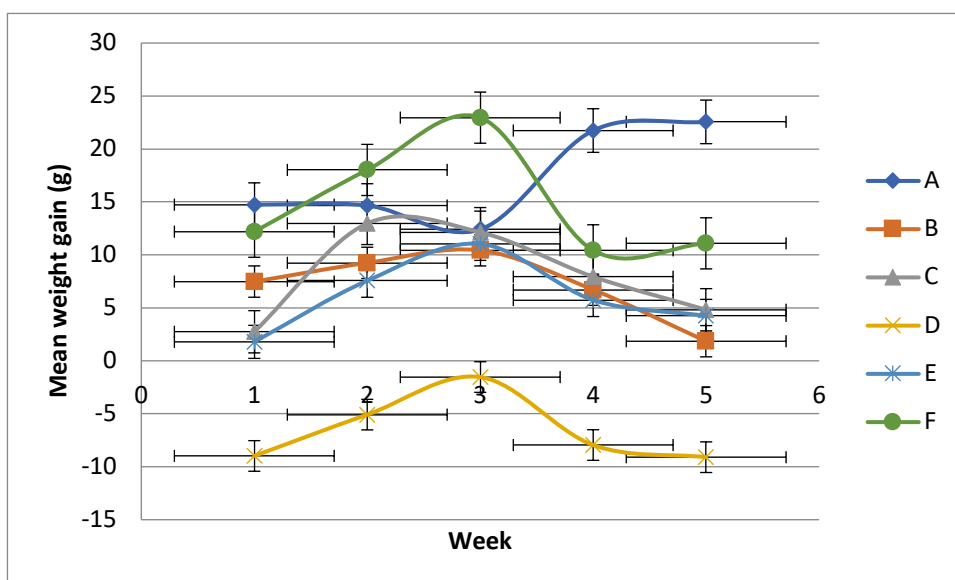


Figure 3: Mean weight gain of rats after five weeks

A: Positive control group fed standard rat feed and water

B: Negative control group fed a high-calorie diet

- C: Treatment group fed high-calorie diet and *Corchorus olitorius* leaves
D: Treatment group fed high-calorie diet and *Crassocephalum crepidioides* leaves
E: Treatment group fed high-calorie diet and *Amaranthus hybridus* leaves
F: Treatment group fed high-calorie diet and *Solanecio bialfrae* leaves

It was observed that the rats in the treatment groups (4.24 – 11.09g) except the group fed *C. crepidioides* (-9.10 g) had higher weight gain than the negative control (1.85 g). This observation may likely be due to the impairment of the metabolic process by the high-calorie diet which was ameliorated by the vegetables. Higher weight gain of rats in the treatment groups compared to the negative control is supported by the report of Onyechi *et al.* [32] who observed an increase in the mean body weight of diabetic rats ($p>0.05$) at different levels of supplementation with the leaf extracts of *C. olitorius*. Also, significant moderate weight gain by the group fed on *S. bialfrae* corroborates the findings of Lienou *et al.* [33] who reported a low body weight gain in rats which confirms the traditional health benefit claim of *S. bialfrae* at reducing plasma total cholesterol, body mass index (BMI) and plasma total triglycerides in pre-obese subjects [34]. Loss of weight recorded in the group fed *C. crepidioides* could be a result of the vegetable possessing significant anti-hyperlipidemic activity as reported by Bahar [9] which was also confirmed by Musa *et al.* [35] who reported a nonsignificant increase in the weight gain of rats fed *C. crepidioides*.

Mean fasting blood sugar

The blood sugar levels of all the groups including the control groups fluctuated throughout the experiment (Figure 4). There was a decline in the blood sugar of all the groups at week 1 when the rats were newly introduced to the high-calorie feed though the decline in the blood sugar of the positive control group was incomprehensible.

