Futo Journal Series (FUTOJNLS)
e-ISSN : 2476-8456 p-ISSN : 2467-8325
Volume-2, Issue-2, pp- 123 - 135
www.futojnls.org

Research Paper December 2016

Lipid Profile of Albino Rats Administered Seed and Pulp Aqueous Extracts of *Citrullus Colocynthis*

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Abstract

The effect of different concentrations of *Citrullus colocynthis* seed and pulp aqueous extracts on serum lipid profile of albino rats was investigated. The concentrations of serum total cholesterol (TC), triacylglycerol (TGs), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), positive atherogenic index (HDL-c/TC) and negative atherogenic index (LDL-c/HDL-c) of the sera samples of albino rats were determined using standard methods. Thirty-five albino rats weighing 156-186g were divided into seven groups of five rats each. Different doses (100, 200, 300 and 400 mg/kg body weight) of *C. colocynthis* seed and pulp aqueous extracts were orally administered daily to groups 2-4 and groups 5-7 respectively, while group 1 (control) received only 0.5 ml of distilled water for a total treatment period of 28 days. Results of the study indicated that there were significant ($p<0.05$) increase in the concentrations of serum total cholesterol (TC), triacylglycerol (TGs), low density lipoprotein cholesterol (LDL-c), positive atherogenic index (HDL-c/TC) and negative atherogenic index (LDL-c/HDL-c) of the sera samples of albino rats in contrast to a slight reduction in the concentration of high density lipoprotein cholesterol (HDL-c), when compared to the control. Oral administration of aqueous seed and pulp aqueous extracts of *C. colocynthis* increased significantly the serum lipid profile of albino rats.

Keywords: *Citrullus colocynthis,* seed, pulp, lipid profile, atherogenic indices, albino rats.

1.0. Introduction

Lipids are group of fats and fat-like substances that are important constituents of cells and sources of energy. Different plasma lipids vary greatly in various populations due to differences in geographical, cultural, economical, social conditions, dietary habits and genetic make-up. Age and gender differences also affect serum lipids (Abubaka, Mabrouk, Gerie, Dikko, Aliku,… & Adama, 2009; Hart & Smith, 1997).

Lipid profile or lipid panel is a panel of blood tests that serve as an initial broad medical screening tool for abnormalities in lipid metabolism, such as cholesterol and triglyceride. The result of this test can identify certain genetic diseases and can also determine approximate risks for cardiovascular diseases, certain forms of pancreatitis, and other diseases (Castelli, Anderson, Wilson & Levy, 1992). Lipid profile is commonly ordered as part of a physical examination, along with other panels such as complete blood count (CBC) and basic metabolic panel (BMP).

The lipid profile typically includes; Cholesterol (CHOL), Triacylglycerols (TGs), Low density lipoproteins (LDLs), High density lipoproteins (HDLs), very low density lipoproteins) VLDLs concentrations and ratios derived from the above values (atherogenic indices). Hyper- or hypo-lipidaemia is an elevation or reduction in the cholesterol esters, triacygylcergl and phospholipids (Iyera, Sharmaa, & Patilb, 2012). The first step in diagnosis of hyper- and hypo-lipidaemia and /or lipoproteinaemias is to define the lipid and lipoprotein patterns by chemical analysis of the serum lipids and lipoproteins (Burtis and Ashwood, 1996).
Traditional medicines support well over 80% of the population in developing countries especially in the rural areas (Klaassen and Eaton, 1991). Available evidence suggests that even in urban areas which are well served by modern healthcare facilities, a good number of people rely on herbal supplements to meet some of their health needs. The medicinal values of these plant parts lie in their phytochemical compositions, which produce definite physiological action on human body (Afolabi, Ibuun, Afor, Obuotor & Farobi, 2007). Plants derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological including antihyperlipidaemic and hepatoprotective activities (Mohammed and Luka, 2013).

Citrullus colocynthis, a member of the *Cucurbitaceae* family is known as Bitter Apple in the English language, Hindal in Arabic, and Abujahl melon in Persian. The extract of the fruit is called *colocynthine* due to its extreme bitter taste. The known compounds found in *Citrullus colocynthis* include glycosides, alkaloids, and flavonoids (Chen, Vaci, & Leupold, 2005; Yoshikawa, Morikawa, Kobayashi, Nakamura & Matsuda, 2007). A number of plant secondary metabolites including Cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids, phenolic compounds have previously been reported from this plant (Chen *et al*., 2005; Yoshikawa *et al*., 2007). However, unknown compounds may also contribute to its therapeutic reputation. The Curcurbitacins (highly oxygenated tetracyclic glycosides) have a broad range of applications due to their wide spectrum of biological activities (Tannin-Spitz, Grossman, Dovrat, Gottlieb & Bergman, 2007). They are found mainly in plants belonging to the *Cucurbitaceae* family, but have also been found in several other families of the plant kingdom (Tannin-Spitz *et al*., 2007).

