Effect of varieties of *Abelmochus esculentus* (Okra) fruits extract on lipid profile of rats fed high-fat diet

ABSTRACT

Background: Okra fruits have been reported as an efficient remedy in the management of hyperlipidemia. But, there are different varieties of Okra, and whether their antihyperlipidemic efficiency varies with the varieties has not been reported.

Aim of the study: The study aimed at investigating antihyperlipidemic efficiency of two commonly consumed okra fruits varieties (NHB-AI-B and Yar Kolon) in Bauchi State.

Methods: The *NHB-AI-B* and *Yar Kolon* okra fruits were sliced, air dried and pulverized into powder then extracted with methanol (80%) using Soxhlet extractor and concentrated at 30°C in a rotary evaporator then finally air dried. Hyperlipidemia was induce by feeding rats with high fats diet for 35 days, then followed by treating with the okra extracts for 21 days. Nine groups of five rats was used: groups 1-3 (hyperlipidemic rats received *NHB-AI-B* okra fruit extract at different doses), group 4-6 (hyperlipidemic rats received *Yar kolon* okra fruit extract at different doses), group 7 (positive control rats treated with 10mg/kg Atorvastatin), group 8 (normal control rats fed basal diet) and group 9 (negative control). Lipid profile were determined from serum of each rat.

Results: The results showed rats fed high fat diet developed hyperlipidemia as evident by their elevated triglyceride, cholesterol, LDL-C, body weight and supressed HDL-C. When Treated with okra fruit extracts from *NHB-AI-B* and *Yar kolon* varieties their altered lipid profile were significantly reversed toward normalcy compared to the untreated rats group. The fiber content of the two okra varieties; *NHB-AI-B* is 12.51±0.00% whereas *Yar kolon* (14.74±0.17%).

Conclusion: The study showed okra fruit extracts from *NHB-AI-B* and *Yar kolon* exert antihyperlipidemic effect where *Yar kolon* variety seem to be the most efficient indicating that antihyperlipidemic potency of okra may varies with their varieties. This, therefore calls for further study to compare more okra varieties to ascertain the most potent.

Keywords: Okra, varieties, *NHB-AI-B, Yar kolon*, fruits, antihyperlidemic, rats, high-fat diet

1. INTRODUCTION

Hyperlipidemia a systemic disorder that impairs the body in a generally unnoticeable, gradual, progressive and systemic way. It accelerate systemic arteriosclerosis, which is an important risk factor for many diseases, such as stroke, coronary artery disease, myocardial infarction and cardiac sudden death [1]. Treatment of any of these diseases with available synthetic drugs is still not satisfactory, they were accompanied with challenges like restricted efficacies, very costly and advanced side effects [2].

Consumption of diet rich in fats had shown to propagate hyperlipidemia in both human and animals leading to the development of obesity and other related complications [3-5]. Increasing rate of obesity in countries such as China, Canada and the USA have been linked to an increase intake of diet rich in fats [6,7]. Literature have shown that modulating dysregulation of lipid metabolism by decreasing the elevated levels of triacylglyceride, cholesterol and low density lipoprotein-cholesterol has paramount beneficiary in the treatment of several cardiovascular related diseases [8].

The plant '*Abelmoschus esculentus L*. (Moench)' known as ladies finger or Okra in English is an important vegetable crop in tropical, subtropical and warm temperate regions around the world with total trade estimated to over $5 billion [9-12]. In Nigeria, it is known as "*Kubewa"* (Hausa), "*O'okro*" (Igbo) and "*L'laa*" (Yoruba). The fresh or dry Okra fruit is used in preparing soup in almost all parts of Nigeria. It was reported that high intake of Okra is associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer [13]. Its fiber content was reported to reduce serum cholesterol resulting in decreasing chances of cardiovascular diseases progression. Generally, consumption of Okra had shown to be efficient in managing

Comment [D1]: Sentence needs to be revised – The manuscript needs to undergo extensive English editing before being considerd for publication

Comment [D2]: Revise the sentence

body's cholesterol level [14]. Research had also shown that Okra improves eye sight and help in maintaining healthy skin due to its vitamin A and beta carotene content [15].

