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Effect of Citrullus colocynthis Juice Pulp Extract on High Fat Induced

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Abstract
Obesity is a chronic disease caused by an imbalance in the amount of energy consumed and the amount of energy expended. Citrullus colocynthis (known as bitter apple) is a desert viney plant that grows in sandy, arid soils. It is rich in various bioactive compounds such as essential oils, glycosides, flavonoids, alkaloids, and fatty acids. This study evaluated the effect of citrus colocynthis on the lipid profile of high-fat diet-induced obese rats. Forty-two male albino rats weighing between 200-250g were divided into six groups of seven rats each. Group 1 contains animals fed with normal feed and water (control group), group 2 contains animals receiving 150mg/kg Citrullus colocynthis and fed with high cholesterol diet, group 3 contains animals receiving 75mg/kg Citrullus colocynthis and fed with high cholesterol diet, group 4 contain animals fed high cholesterol diet only, group 5 contain animals receiving 150mg/kg Citrullus colocynthis and fed with normal diet and group 6 contain animals receiving 75mg/kg Citrullus colocynthis and fed with normal diet. The animals were sacrificed after three months, blood was collected for biochemical evaluation. The administration of high-cholesterol diet-induced obesity was indicated by an increase in body mass index, Lee index, and specific rate of body mass gain in the group fed with high cholesterol diet only when compared to the control group. The serum was analyzed for cholesterol, triglyceride, low-density lipoprotein, and high-density lipoprotein, significant decrease in high-density lipoprotein and an increase in cholesterol, triglyceride, and low-density lipoprotein levels was observed in the group that fed with high cholesterol diet only when compared to the control group and the group that is fed with high cholesterol diet and treated with 150mg/kg of the extract, while significance increase in high-density lipoprotein and decrease in cholesterol, triglyceride, low-density lipoprotein levels were observed in the group that was given 150mg/kg of the extract only when compared to the control group and the group that was fed high cholesterol diet only. In conclusion this study suggested that Citrullus colocynthis juice pulp extract is effective in the treatment of obesity as evidenced by a reduction in the levels of cholesterol, triglycerides, and LDL, while there is an increase in the level of HDL, due to the presence of phytochemical constituents in the plant.

Keywords: *Citrullus Colocynthis, obesity, overweight, genetics, cardiovascular dysfunction*

INTRODUCTION

Obesity has become a global health crisis, surpassing undernutrition and infectious diseases as the primary contributor to ill health. In Nigeria, the prevalence of overweight and obese individuals has reached epidemic proportions, with 20.3%-35.1% being overweight and 8.1%-22.2% being obese Chukwuonye *et al*. (2013). The World Health Organization reports that the worldwide prevalence of obesity has almost tripled since 1975, with nearly 2 billion adults now overweight, and estimates that 2.7 billion adults will be overweight and over 1 billion will be affected by obesity by 2025 (WHO, 2016).

Obesity is a chronic disease caused by an imbalance in the amount of energy consumed and the amount of energy expended. Excess energy is stored in fat cells, which grow in size and/or number. Obesity is a pathological lesion caused by fat cell hyperplasia and hypertrophy (George 2004). Obesity causes or worsens a wide range of health problems, both independently and in conjunction with other diseases (Kopelman 1994). It is linked to the development of type 2 diabetes, coronary heart disease (CHD), an increased incidence of certain types of cancer, respiratory complications (obstructive sleep apnea), and osteoarthritis of large and small joints (Kopelman 2000).

Excess energy is stored in fat cells, which grow in size and/or number. Obesity is a pathological lesion caused by fat cell hyperplasia and hypertrophy. Because of the weight or mass of the extra fat, or because of the increased secretion of free fatty acids and numerous peptides from enlarged fat cells, enlarged fat cells because of the clinical problems associated with obesity. Diabetes mellitus, gallbladder disease, osteoarthritis, heart disease, and some forms of cancer are the result of these two mechanisms (George 2004)

Although the etiology of obesity is highly complex, with genetic, physiologic, environmental, psychological, social, economic, and even political factors all interacting to varying degrees to promote the development of obesity (Aronne *et al*. 2009), the most common cause is excess energy consumption (dietary intake) relative to energy expenditure (energy loss via metabolic and physical activity). Because, unlike alcohol, carbohydrate, and protein, the body has a very poor auto-regulatory system for fat and an almost unlimited ability to store fat, there are several important ways in which fat intake and the consumption of high-fat diets play a significant role in the development of obesity. As a result, high-fat, low-carbohydrate diets are effective at causing obesity (Ellis *et al*. 2002; Ghibaudi *et al*. 2002; Harrold *et al*. 2000). Obesity being a worldwide problem, many medications have been developed to treat it. However, the majority of anti-obesity medications that were approved and marketed have now been withdrawn due to serious side effects. The European Medicines Agency (EMA) recommended the withdrawal of several anti-obesity drugs from the market in 2000, including phentermine, diethylpropion, and mazindol, due to an unfavorable risk-benefit ratio (Glazer 2000). This has resulted in the search for alternative and medicinal plants with anti-obesity properties, one of which is *Citrullus colocynthis*.