In folk medicine, *Citrullus colocynthis* is widely used by rural inhabitants as a purgative, anti-diabetic, anti-neoplastic, anti-rheumatic, and anti-allergic agent (Tannin-Spitz *et al*., 2007). Although the whole fruit is often used for the treatment of the aforementioned diseases, but some particular parts of the fruit are also used for specific purposes. One of such example is the traditional application of the dried-pulp and seed extracts of *Citrullus colocynthis* for the treatment of constipation and diabetes (Kumar, Kumar, Saroha, Singh & Vashishta, 2008; Nmila, Gross, Rchid, Roye, Manteghetti, ..& Sauvaire, 2000). Some adverse effects include bloody diarrhea and toxic colitis, and are responsible for *Citrullus colocynthis* classification as a toxic plant, where it is considered among the top ten (Nmila *et al*., 2000). Interestingly, most of the studies on the toxic effects of this medicinal plant are performed on the whole fruit extract (*colocynthine*). Since different parts of the fruit, such as the pulp or the seed, are claimed to exert different therapeutic effects, it is reasonable to suggest that a particular therapeutic benefit, or toxic side effect could be attributed to one part of the fruit, and not another. Given the varied ethno-medicinal uses of *Citrullus colocynthis* in Nigeria, when used singly or in combination with other plants, there is the need to evaluate the effect of these aqueous extracts on lipid profile of albino rats at varied concentrations, this prompted the bases of this study.

2. 0. Materials and Methods

2.1 Collection, Identification and Preparation of Plant Extract

The fruit of *Citrullus colocynthis* used for this work was bought at Ekeonuwa market, in Owerri Municipal Council, Imo State. The plant was identified by Mr. Francis Iwueze of the Department of Forestry and Wildlife, School of Agricultural Technology, Federal University of Technology, Owerri. The seeds of *Citrullus colocynthis* were carefully removed from the fruit rinds and air-dried. The fruits pulps were removed carefully removed from the epicarp and air-dried. The extracts of the ground seed and pulp were made as follows: A 100g portion of the ground seed and pulp of *Citrullus colocynthis* was each soaked in 500ml of water for 24 hours, filtered and then exhaustively extracted with the aid of soxhlet extractor. The solvent from each extract was then distilled off in a distillatory and evaporated to dryness at 40 ^{O}C. The solid extract was each placed in a sterile container, labeled and stored at 4^{O}C in a refrigerator from where portions were taken and prepared for the study. Rats in Group 1 (Control) were orally administered 0.5ml distilled water (the vehicle) while those in Groups 2 to 4 and Groups 5 to 7 were administered the same volume of *Citrullus colocynthis* seed and pulp extracts at 100, 200 and 400 mg/kg body weight/day, respectively and the treatment continued for 28 days.

2.2 Animal Handling:

Thirty-five (35) male albino rats, weighing between 156-186g were used for the study were purchased from Animal Unit, Abia State University, Uturu, Abia State, Nigeria. The animals were treated and handled humanely in accordance with the standard principle of the laboratory animal care of the National Institute of Health (NIH, 1995). They were supplied with feed and water *ad libitum*.

2.3 Animal Grouping and Treatment

The animals were divided into seven groups of five (5) in each cage according to their relative body weights. The weights of the rats before the administration of feeds were recorded. The animals were allowed to acclimatize to the environment for one (1) week on a regular water and feed. After acclimatization, each group was administered their respective concentrations. Rats in Group 1 (Control) were orally administered of 0.5ml distilled water (the vehicle) while those in Groups 2 to 4 and

Groups 5 to 7 were administered the same volume of *Citrullus colocynthis* seed and pulp extracts at 100, 200 and 400 mg/kg body weight/day, for a total of treatment period of treatment 28 days.

2.4 Collection of Blood Samples

After 28 days, the rats were an anesthetized by exposure to dichloromethane vapor in covered transparent plastic container. Incisions were then made into the thoracic regions and blood collected by cardiac puncture using 5mL hypodermic syringes and needles. The blood samples were dispensed into sterile sample bottles, allowed to clot and centrifuged at 3000 rpm for 10mins. The serum was separated using micropipette and the serum from each rat was used for the various parameters.