In addition, extracts from Okra have been reported to possess antihyperlipidemic properties [16,17]. Literature survey showed that there are different varieties of okra [18], and whether their antihyperlipidemic properties varies with their varieties has not been studied. This study therefore investigated antihyperlipidemic efficiency of two commonly consumed varieties of Okra fruits (NHB-AI-B and Yar Kolon) in Bauchi State, Nigeria on rats fed high-fat diet. The study confirmed that consumption of high-fat diet for a long-time could induces hyperlipidemia in rats, and treatment with fruit extracts from *NHB-AI-B* and *Yar Kolon* okra varieties led the reversal of the altered lipid profile toward normalcy by a varied degrees. *Yar kolon* variety emerge as the most efficient, and highest in fiber content suggesting possible difference by okra varieties in their antihyperlipidemic efficiency.

Comment [D3]: Utility of the sudy

Comment [D4]: This paragraph seems like the conclusion and has to be moved to the appropriate section

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals

All chemicals used for the study were of analytical grade and were obtained from Sigma Aldrich, England and British Drug House (BDH), London. Reagent kits for assaying lipid profile were obtained from Randox Lab., UK.

2.1.2 Experimental Animals

Forty-five Wistar albino rats about 4 weeks old were obtained from the Animal House, Department of Biology, Bayero University Kano, Nigeria. They were kept in cages with free access to water and feed for two weeks for acclimatization. The principles of laboratory animal care [19] and ethical guidelines for investigation of experimental pain in conscious animals [20] were observed.

Comment [D5]: Kindly provide the ethical approval number

2.1.3 Plant Collection/Identification:

The two Okra fruit varieties: *NHB-AI-B* and *Yar Kolon* were obtained from the Teaching and Research Farm of the Faculty of Agricultural Science Technology, Abubakar Tafawa Balewa University, Bauchi. They were authenticated and identified with Voucher number: 1914.

2.2 Methods

2.2.1 Plant Extraction:

The *NHB-AI-B* and *Yar Kolon* Okra fruit varieties were each extracted following the method described by Doreddula *et al* [21] with modification in the extraction time (12 hours). The Okra fruits were sliced, air dried at 25^{o}C and then pulverized using pestle and mortar. The powdered *NHB-AI-B* (254g) and *Yar Kolon* (282g) were extracted with methanol (80%) using Soxhlet extractor for 12 hours at 25^{o}C and concentrated at 30^{0}C in a rotary evaporator then air dried. The dried extracts: *NHB-AI-B* (58.03g) and *Yar Kolon* (48.24g) were separately kept in an air-tired containers in a refrigerator at 4^{o}C until used.

2.2.2 Determination of fiber content

Fiber content of extracts from Okra varieties were determined following standardized method of the Association of Official Analytical Chemists. Where percent fiber was calculated as follows;

$$(\%)\ \text{Crude fiber content} = \frac{\text{Weight loss on ignition}}{\text{Initial weight of sample}} \times 100$$

2.2.3 Formulation of high fat diet

The high fat diet was formulated using Super starter animal feed composed of the following: maize (46%), soybean meal (18.5%), groundnut cake (15%), fishmeal (2%), wheat offal (12.45%), bone meal (2%), oyster shell (3%), salt (0.25%), premix (0.25%), methionine (0.3%), and lysine (0.25) respectively. The basal diet was 100% super starter feed while the formulated high fat diet composed of 75% super starter feed, 5% egg yolk and 20% palm oil.

2.2.4 Experimental Design

The study was conducted with a total of forty-five rats randomly divided into nine groups of five each. Rats were allowed to consume high-fat diet for a period of 35 days in order to induce hyperlipidemia as done by Karam *et al [*22]. After the 35 days feeding exercise, blood sample were collected from their tails after immersed in water bath at 45^{o}C for 5 min to make the blood vessel swell in order to get the desire volume (1-1.5ml). Serum separated from blood was used to ascertain lipid fractions like TG, TC, LDL-C, VLDL-C and HDL-C which were compared to normal control rats to make sure the success of hyperlipidemic animal model [23]. The animals groups were treated with the extracts as follows;

Comment [D6]: Was the blood volume sufficient to carry out the experiment

Comment [D7]: Animals were treated for with extract for how many days?

Group I: Hyperlipidemic rats + 250mg/kg body wt. *NHB-AI-B* okra fruit extract variety

Group II: Hyperlipidemic rats + 500mg/kg body wt. *NHB-AI-B* okra fruit extract variety

Group III: Hyperlipidemic rats + 750mg/kg body wt. *NHB-AI-B* okra fruit extract variety.

