EFFECT OF FLAVONOIDS ISOLATED FROM GARDEN EGG VEGETABLES (*SOLANUM MACROCARPUM*) IN DIET INDUCED OBESE (DIO) WISTAR RATS.

Background and objectives: Increased consumption of fatty foods and refined carbohydrates are poor feeding habit of people which leads to increased incidence of obesity, diabetes, cardiovascular diseases, high blood pressure and cancer. There is low consumption of vegetables which lead to prevalence of micronutrient deficiency. This study was designed to determine the effect of flavonoids isolated from (*Solanum macrocarpum*) in DIO Wistar Rats. **Methods:** Four groups of twenty male Wistar rats were fed a highly palatable diet for 2 weeks to induce obesity resembling mild obesity condition in human population. DIO rats received rat chow and flavonoids extract daily for 6 weeks. Group 1 received rat chow alone; Group 2- 0.05% of flavonoids extract and rat chow; Group 3- 0.15% of flavonoid extract and rat chow; and Group 4- 0.25% of flavonoid extract and rat chow. BMI, Total cholesterol, HDL, LDL and Triglyceride were evaluated using standard analytical technique. The data were statistically analyzed using ANOVA and mean separated using LSD. **Results:** Feeding the rats with palatable diet showed increased in BMI, total cholesterol, LDL and triglyceride levels along with decrease in HDL ($p<0.05$). Consumption of flavonoids resulted in the significant reduction in BMI, LDL, total cholesterol and triglyceride level and exhibited significant elevation in HDL cholesterol compared to the rats fed only rat chow ($p<0.05$). It was observed that the decrease in BMI, Triglyceride, total cholesterol and LDL cholesterol level of rats fed 0.25% of flavonoids were significantly different ($p<0.05$) from those fed 0.15% and 0.05% flavonoids. **Conclusions:** The results suggest that flavonoids extract from *Solanum macrocarpum* has atherogenic effect which can help to reduce obesity.

Key words: Flavonoids, *Solanum Macrocarpum,* lipid profile, Obesity and Rats.

Introduction

Quite large number of vegetables have long been known and reported to have health protecting properties and uses. Vegetables are important sources of protective substances, which are highly beneficial for the maintenance of good health and prevention of diseases (Sheela et al., 2004; Nnamani, Oselebe, & Agbatutu, 2007). The indigenous knowledge of the health promoting and protecting attributes of vegetables are clearly linked to their nutritional and non- nutrient bioactive properties. Vegetables have long been, and continue to be reported to significantly contribute to the dietary vitamin and mineral intakes of local populations (Oboh and Akindahunsi, 2005). More recent reports have shown that they also contain non- nutrient bioactive phytochemicals that have been linked to protection against cardiovascular and other degenerative diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (http//www.phytochemicals.info). There are many phytochemicals and each works differently. The feeding habits of people have deteriorated leading to high consumption of fatty foods and refined carbohydrates. The poor feeding habits lead to increased incidence of obesity, diabetes, cardiovascular diseases, high blood pressure and cancer that have been previously rare in the society. These problems are more prominent in urban areas where there is increased preference for a few exotic foods. The food base for the rural population has become narrower, leaving communities more vulnerable to food shortages and nutrient-deficiency diseases. There is low consumption of green leafy vegetables in the diet and these lead to prevalence of micronutrient deficiency. These deficiency diseases lead to retarded physical growth, low intellectual development and a variety of other conditions. The major

problem that leads to this study is that there is high prevalence of obesity in the society. The effect of flavonoid levels on obesity induced rats would be ascertained.

MATERIALS AND METHODS

The study design used was experimental des The fresh leaves of *Solanum ~~macrocarpum~~* was purchased from Ogige market, in Nsukka L.G.A of Enugu State Nigeria. They were separately plucked and sorted by removing extraneous materials and rinsed with deionized water.

Extraction of Flavonoids

Petu um ether, ethanol (aqueous solution: 70 vol.%) and distilled water was used as the extracting solvents for the extraction of flavonoid (Velickovic et al., 2006).