2.5 Lipid Profile Determination

Total cholesterol and triacylglycerol concentrations were determined according to the method of Allian *et al,* (1974). Low density lipoprotein concentration was determined using the method of Assmann *et al,* (1984). The method of Albers *el al,* (1978) was used to determine the concentration of high density lipoprotein, while positive and negative atherogenic indices (AI) were determined as described by Igwe *et al,* (2007)

2.6 Statistical Analysis

All data generated were expressed as mean ± standard deviation and analysed for statistical significance by using one way Analysis of Variance (ANOVA). Values were considered significant at $p<0.05$.

3. 0. Results and Discussion

.The study revealed the extent to which the oral administration of different concentrations of *Citrullus colocynthis* seed and pulp extracts affected serum lipid profile (serum total cholesterol (TC), triacylglycerol (TGs), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c). positive atherogenic index (HDL-c/TC) and negative atherogenic index (LDL-c/HDL-c) of albino rats.

The result of serum total cholesterol concentration as presented in Figure 1 indicated that there was a significant ($p<0.05$) increase when compared to the control. This may be attributed to the gut intra-lumenal interraction of the phytochemical component saponin in the extracts. This result agrees with the report of Nwaogu *et al* (2015) who reported a significant ($p<0.05$) increase in serum cholesterol on albino rats when administered ground seeds of *Picralima nitida*- incorporated diets.

Slide 1: The fruits of *Citrillus colocynthis*

Slide 2: The seeds and pulp of *Citrillus colocynthis*..

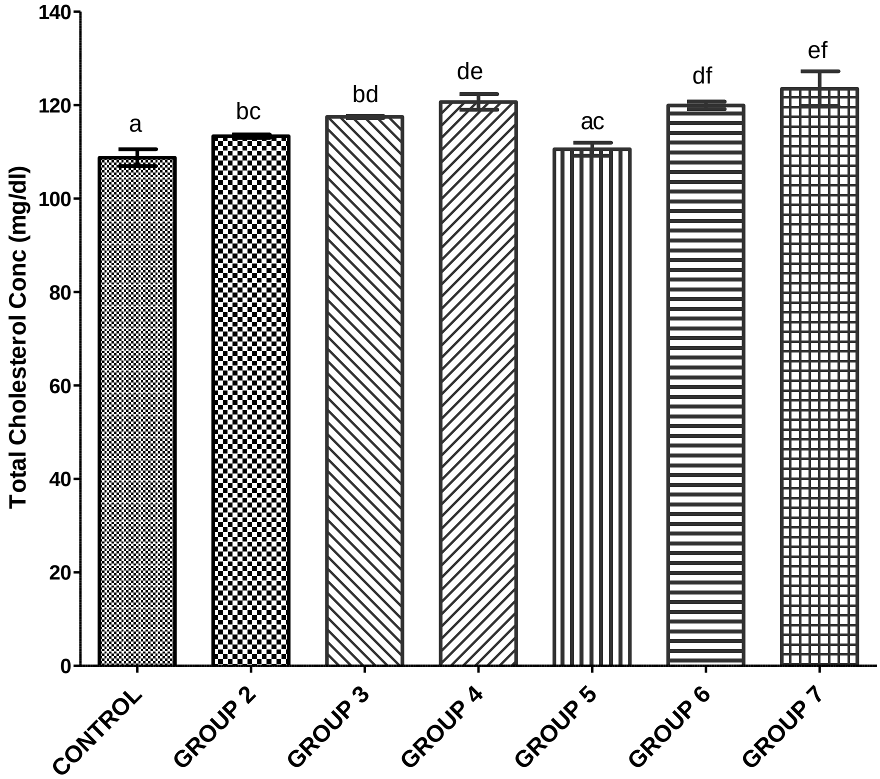

Fig. 1: Total cholesterol concentration (mg/dl) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