Group IV: Hyperlipidemic rats + 250mg/kg body wt. *Yar kolon* okra fruit extract variety

Group V: Hyperlipidemic rats + 500mg/kg body wt. *Yar kolon* okra fruit extract variety

Group VI: Hyperlipidemic rats + 750mg/kg body wt. *Yar kolon* okra fruit extract variety

Group VII: Hyperlipidemic rats + 10mg/kg body wt. Atorvastatin (Positive control)

Group VIII: Non-hyperlipidemic rats + Distilled water (Normal control)

Group IX: Hyperlipidemic rats + Distilled water (Negative control)

The extract doses were determined based from the studies by Ngoc *et al [*14] and Sabitha *et al* [17].

2.2.5 Determination of water and feed intake:

Water and feed intake of the experimental animals were determined daily, water was measured using measuring cylinder before giving to the animals and after 24 hours. Feed was also weighed using weighing balance before giving to the animals and after 24 hours.

2.2.6 Determination of body weight:
The weight of the animals was determined weekly throughout the experimental period. The weight was taken in the morning before feeding the rats by properly placing each rat in the weighing pan of the weighing scale and then the weight recorded.

2.2.7 Collection of Blood Samples
Animals were sacrificed after 21 days treatment, they were anaesthetized by putting in a plastic jar saturated with chloroform vapor followed by cervical dislocation. Blood was collected in a labelled test tubes and serum removed was used for lipid profile determinations.

2.2.8 Lipid profile determinations
Serum triglyceride (TG) was determined using the method described by Fossati and Prencipe [24]. Serum total cholesterol (TC) was determined spectrophotometrically according to the method of Roeschlau *et al [*25], and high density lipoprotein Cholesterol (HDL-C) was measured according to the Lopes-Virella *et al [*26] method. LDL-cholesterol and VLDL-cholesterol (VLDL-C) were determined by the formula described by Friedewald *et al* [27]. LDL-cholesterol concentration (mg dLG1) = [TC-(HDL-C + Triglycerides / 5)] and VLDL-cholesterol concentration (mg dLG1) = [Triglycerides / 5].

2.3 Statistical analysis:
Data from the experiments were expressed as mean ± standard deviation (SD) but presented in a bar chart graphical format with error bars. Percentage difference between mean values were also presented alongside. Significant difference was accepted at $p < .05$ as analyzed by ANOVA using SPSS software version 23.

Comment [D8]:
1.What was the need for the animal to be sacrificed after treatment with okra extract?

2.Did ethical clearance include sacrificing the animal

Comment [D9]: Method needs to be described

3. RESULTS

3.1 Fiber Content of *NHB-AI-B and Yar Kolon* Okra Fruit Varieties

The percent crude fiber content of the varieties of *Abelmochus esculentus* fruit; *NHB-AI-B and Yar Kolon* showed, *Yar kolon* variety highest with 14.74 ±0.17% whereas *NHB-AI-B* has 12.51 ±0.00% respectively.

3.2 Effect of Okra Fruit Varieties on Triglycerides of Rats Fed High-Fat Diet

A significant increase ($p<0.05$) in the level of triacylglyceride (TG) was shown by the rats' groups that were fed high fat diet compared to the rats fed normal feed. Treated hyperlipidemic rats with either methanol extract from *NHB-AI-B* and *Yar Kolon* okra fruit varieties showed a reversal effect on the TG levels. Where, extract from *Yar Kolon* okra fruit variety seem to the most effective by lowering TG levels above 70% compared to that of *NHB-AI-B* variety as presented in Figure I.

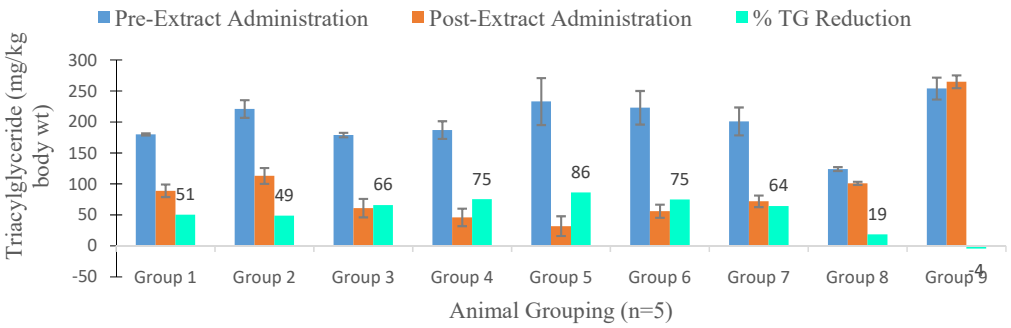

Fig. I. Effect of Administration of Methanol Extracts of *NHB-AI-B and Yar Kolon* Okra Fruit Varieties on Triacylglyceride Level of Rats Fed High Fats Diet