Sourcing of animals

Twenty male adult rats were purchased from the Department of Veterinary Pathology, University of Nigeria, Nsukka. The animals were divided into 4 groups of 5 rats each on the basis of body weight such that the difference in mean body weight of each group did not exceed 5g (AOAC, 1995). The rats were housed individually in cages equipped to separate urine and feaces in the Department of Home Science, Nutrition and dietetics, University of Nigeria Nsukka animal house.Twenty male adult rats were fed a highly p able diet for 3 weeks to induce mild obesity. The composition of rodent pelleted chow, are 60% of ~~energy as~~ carbohydrate, 30% ~~as~~ protein and 10% ~~as~~ fat. The palatable diet consisted of 33% chow, 33% condensed milk and 7% sucrose by weight, with the remainder being added water. This provided 65% ~~of energy as~~ carbohydrate, 19% ~~as~~ protein and 16% ~~as~~ fat. This diet was designed to promote weight gain through hyperphagia, without employing major changes in macronutrient composition, compared with normal rat chow. This is a reliable method of inducing weight gain and insulin resistance (Widdowson *et al*., 1997; Wilding *et al*., 1992; Pickavance *et al*., 1999). Rats were allowed free access to water throughout the study and were maintained on a 12 : 12 h light k phase schedule.

At the end of the 2-weeks period, when the palatable diet-treated rats had developed significant weight gain. The extracts were given orally with stringe daily for 6 weeks to the animals. The groups were treated as follows- Group 1 received rat chow alone; Group 2- (0.05% ie 100g) of flavonoids extract; Group 3- (0.15% ie 300g) of flavonoid extract; and Group 4 (0.25%ie 500g) of flavonoid extract. The weights and length of animals was recorded each day. Daily food intake and extract was also recorded to calculate nutrient intake.

Table 1: Diet Composition

The composition of the diet is presented in the table below.

GROUP1	GROUP 2	GROUP 3	GROUP4

Rat chow	Rat chow + 0.05% flavonoids	Rat chow + 0.15% flavonoids	Rat chow + 0.25%flavonoids

Blood sample collection and biochemical indices determination

Blood was collected from the retro-bulba plexus of the medial canthus of the eye of the rats. A nucrocapillary tube was carefully inserted into the canthus of the eye to puncture the retro-bulbar plexus and thus enable outflow of about 2ml of blood into a clean glass test tube. The blood sample was kept at room temperature for 30 minutes to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3,000 revolutions per minute for ten minutes using a table centrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernant was then carefully aspirated with syringe and needle and stored in a clean sample bottle for the clinical chemistry determination. Blood were collected on weeks 0, 2, 4, 6 and 8 for hematological determinations. The serum was used to determine the cholesterol, LDL, HDL and triglyceride.

Hematological determination

Enzymatic colorimetric test (CHOD- PAP method) for the in-vitro determination of cholesterol in serum, using Quimica Clinica Applicada (QCA) cholesterol test kit was used to determine serum cholesterol (Allain, Poon, Chan, Richmond and Fu, 1974).

Dextran sulphate-mg (II) method for the in-vitro determination of HDL-cholesterol in serum, using Quimica Clinica Applicada (QCA) HDL test kit was used to determine HDL (Albers, Warnick and Cheung, 1978).

Polyvinyl sulphate method for the in-vitro determination of LDL-cholesterol in serum using Quimica Clinica Applicada (QCA) LDL test kit was used to determine LDL (Assman, Jab and Hohnert, 1984).

The glycerol-phosphate oxidase method (enzymatic test) for the in-vitro determination of triglycerides in serum, using Quimica Clinica Applicada (QCA) Triglyceride test kit was used to determine triglyceride (Jacobs and VanDemark, 1960).