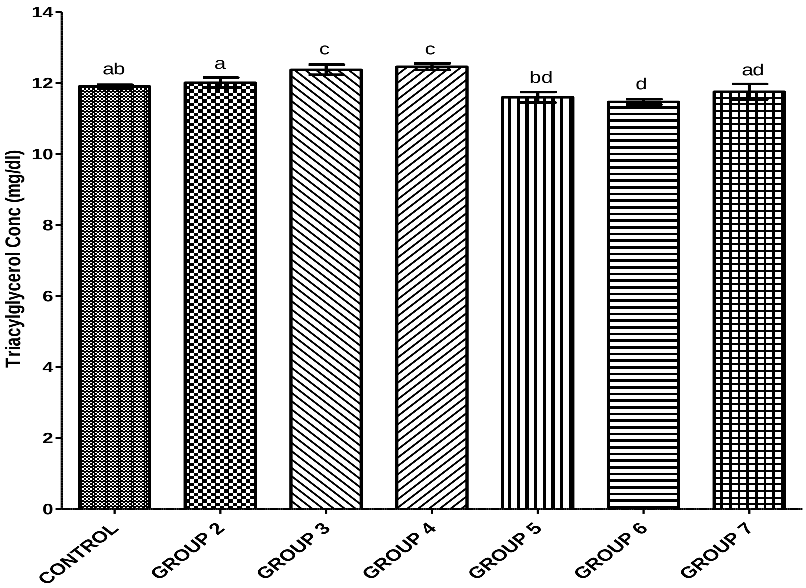

Fig. 2: Triacylglycerol concentration (mg/dl) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

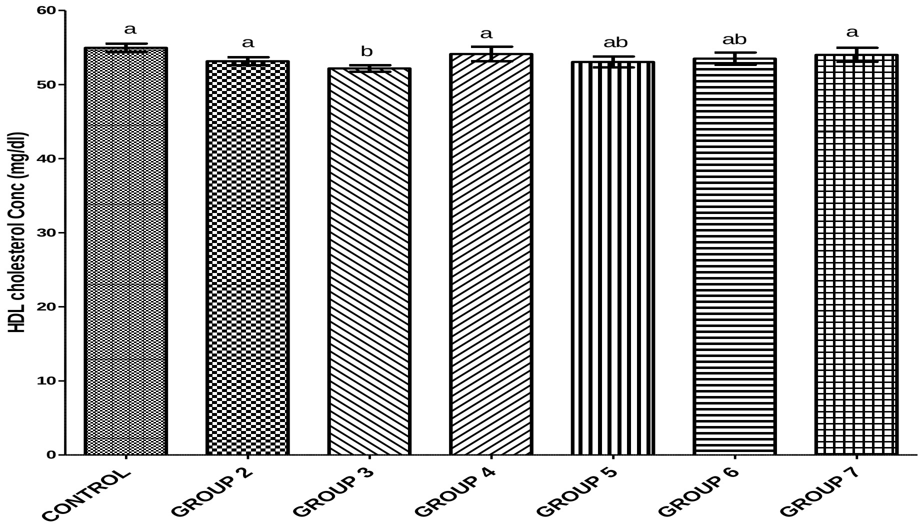

Fig. 3: High density lipoprotein (HDL) cholesterol concentration (mg/dl) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