Group 1, 2 and 3 = Hyperlipidemic rats + *NHB-AI-B* (250, 500 and 750mg/kg Body wt.).
Group 4, 5, and 6 = Hyperlipidemic rats + *Yar Kolon* (250, 500 and 750mg/kg Body wt.)
Group 7 = Positive Control, Group 8 = Normal Control, and Group 9 = Negative Control

Comment [D10]: Tables for baseline levels of lipid parameters for all the different groups with their mean, SD and significane is required.

Comment [D11]: What the author means by the word "reversal effect"
?
Atmost they can say that there is lowering in the levels of triglycerides

3.3 Effect of Okra Fruit Varieties on Cholesterol of Rats Fed High-Fat Diet

The result of total cholesterol levels of rats fed high fat diet which were later administered extracts from okra fruit varieties is shown in Figure II. The study recorded a significant increase in total cholesterol (TC) levels from rats' groups fed high fat diet for 35 days period. Administration of methanolic extracts from both *NHB-AI-B* and *Yar Kolon* okra fruit varieties induces a positive change on the elevated cholesterol. The median and high doses of *Yar Kolon* okra fruit variety lower cholesterol level by 54% as recorded from the treated rats groups while the standard drug was able to reduced cholesterol level by 45% and *NHB-AI-B* okra fruit variety had less reduction of cholesterol level.

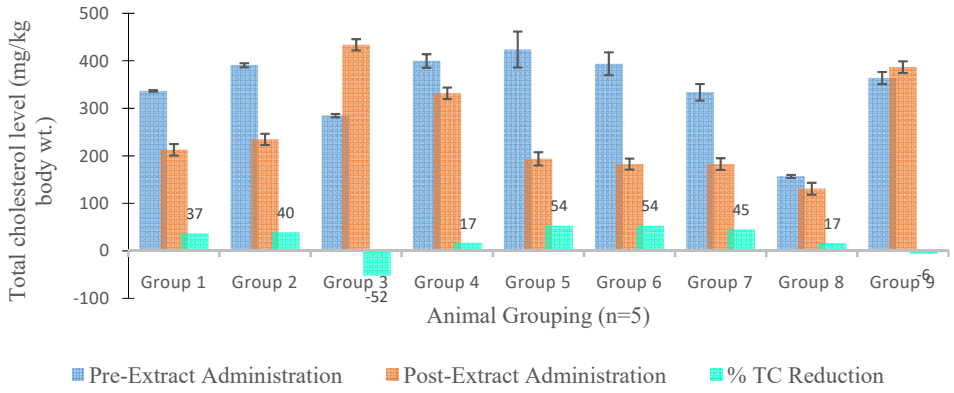

Fig. II. Effect of Administration of Methanol Extracts of *NHB-AI-B and Yar Kolon* Okra Fruit Varieties on Cholesterol Level of Rats Fed High Fats Diet

Group 1, 2 and 3 = Hyperlipidemic rats + *NHB-AI-B* (250, 500 and 750mg/kg Body wt.).
Group 4, 5, and 6 = Hyperlipidemic rats + *Yar Kolon* (250, 500 and 750mg/kg Body wt.)
Group 7 = Positive Control, Group 8 = Normal Control, and Group 9 = Negative Control

3.4 Effect of Okra Fruit Varieties on LDL-Cholesterol of Rats Fed High-Fat Diet

Change in the levels of low density lipoprotein cholesterol was recorded in the study from rats' groups fed high fat diet which were significant different ($p<0.05$) compared to the level from rats

fed normal feed. Following extracts administration to various rats' groups showed a reduced level in the LDL-C when compared with the value from the untreated rats group. Rats from group 5 and 6 that received varied doses of methanol extract from *Yak Kolon* okra fruit variety and group 7 treated with standard drug had their LDL-C reduction level above 25% while those treatment with *NHB-AI-B* okra variety had less than 25% as seen in figure III.