The Body mass index was determined as follows:

Instruments

1. Weighing scale.
2. Measuring tape

Method

1. The length of the rat was determined by measuring the rat with measuring tape from the head to the tail and it was then recorded in centimeters.
2. The weight of the rat was also determined with a weighing scale and the weight recorded in grammes

BMI was calculated by the formula:

$$\frac{\text{Weight (g)}}{\text{Length (cm)}^2}$$

The result was expressed as $g/cm^{2.}$

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) version 22. Means were separated using Least Significance Difference (LSD) and

Results

Table 2 shows the mean Body Mass Index (BMI) of rats from day 0-week 8. At day 0, which was the first day after acclimatization, the BMI of rats ranged from 0.35-0.40g/cm^2. At week 2 when obesity was confirmed the BMI level was between 0.60- 0.64g/cm^2. The mean BMI of rats fed rat chow and varied level of flavonoid extract decreased as the week progresses with the group fed 0.25% flavonoid having the highest decrease.

Table 2: Effect of flavonoid extract on the BMI of rats.

Days	Group 1 Rat chow alone	Group2 Rat chow + 0.05% Flavoniods	Group3 Rat chow+ 0.15% Flavoniods	Group 4 Ratchow+ 0.25% Flavoniods
Day 0	0.40	0.36	0.36	0.35
Week 2	0.61	0.64	0.65	0.60
Week 4	0.63 (3.28%)↑	0.63 (1.56%)↓	0.62 (4.60%)↓	0.50 (10.00%)↓
Week 6	0.63 (0%)	0.62 (1%)↓	0.52 (16.13%)↓	0.46(8.00%)↓
Week 8	0.61 (3.28%)↓	0.54 (10.59%)↓	0.45 (13.46%)↓	0.38 (17.39%)↓
Total % ↓&↑	0%	13.15%↓	34.19%↓	35.39%↓

Value in bracket is the percentage decrease and increase.
Day 0: First day after acclimatization,Week 2: The day obesity was confirmed.Week 4: First test of recovery. Week 6 : Second test of recovery. Week 8: Last test of recovery.
Key: ↑-increase
↓-decrease

Table 3 shows the mean cholesterol level of rats from day 0 to week 8. At day 0, which was the first day after acclimatization, the total cholesterol of rats ranged from 1.59-1.63mmol/L. At week 2 when Obesity was confirmed the total cholesterol level was between 2.70-2.74mmol/L. The mean cholesterol level of rats fed rat chow and varied level of flavonoids extract decreases as the week go by with the group that received 0.25% flavonoid extract having the highest level of increase.

Table 3: Effect flavonoid extract on the Cholesterol level of rats

Days	Group 1 Rat chow alone	Group2 Rat chow + 0.05% Flavoniods	Group3 Ratchow+0.15% Flavoniods	Group4 Ratchow+0.25% Flavoniods
Day 0	1.60	1.63	1.59	1.61
Week 2	2.72	2.73	2.70	2.74
Week 4	2.70 (0.74%)↓	2.61 (4.40%)↓	2.39 (11.48%)↓	2.10 (23.36%)↓
Week 6	2.72 (0.74%)↑	2.39(12.26%)↓	2.14 (10.46%)↓	1.96 (6.67%)↓
Week 8	2.73 (0.37%)↑	2.07(9.61%)↓	1.88 (12.15%)↓	1.64 (16.33%)↓
Total % ↓&↑	0.37%↑	26.27% ↓	34.09%↓	46.36%↓

Value in bracket is the percentage decrease and increase.
Day 0: First day after acclimatization,Week 2: The day obesity was confirmed.Week 4: First test of recovery. Week 6 : Second test of recovery. Week 8: Last test of recovery.
Key: ↑-increasen
↓-decrease

Table 4 shows the mean Low Density Lipoprotein cholesterol (LDL) of rats from day 0 to week 8. At day 0, which was the first day after acclimatization, the LDL cholesterol of rats ranged from 0.48-0.50mmol/L. At week 2 when Obesity was confirmed the LDL cholesterol level was between 0.92-0.99mmol/L. The mean LDL cholesterol of rats fed rat chow and varied level of flavonoid extract decreased with increase in weeks while the group that received 0.25% flavonoid extract having the highest level of decrease.