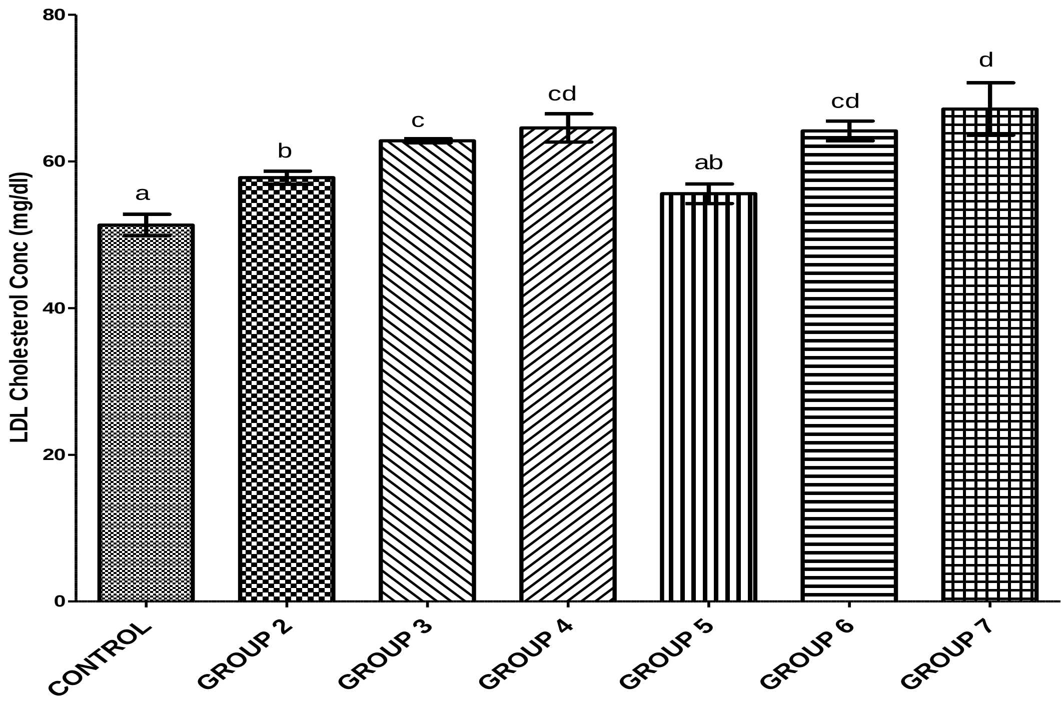

Fig. 4: Low density lipoprotein (LDL) cholesterol concentration (mg/dl) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

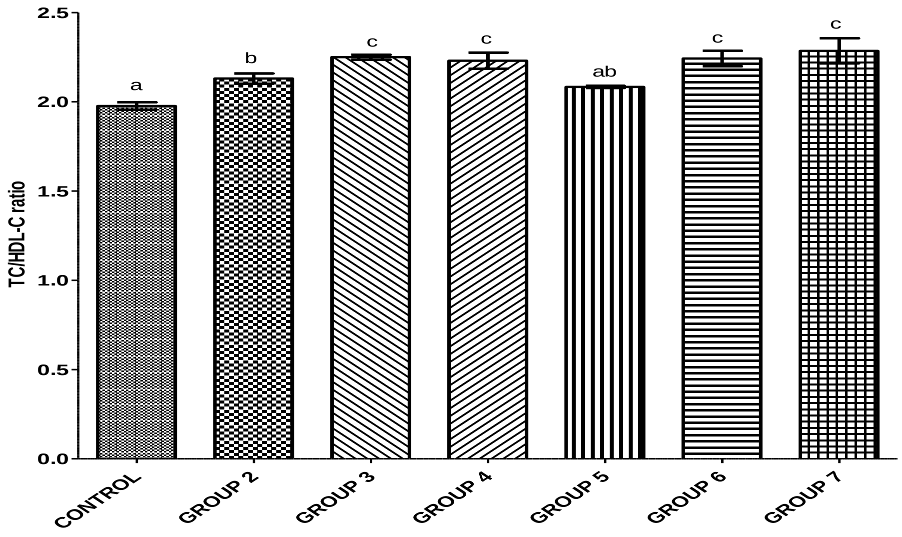

Fig. 5: Total cholesterol/HDL cholesterol ratios of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant ($p<0.05$).

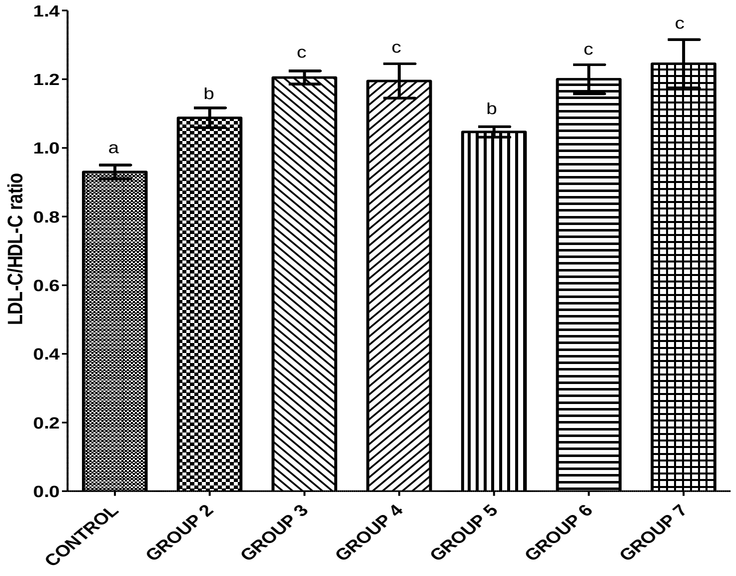

Fig. 6: LDL-c/HDL-c ratios of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant ($p<0.05$).