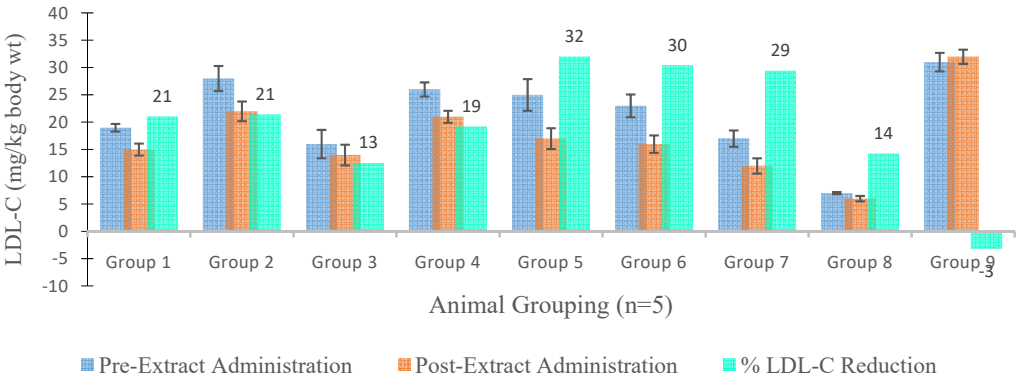

Fig. III. Effect of Administration of Methanol Extracts of *NHB-AI-B and Yar Kolon* Okra Fruit Varieties on Low Density Lipoprotein-Cholesterol Level of Rats Fed High Fats Diet

Group 1, 2 and 3 = Hyperlipidemic rats + *NHB-AI-B* (250, 500 and 750mg/kg Body wt.).
Group 4, 5, and 6 = Hyperlipidemic rats + *Yar Kolon* (250, 500 and 750mg/kg Body wt.)
Group 7 = Positive Control, Group 8 = Normal Control, and Group 9 = Negative Control

3.5 Effect of Okra Fruit Varieties on VLDL-Cholesterol of Rats Fed High-Fat Diet

High levels of very low density lipoprotein cholesterol pre-extract treatment were recorded in the study from rats' groups fed high fat diet which are significantly different ($p<0.05$) compared to the level from rats fed normal feed. Post-treatment with methanol extracts from the two Okra fruit varieties; *NHB-AI-B* and *Yar Kolon* showed reduction in the VLDL-C levels where the rats groups that received varied doses of *Yar kolon* okra fruit variety had their VLDL-C reduction

level above 70% but *NHB-AI-B* okra fruit variety and standard drug treated rats groups had less than 70% of VLDL-C reduction as presented in figure IV.

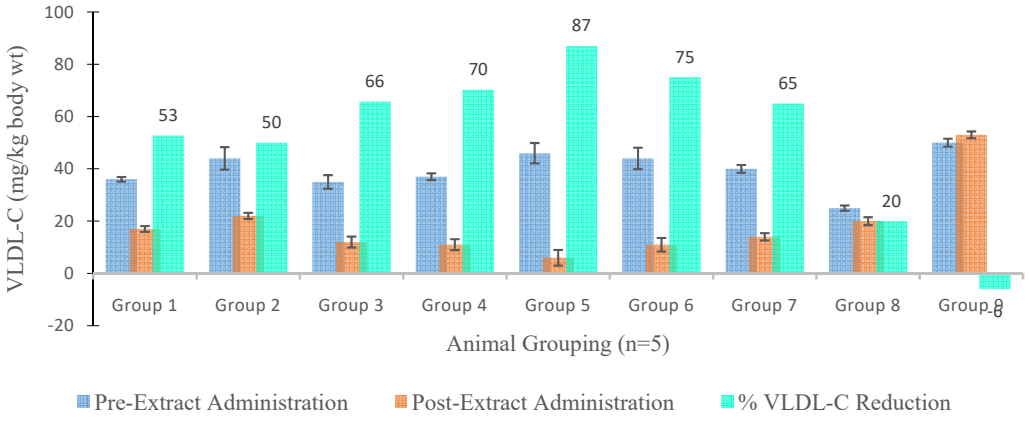

Fig. IV. Effect of Administration of Methanol Extracts of *NHB-AI-B and Yar Kolon* Okra Fruit Varieties on Very Low Density Lipoprotein-Cholesterol Level of Rats Fed High Fats Diet