Table 4: Effect of flavonoid extract on the Low Density Lipoprotein cholesterol level of rats.

Days	Group 1 Rat chow alone	Group2 Rat chow+ 0.05% Flavoniods	Group3 Rat chow+ 0.15% Flavoniods	Group4 Ratchow+ 0.25% Flavoniods
Day 0	0.50	0.50	0.48	0.50
Week 2	0.95	0.99	0.92	0.92
Week 4	0.94 (1.05%)↓	0.86 (13.13%)↓	0.83(9.78%)↓	0.77 (16.30%)↓
Week 6	0.95 (1.06%)↑	0.73(1%)↓	0.79 (3.32%)↓	0.67 (12.99%)↓
Week 8	0.93 (2.11%)↓	0.72 (10.59%)↓	0.45 (15.18%)↓	0.49 (26.87%)↓
Total % ↓&↑	2.10%↓	24.72%↓	28.28%↓	55.26%↓

Value in bracket is the percentage decrease and increase.
Day 0: First day after acclimatization,Week 2: The day obesity was confirmed.Week 4: First test of recovery. Week 6 : Second test of recovery. Week 8: Last test of recovery.
Key: ↑-increase
↓-decrease

Table 5 shows the mean High Density Lipoprotein cholesterol (HDL) of rats from day 0 to week 8. At day 0, which was the first day after acclimatization, the HDL of rats ranged from 1.14-1.17mmol/L. At week 2 when Obesity was confirmed the HDL cholesterol level was between 0.47-0.48mmol/L.The mean HDL of rats fed varied level of flavonoid extract inceased as the weeks increased with the group fed 0.25% having the highest level of increase.

Table 5: Effect of flavonoid on the High Density Lipoprotein cholesterol level of rats

Days	Group 1 Rat chow alone	Group2 Rat chow + 0.05% Flavoniods	Group3 Ratchow + 0.15% Flavoniods	Group4 Ratchow+0.25% Flavoniods
Day 0	1.15	1.14	1.14	1.17
Week 2	0.48	0.48	0.47	0.47
Week 4	0.46 (4.17%)↓	0.68 (41.67%)↑	0.67 (42.55%)↑	0.79(68.09%)↑
Week 6	0.48 (4.38%)↑	0.70 (2.94%)↑	0.85 (26.87%)↑	0.93 (17.72%)↑
Week 8	0.47 (2.08%)↓	0.96(37.14%)↑	1.05 (23.53%)↑	1.12 (20.43%)↑
Total % ↓&↑	0.87%↓	81.75%↑	92.95%↑	106.24%↑

Value in bracket is the percentage decrease and increase.
Day 0: First day after acclimatization,Week 2: The day obesity was confirmed.Week 4: First test of recovery. Week 6 : Second test of recovery. Week 8: Last test of recovery.
Key: ↑-increase
↓-decrease

Table 6 shows the mean Triglyceride of rats from day 0 to week 8. At day 0, which was the first day after acclimatization, the triglyceride level of rats ranged from 0.60-0.66mmol/L. At week 2 when Obesity was confirmed the triglyceride level was between 1.91-1.96mmol/L. The mean triglyceride level of rats fed rat chow and varied level of flavonoid extract varied immensely with the group that received 0.25% extract having the highest decrease in triglyceride.

Table 6: Effect of flavonoid on the Triglyceride level of rats

Days	Group 1 Rat chow alone	Group2 Rat chow + 0.05% Flavoniods	Group3 Rat chow + 0.15% Flavoniods	Group4 Rat chow+ 0.25% Flavoniods

Day 0	0.66	0.64	0.66	0.60
Week 2	1.91	1.95	1.91	1.96
Week 4	1.94 (1.57%)↑	1.92 (1.54%)↓	1.65 (13.61%)↓	1.59 (18.88%)↓
Week 6	1.90 (2.06%)↓	1.72 (10.42%)↓	1.59 (3.64%)↓	1.26 (20.75%)↓
Week 8	1.91 (0.52%)↑	1.60 (6.98%)↓	1.29 (18.88%)↓	0.72 (42.86%)↓
Total % ↓&↑	0.03%↑	18.94%↓	36.13%↓	82.49%↓