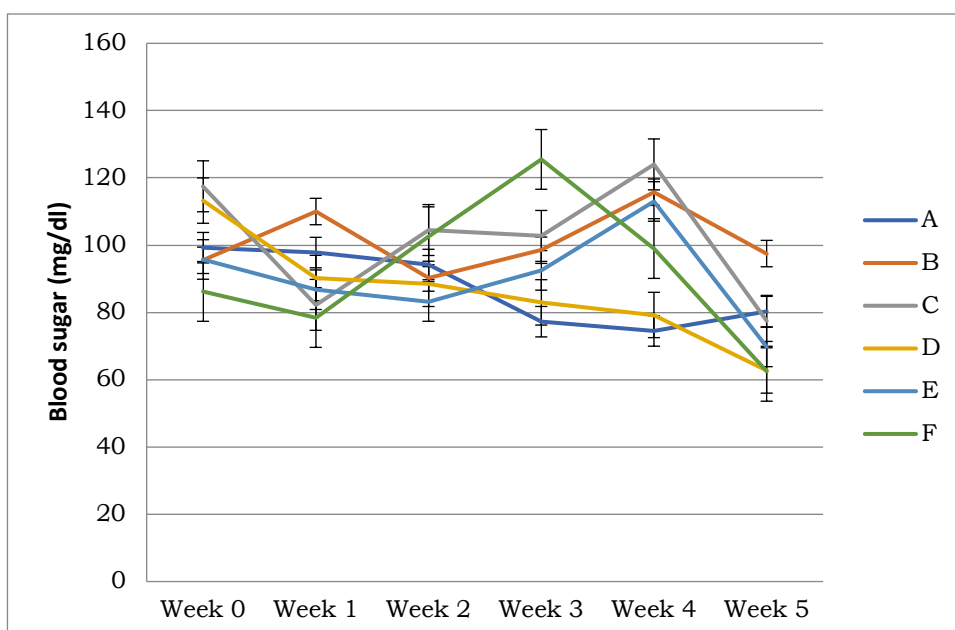


Figure 4: Mean blood sugar of rats during five weeks of the experiment

A: Positive control group fed standard rat feed and water

B: Negative control group fed a high-calorie diet

C: Treatment group fed high-calorie diet and *Corchorus olitorius* leaves

D: Treatment group fed high-calorie diet and *Crassocephalum crepidioides* leaves

E: Treatment group fed high-calorie diet and *Amaranthus hybridus* leaves

F: Treatment group fed high-calorie diet and *Solanecio biafrae* leaves

The blood sugar of the four treatment groups and control A was significantly ($p < 0.05$) lower than that of the negative control at the end of the experiment. This corroborates the report of Okoro [36] of 125 and 62.5 mg/kg body weight of *S. biafrae* efficacy in reducing significantly ($p < 0.05$) postprandial hyperglycemia after administration. Also, the finding about the effect of *C. olitorius* on blood sugar is in agreement with the report of Onyechi *et al.* [32] while the stimulating property of *A. hybridus* and *C. crepidioides* on the stomach and digestion is likely to be responsible for the blood sugar lowering effect of these vegetables observed in this study [14]. The blood sugar level of the positive control fed standard rat feed being comparable to that of the treatment groups could be attributed to higher feed consumption and higher weight gain of rats in this group.

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) is used to determine resistance to insulin and Fig. 5 shows the results of the OGTT carried out. At baseline, the mean glucose level of the groups was between 62.50 ± 7.14 and 81.75 ± 12.09 , while at 120 min, it was from 77.25 ± 11.98 to 107.25 ± 3.59 mg/dL. The study showed that the treatment groups and the negative control peaked at 60 min except for the

group fed *A. hybridus*, which attained its peak at 30 min alongside the positive control. This observation strongly supports the report that *A. hybridus* possesses a significant protective effect against oxidative damage in diabetic rats as reported by Balasubramanian and Karthikeyan [37].

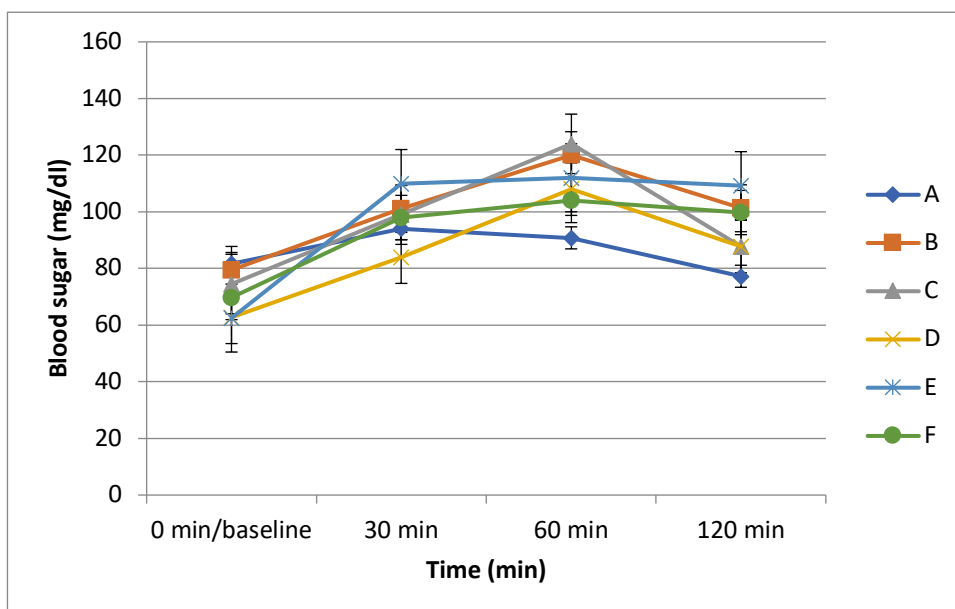


Figure 5: Oral glucose tolerance test of the rats

A: Positive control group fed standard rat feed and water

B: Negative control group fed a high-calorie diet

C: Treatment group fed high-calorie diet and *Corchorus olitorius* leaves

D: Treatment group fed high-calorie diet and *Crassocephalum crepidioides* leaves

E: Treatment group fed high-calorie diet and *Amaranthus hybridus* leaves

F: Treatment group fed high-calorie diet and *Solanecio biafrae* leaves

The peaks of the treatment groups (except the group fed *A. hybridus* and the negative control at 60 min) were not significantly different. Attainment of the peak at 60 min is an indication that the cells of all the rats in the negative control and the treatment groups except the group fed *A. hybridus* were already getting resistant to insulin as a result of the high-calorie diet and there was likely to be an impairment of glucose metabolism [38].