Group 1, 2 and 3 = Hyperlipidemic rats + *NHB-AI-B* (250, 500 and 750mg/kg Body wt.).
Group 4, 5, and 6 = Hyperlipidemic rats + *Yar Kolon* (250, 500 and 750mg/kg Body wt.)
Group 7 = Positive Control, Group 8 = Normal Control, and Group 9 = Negative Control

3.6 Effect of Okra Fruit Varieties on HDL-Cholesterol of Rats Fed High-Fat Diet

The study recorded an elevation in high density lipoprotein cholesterol levels from rats' groups fed high fat diet pre-extract administration. When treated with the methanolic extracts from *NHB-AI-B and Yar Kolon* okra fruit varieties, the elevation HDL-C levels were slightly promoted where rats in group 3 which received high dose of *NHB-AI-B* okra fruit variety had their HDL-C level elevated by 21% compare to other extract-treated rats groups and the standard drug which had less while untreated rats had 45% reduction of HDL-C levels as can be seen in figure V.

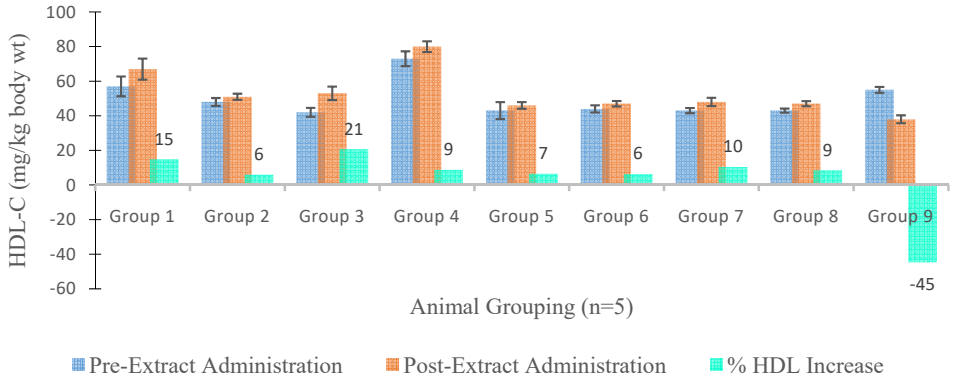

Fig. V. Effect of Administration of Methanol Extracts of *NHB-AI-B and Yar Kolon* Okra Fruit Varieties on High Density Lipoprotein-Cholesterol Level of Rats Fed High Fats Diet

Group 1, 2 and 3 = Hyperlipidemic rats + *NHB-AI-B* (250, 500 and 750mg/kg Body wt.).
Group 4, 5, and 6 = Hyperlipidemic rats + *Yar Kolon* (250, 500 and 750mg/kg Body wt.)
Group 7 = Positive Control, Group 8 = Normal Control, and Group 9 = Negative Control

3.7 Effect of Okra Fruit Varieties on Water and Feed Intake of Rats Fed High-Fat Diet

The water intake of all rats in the various groups was in the range of 30.00±0.01ml/day/rat before the extracts administration. During extracts administration, the water intake were elevated at the average of 50.00±0.20 ml/day/rat. The study recorded a little or no significant changes in the levels of feed intake (18.39±0.12g/day/rat) by rats from all the various groups pre/post-administration of the methanol extracts of the two okra fruit varieties.

3.8 Effect of Okra Fruit Varieties on Body Weight of Rats Fed High-Fat Diet

The result of the rats' body weights is presented in figure VI. There was an increase in weight gain by rats fed high fats diet pre-treatment compare with those that fed basal diet. Continuous feeding rats with high fats diet alongside extract administration does not change in body weight gain of some rats groups except those that received *Yar kolon* extract that seem to be suppressed.

Comment [D12]: There should have been a comparison between baseline body weight, body weight after fat diet and body weight after okra treatment.

The calorie content of 100 grms of okra is around 33 kcals. Do the authors have an explanation as to how the body weight can increase after okra treatment. Considering the beneficial effects on the lipid parameters there should have been a decrease or no change compared to pretreatment levels.

Was the change in body weight found to be statistically significant. As seen by the graph this is contrary as the okra in the group 1,2,3 has increase and does the author have explanation for this increase?