Value in bracket is the percentage decrease and increase.
Day 0: First day after acclimatization,Week 2: The day obesity was confirmed.Week 4: First test of recovery. Week 6 : Second test of recovery. Week 8: Last test of recovery.
Key: ↑-increase
↓-decrease

Discussion

Body mass index (BMI): There was an increase in the BMI level of the rats fed fat diet from day 0 which was 0.35- 0.40g/cm^2 to 0.60-0.65g/cm^2 on week 2. These were as a result of gradual build up of fatty substances, including cholesterol on the walls of the arteries. This build-up reduces the blood flow to the heart, brain and other tissues which is known as hardening of the arteries (Patel, 2008). These result to athereosclerosis and obesity. There was a decrease in the BMI level of rats fed varied levels of flavonoids extracts from week 4 to week 8.The decrease in BMI observed may likely be due to the flavonoids extract that was added to the rat chow. This observation is in line with the findings of Huxley and Neil (2003) who opined that high dietary intake of flavonoids from fruits and vegetables as well as from tea and wine, may be associated with a decrease in cardiovascular diseases (CVD) mortality in free living population, where obesity/atherosclerosis is a risk factor. The result showed a higher decrease (35.39%) in BMI of the rats fed 0.25% flavonoids than those fed 0.15%, 0.05 % flavonoids and those that received rat chow alone. It was observed that the total percentage decrease in BMI of rats fed 0.25% flavonoid extract was (35.39%) which is higher than those rats fed 0.15% flavonoid extract (34.19%), 0.05% flavonoid extract (13.15%) and those received rat chow alone (0%).

Cholesterol: There was an increase in the total cholesterol level of the rats from day 0 (1.59-1.63mmol/L) to 2.70-2.74mmol/L at week 2 after consumption of high fat diet. Nordqvist (2009) reported that when both blood cholesterol and triglyceride level are high, the risk of developing coronary heart disease rises significantly. There was a decrease in the total cholesterol level of rats fed varied levels of flavonoids extract from week 4 to week 8. The decrease in total cholesterol observed may be due to the addition of flavonoids to the rat chow. A Japanese study reported an inverse correlation between flavonoid intake and total plasma cholesterol concentration. Nordqvist (2009) also observed that low level of cholesterol aids in the production of bile, which converts ~~sunshine~~ to vitamin D. Bile is also important for the metabolism of fat soluble vitamins, including vitamin A, D, E and K. The result showed higher decrease in cholesterol level of rats fed 0.25% flavonoids (46.36%) than the other three experimental groups. It was observed that the total percentage decrease in cholesterol levels of rats fed 0.25% flavonoid extract was ~~(~~46.36%) which is higher than those of rats fed 0.15% flavonoid extract (34.09%), 0.05% flavonoid extract (26.27%) and those received rat chow

alone had 0.37% increase. ~~The result is in line with the findings of Huxley and Neil (2003) who opined that high dietary intake of flavonoids from fruits and vegetables as well as from tea and wine, may be associated with a decrease in CVD mortality in free living population which obesity/atherosclerosis is a risk factor~~.