Mean serum insulin of the test rats

Figure 6 shows the results of serum insulin, the negative control had the highest amount of insulin in the serum with 35.20 μ U/ml while group C (fed *C. olitorius*) had the lowest with 24.75 μ U/ml. It has been shown that high insulin levels are usually observed in hyperglycemia as reported by Ensling *et al.* [39].

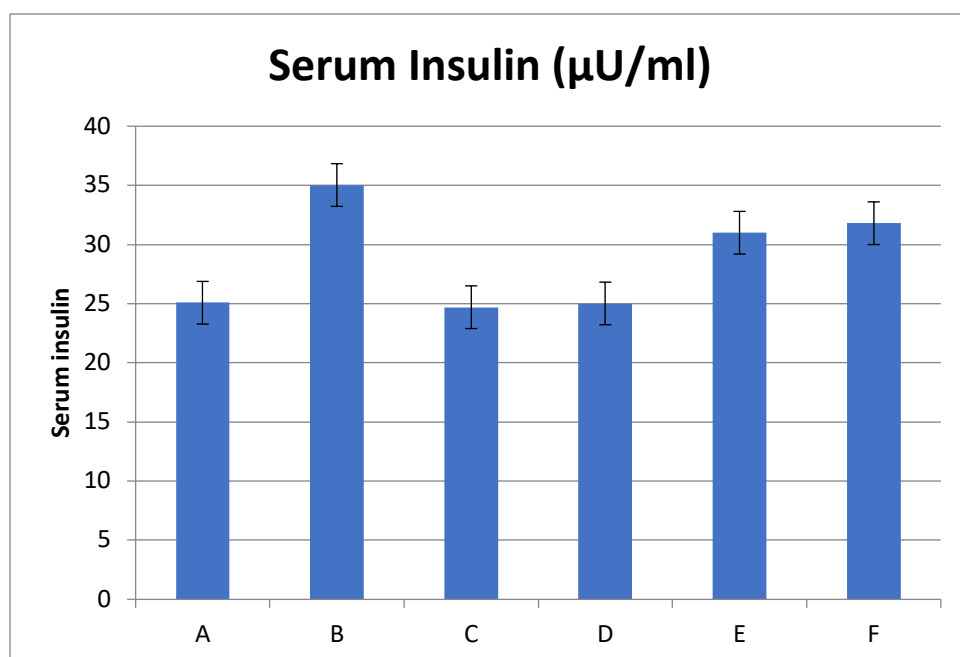


Figure 6: Serum Insulin of the test rats (µU/ml)

A: Positive control group fed standard rat feed and water

B: Negative control group fed a high-calorie diet

C: Treatment group fed high-calorie diet and *Corchorus olitorius* leaves

D: Treatment group fed high-calorie diet and *Crassocephalum crepidioides* leaves

E: Treatment group fed high-calorie diet and *Amaranthus hybridus* leaves

F: Treatment group fed high-calorie diet and *Solanecio biafrae* leaves

The serum insulin of the groups fed *C. olitorius* (24.75 µU/ml) and *C. crepidioides* (25.28 µU/ml) was not far from each other and was not significantly lower than that of the positive control group (25.78 µU/ml). This finding about the effect of vegetables on serum insulin in the hyperglycemic condition support the earlier reports of Onyechi *et al.* [32], Adelakun and Ogunlade [40], Balasubramanian and Karthikeyan [37], and Okoro [34].

CONCLUSION

The rats did not show an innate preference for sweet foods but got adapted to the consumption of a high-calorie diet before the end of the experiment. The effect of consumption of high-calorie feed on blood sugar was ameliorated by the simultaneous consumption of vegetables. The consumption of vegetables has to be consistent over some time for the hypoglycemic effect to be expressed. There was a loss of weight with the consumption of *C. crepidioides* and both *C. crepidioides* and *S. biafrae* showed the highest blood sugar-reducing properties. *A. hybridus* prevented resistance to insulin and all the vegetables prevented increased insulin production as a result of hyperglycemia.

Table 1: Nutrient composition of the standard rat feed and high-fat feed

Sample	Feed A	Feed B	T-Value	P-Value
Crude protein (%)	17.78±0.03	13.77±0.08	84.924	0.11
Fat (%)	5.41±0.03	26.79±0.04	-717.107	0.52
Moisture (%)	7.68±0.02	6.19±0.05	51.615	0.22
Fiber (%)	9.38±0.04	14.83±0.10	-88.932	0.11
Ash (%)	9.71±0.05	6.79±0.05	78.040	1.00
Carbohydrate (%)	50.03±0.07	31.62±0.09	274.052	0.53
Energy (kcal)	319.89±0.11	422.67±1.04	-169.715	0.11

Feed A: Standard rat feed

Feed B: High fat feed

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