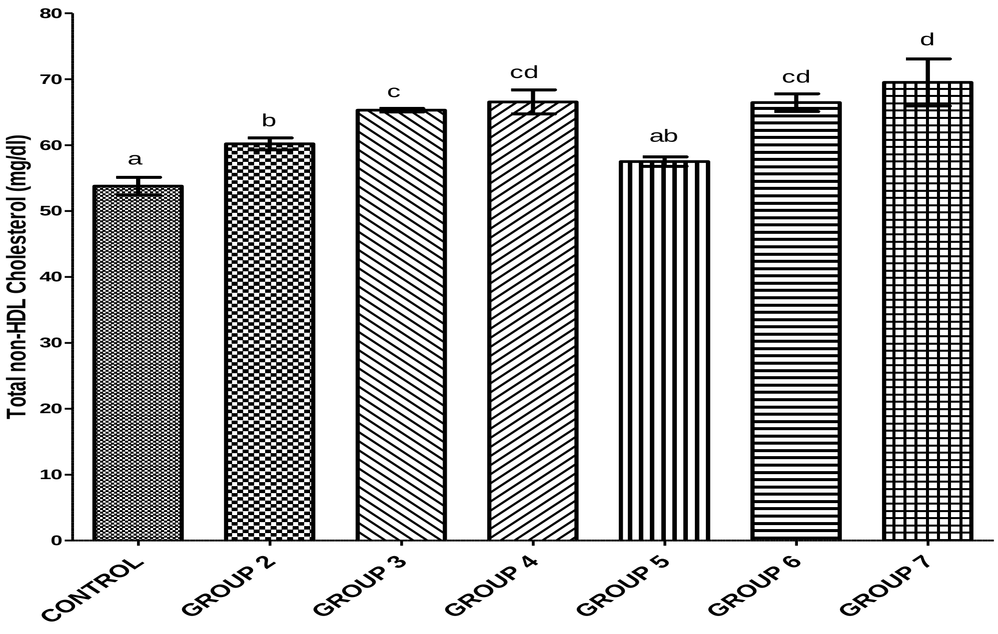

Fig. 6: Total non-HDL cholesterol concentration (mg/dl) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant ($p<0.05$).

The result of triacylglycerol concentration as shown in Table 2 indicated that there was no significant ($p>0.05$) difference in triacylglycerol concentration in the rats administered different concentrations of the extracts when compared to the control. This is in contrast to the report of Ujowundu *et al,* (2014) who reported an increase in serum triacylglycrerol due to treatment with *Ocimum gratissimum* and *Gongronema latifolium*-formulated diet of albino rats.

Result obtained from High Density Lipoprotein cholesterol (HDL-c) concentration, Figure 3 revealed that there was no significant ($p>0.05$) difference in the HDL-c concentration of rats in Groups 2, 4, and 7 when compared to the control, but there was a slight reduction when compared with other groups. The observed decrease in HDL concentration in the groups 3, 5 and 6 may have resulted from the accumulation of cholesterol released into the serum from the dying cells and from membranes undergoing turnover (Ubani *et al*., 2010). HDL acts as a shuttle that moves cholesterol throughout the body and also binds esterified cholesterol released from the peripheral tissues and then transfer cholestryl esters to the liver or to tissues that use cholesterol to synthesize steroid hormones (Berg *et al*., 2007).

Results obtained in this study, Figure 4 also show that there was a significant ($p<0.05$) increase in the Low Density Lipoprotein cholesterol (LDL-c) concentrations of rats administered the extracts when compared to the control. This result also agrees with the report of of Ujowundu *et al,* (2014) who reported an increase in serum low density lipoprotein cholesterol (LDL-c) concentration of albino rats due to administration of *Ocimum gratissimum* and *Gongronema latifolium*-formulated diet.

Atherogenic indices are strong indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardioivascular diseases and vice versa. The results of both positive atherogenic index (TC/HDL) Figure 5, and negative atherogenic index (LDL-C/HDL-c Figure 6 show slight significant ($p<0.05$) increase in concentrations of rats administered the extracts when compared to the control. These ratios have been variously applied in assessing the risk of cardiovascular diseases in humans, however, their applications have been limited towards monitoring experimentally induced diseases or laboratory conditions associated with CVD.

4.0 Conclusion.

The study revealed that oral administration of seed and pulp aqueous extracts of *Citrullus colocynthis* caused a significant ($p<0.05$) increase in lipid profile concentrations (total cholesterol, triacylglycerol, low density lipoprotein, positive atherogenic TC/HDL and negative atherogenic LDL/HDL indices but a slight reduction in HDL-c). This observation points to the fact that the extracts at high concentrations over a long period of time can precipitate cardiovascular diseases in animals and possible induction of dyslipidaemia.

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