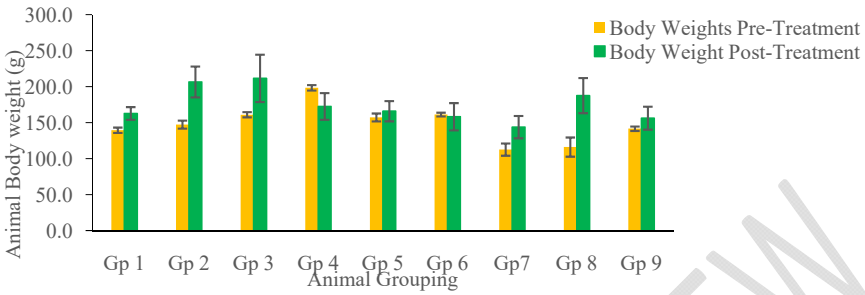

Fig. VI. Effect of Administration of Methanol Extracts of *NHB-AI-B and Yar Kolon* Okra Fruit Varieties on Body Weight of Rats Fed High Fats Diet

Group 1, 2 and 3 = Hyperlipidemic rats + *NHB-AI-B* (250, 500 and 750mg/kg Body wt.).
Group 4, 5, and 6 = Hyperlipidemic rats + *Yar Kolon* (250, 500 and 750mg/kg Body wt.)
Group 7 = Positive Control, Group 8 = Normal Control, and Group 9 = Negative Control

3. DISCUSSION

Hyperlipidemia is an important risk factor for the development of atherosclerosis and subsequent cardiovascular diseases and stroke [10]. It is a major health problem among populations of both affluent and non-affluent societies [28, 29]. Several treatments like dietary restriction and exercise, pharmacological intervention with drugs like fibrates, statins and bile acid sequestrants have been in used but shown to have a spectrum of adverse effects in patients, and are also very costly [30]. Research has been directed toward finding safer, inexpensive, and effective agents to combat the disorder [13].

In this study, antihyperglycemic efficiency of two commonly consumed varieties of Okra fruits: *NHB-AI-B* and *Yar Kolon* in Bauchi State, Nigeria was investigated on rats fed high-fat diet. After feeding rats with high-fat diet for a period of 35 days, the study observed elevation in

Comment [D13]: As per the title of the study and the results I think the authors were investigating hyperlipidaemia and not hyperglycemia – isn't that the case?

Comment [D14]: What is the reason why the authors decided to give the treatment for 35 days. Is there a scientific reason for the same. What is the reference?.
Pls provide the table mentioning the baseline value and increase in lipid concentration after feeding as mentioned above. Further was this increase significant statistically?

serum lipid components like triacylglyceride, cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol and diminished high density lipoprotein cholesterol level. When the various rats groups were treated with different doses of methanol extracts from *NHB-AI-B* and *Yar kolon* okra fruit varieties alongside the standard drug for 21 days period. The study found that the altered lipid components were reversed toward normalcy by the both varieties of okra fruit extracts in a varied degrees. *Yar kolon* okra fruit variety emerge as the most efficient, and also highest in fiber content attesting to the fact that fiber plays a major role, and a reflection of the possibility that antihyperlipidemic efficiency of okra may varies with their varieties.

Comment [D15]: What is the reason why the authors decided to give the treatment for 21 days. Is there a scientific reason for the same. What is the reference?.

The alternations in the measured lipid components; triglyceride, cholesterol, low density lipoproteins and high density lipoproteins in rats fed high fat diet in the study agrees with the report from a study conducted by Karam *et al* [22] , where it was found that diet rich in fats could induces hyperlipidermia in animals' when consumed for long-time period. The ability for *Abelmochus esculentus* variety; *Yar kolo*n and *NHB-AI-B* fruit extracts to lower the elevated triacylglyceride, cholesterol, low density lipoprotein and very low density lipoprotein levels in rats fed high fats diet has been experimentally demonstrated with plant extracts as reported by several studies. In an earlier study, it was reported that high intake of Okra products is associated with a reduced risk of a number of chronic diseases, such as atherosclerosis and cancer [13].

Comment [D16]: Please provide the references for the same

The high levels of triglycerides from rats fed high fat diet in the study is an indication of fats deposition which resulted in the increase accumulation of the lipid. Literature survey shown that intake of high fats diet promotes deposition of fats in tissues like the adipose tissue which may alters lipid metabolism [31, 32]. Declined in serum triglyceride level after rats were treated with

extracts from *Yar kolo*n and *NHB-AI-B* okra fruit varieties is an indication that they contain components that are extractable with methanol and likely retaining their antihypertriglyceridemic properties. Antihypertriglyceridemic effect of okra extract have been reported in a study conducted by Esan *et al* [33] in which the present study is in tandem with their findings.