Low Density Lipoprotein (LDL): There was an increase in the LDL cholesterol level of the rats from day 0 (0.48-0.50mmol/L) to 0.92-0.99mmol/L at week 2 after consumption of high fat diet. The decrease in the LDL cholesterol level of rats fed varied levels of flavonoids extract from week 4 to week 8 is of interest. The decrease in the level of LDL cholesterol level may be due to the consumption of flavonoids, which was added to the rat chow. Middleton, Kandaswani and Theoharides (2000) observed that elevated plasma low density lipoprotein (LDL) cholesterol concentration is a primary risk factor for the development of atherosclerosis and coronary artery diseases. Nordqvist (2009) observed that high LDL cholesterol increased the risk of arterial disease. Hertog, Fesich and Hollman (1993) observed that flavonoids seem to suppress LDL cholesterol oxidation and inflammatory progression in the artery wall. The result showed a higher decrease in LDL cholesterol level of rats fed 0.25% flavonoids than the other three experimental groups. It was observed that the total percentage decrease in LDL of rats fed 0.25% flavonoid extract was (55.26%) which was higher than those of rats fed 0.15% flavonoid extract (28.28%), 0.05% flavonoid extract (24.72%) and those that received rat chow alone (2.10%). ~~The results were in line with the findings of Huxley and Neil (2003) who opined that high dietary intake of flavonoids from fruits and vegetables as well as from tea and wine may be associated with a decrease in CVD mortality in free living population where obesity/atherosclerosis is a risk factor.~~

High Density Lipoprotein (HDL): There was a decrease in the total HDL cholesterol level of the rats from day 0 (1.14-1.17mmol/L) to 0.47-0.48mmol/L at week 2 after consumption of high fat diet. There was an increase in the HDL cholesterol level of rats fed varied levels of flavonids extract from week 4 to week 8.The increase in the level of HDL cholesterol level may be due to the addition of flavonoids to the rat chow. Nordqvist (2009) noted that HDL cholesterol takes cholesterol away from the cells. Sonoyama et al. (1995) reported that plasma cholesterol concentration was significantly lowered in rats fed beet fibre and this difference was due mainly to a higher HDL cholesterol concentration. The result showed higher increase in HDL cholesterol level of rats fed 0.25% flavonoids than those fed 0.15%, 0.05 % flavonoids extract and rat chow alone. It was observed that the total percentage increase in HDL of rats fed 0.25% flavonoid extract was (106.24%) which was higher than those of rats fed 0.15% flavonoid extract (92.95%), 0.05% flavonoid extract (81.75%) and those that received rat chow alone had 0.87% decrease. Dauchet, Amonyel, Herberg and Dallongeville (2006) observed that the risk of CVD was dose dependent and decreased by 4% for each additional portion per day of vegetables and by 7% for fruits consumption.

Triglycerides: There was an increase in the triglyceride level of the rats from day 0 (0.60-0.66mmol/L) to 1.80-2.20mmol/L at week 2 after consumption of high fat diet. There was a decrease

in the triglyceride level of rats fed varied levels of flavonoids extract from week 4 to week 8.The decrease in the level of triglyceride level was probably due to the addition of flavonoids to the rat chow. Nelson, Cox and Lehninger (2000) observed that in human body, high triglycerides in the blood stream have been linked to atherosclerosis and by extension, the risk of heart disease and stroke. The risk can partly be accounted for by a strong inverse relationship between triglyceride and HDL cholesterol level (Nelson et al., 2000). The result showed a higher decrease in triglceride level of rats fed 0.25% flavonoids than those fed 0.15%, 0.05 % flavonoids extract and rat chow alone. It was observed that the total percentage decrease in triglyceride of rats fed 0.25% flavonoid extract was 82.49% which was higher than those of rats fed 0.15% flavonoid extract (36.13%), 0.05% flavonoid extract (18.94%) and those that received rat chow alone had 0.03% increase.

Conclusion

This study showed that flavonoid extract from *Solanum ~~macranthum~~* significantly reduced the lipid profile (Body mass index (BMI), low density lipoprotein (LDL), cholesterol and triglyceride) of diet induced obesed rats. Consumption of 500g portion size of *Solanum ~~macranthum~~* daily is of great importance since reduction of obesity and atherosclerosis is dose depenedent. Consumption of small quantity of vegetables will be of little or no benefit to the body. These extract however caused significant decreases in BMI, LDL, Total cholesterol and Triglyceride levels with increase in HDL cholesterol of the rats. The result suggests a great atherogenic potential of *Solanum ~~macranthum~~*.

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