The reduction in levels of cholesterol by the two varieties of okra fruit extract in the study could be probably be due to their fiber content. It was reported that okra contain fibers which could play a vital role in supressing lipid fractions such as cholesterol level leading to the decreasing risk of cardiovascular disease [14]. In the present study, *Yar kolo*n and *NHB-AI-B* fruit extracts were able to lower cholesterol in a vary degree where *Yar kolon* okra fruit variety was seen to be the most effective by lowering cholesterol by 54% in a dose dependent manner. The antihypercholesterolemic activity of *Yar kolon* okra fruit variety could be a reflection of its high fiber content as determined in the study compare to that of *NHB-AI-B* fruit extract. Antihypercholesterolemic effect of okra fruit extract had been reported in a recent study by Djamil *et al* [34].

The hypothetic mechanisms of antihypercholesterolemic effect of Okra plant extract have been postulated by some scholars. A report from Esan *et al* [33] indicated that okra extract may act by altering HMG-CoA activity resulting in the decreasing hypercholesterolemia. Another researcher have pointed out that reduction of cholesterol by the okra extract is associated with a decrease of its LDL fraction, a target of several hypolipidemic drugs [35]. Decrease in LDL-C levels of rats fed high fat diet after treated with the okra fruit varieties particularly the *Yar kolon* variety might have contributed to their cholesterol reduction possibly agreeing with the suggestion by Poorva and Sunita [35]. A reduction in serum LDL cholesterol levels have been reported from a study

conducted by Esan *et al* [33] on hypercholesterolemic mice treated with two different doses of okra seeds extract for 42 days.

High density lipoprotein (HDL) was reported to aid in the translocation of cholesterol from the peripheral tissue, such as arterial walls to the liver for catabolism, this shows that when plasma HDL level reduces, the rate of risk factor for developing atherosclerosis increases [36]. In the present study, decreased levels of HDL-C recorded in rats fed high-fat diet pre-extract treatment is an indication of increase risk of atherosclerosis development. But, when treated with different doses of *Yar kolo*n and *NHB-AI-B* fruit extracts, their HDL-C levels were slightly elevated. Several findings have reported an increased level of HDL-C after treatment, where it was correlated with a decrease in cholesterol level along with its LDL fraction and suggested to be due to increasing cholesterol excretion and decreasing absorption through gastro intestinal tract [37-38]. Relating this scenario to the present study, it could be stated that elevated HDL-C couple with the decreased level of cholesterol from rats received okra fruit varieties is a resultant effects of increasing cholesterol elimination and supressing absorption which might have led to decrease hyperlipidemia progression in those rats. Several other studies have also found that increase in HDL-C is associated with a decrease in coronary risk [39-40]

Comment [D17]: Provide details in the tabular format

Comment [D18]: Author could have easily investigated this by measuring the stool fat content. Why was it not carried out in the present study?

Literature survey shows that the growth rate of animals is influenced by species, individuals, sex, age, feeding, and diet consumed [41]. The present study had observed changes in both growth and body weights gain throughout the experimental period by rats fed high fat diet compared with those that fed basal (normal) feed. Study have shown that weight gain is caused by an increase in the amount of fat deposited in adipose tissue, especially those under the skin and the abdominal cavity [32]. Continuous feeding rats with high-fat diet might have contributed to their increasing body weight. However, in the rats groups treated with extracts from *Yar kolo*n and

Comment [D19]: Changes in growth and body weights needs to be presented by the authors in the tabular format

NHB-AI-B fruit varieties particularly the *Yar kolo*n extract, gain in their body weight was retarded possibly due to the effect of the extract against fats deposition.

Comment [D20]: Was the finding statistically significant?

Does the other variety enhance the fat deposition?

Conclusion:

The study confirmed that feeding rats with high-fat diet for a long-time induces hyperlipidemia, and extracts from *NHB-AI-B* and *Yar kolon* okra fruit varieties exert antihyperlipidemic effect in a varied degrees. *Yar kolon* variety seem to be the most efficient, and highest in fiber content suggesting possible difference by okra varieties in their antihyperlipidemic efficiency. This, therefore calls for further study to compare more okra varieties to ascertain the most potent for pharmaceutical utilization.

Ethical Approval: Authors hereby declare that the experiment was performance in accordance with "Principles of laboratory animal care" (NIH publication No: 85-23, revised 1985) and ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983) were observed.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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