Citrullus colocynthis (*Citrullus colocynthis*, Cucurbitaceae) is a plant known as the "bitter apple" that grows abundantly in the Arabian deserts and other parts of the world. This plant has traditionally been used to treat constipation, diabetes, leukemia, and bacterial infections (Alkofahi *et al*. 1996). Recent research has confirmed the hypoglycemic, antidiabetic, hypolipidaemic, and antihyperlipidaemic effects of *Citrullus colocynthis* aqueous extract in both diabetic and non-diabetic animal models (Dallak 2011, Rajangam and Christina 2013, Akinleye *et al*. 2016). As a result, the purpose of this study is to examine the anti-obesity effect of *Citrullus colocynthis* fruit juice pulp on high-fat-induced obese rats.

Justification Of The Study

Obesity is a global health concern that is associated with an increased risk of developing chronic diseases, including cardiovascular diseases, type 2 diabetes, and some types of cancer (World Health Organization, 2020), which is characterized by excessive fat deposition in adipose tissue and other internal organs such as the liver, heart, skeletal muscle, and pancreatic islet (World Health Organization, 1997; Ahima, 2006). The high prevalence of obesity and its associated health risks has led to the search for safe and effective therapeutic interventions. At present, because of the high cost and potentially hazardous side effects of synthetic drugs, the need for natural products against obesity is under exploration which may be an alternative strategy for developing effective, safe antiobesity drugs (Moro and Basile, 2000). Hence, the a need to source natural products of plant origin that are believed to have little or no side effects (Adedosu et al., 2012). Citrullus Colonynthis (CC) is a fruit that is widely used in traditional medicine for the treatment of various diseases, including obesity (Bouriche, Derdiche, & Sahli, 2018). CC is rich in bioactive compounds, including flavonoids, alkaloids, and phenolic compounds, which have been shown to possess antioxidant and hypolipidemic effects (Amira, Belhadj, Hamrouni, & El-Benna, 2018; Bouriche et al., 2018). Therefore the study aims to investigate the effect of Citrulus Colonynthis on the lipid profile of High fat induced obese rats.

LITERATURE REVIEW

Obesity

Obesity is now so common within the world's population that it is beginning to replace undernutrition and infectious diseases as the most significant contributors to ill health. Obesity causes or exacerbates many health problems, both independently and in association with other diseases (Kopelman 1994). In particular, it is associated with the development of type 2 diabetes mellitus, coronary heart disease (CHD), an increased incidence of certain forms of cancer, respiratory complications (obstructive sleep apnoea), and osteoarthritis of large and small joints. The Build and Blood Pressure Study has shown that the adverse effects of excess weight tend to be delayed, sometimes for ten years or longer. Life insurance data and epidemiological studies confirm that increasing degrees of overweight and obesity are important predictors of decreased longevity (Lew 1985). In the Framingham Heart Study, the risk of death within 26 years increased by 1% for each extra pound (0.45 kg) increase in weight between the ages of 30 years and 42 years, and by 2% between the ages of 50 years and 62 years (Hubert 1986). Despite this evidence, many clinicians consider obesity to be a self-inflicted condition of little medical significance. Here I will review the epidemiology and factors influencing obesity and the health consequences of excessive body fat.

Definition Of Obesity And Overweight

In clinical practice, body fat is most commonly and simply estimated by using a formula that combines weight and height. The underlying assumption is that most variation in weight for persons of the same height is due to fat mass, and the formula most frequently used in epidemiological studies is body mass index (BMI). A graded classification of overweight and obesity using BMI values provides valuable information about increasing body fatness. It allows meaningful comparisons of weight status within and between populations and the identification of individuals and groups at risk of morbidity and mortality. It also permits the identification of priorities for intervention at an individual or community level and for evaluating the effectiveness of such interventions. It is important to appreciate that, owing to differences in body proportions, BMI may not correspond to the same degree of fatness across different populations. Nor does it account for the wide variation like obesity between different individuals and populations (WHO, 1995; WHO, 1997)

Epidemiology Of Overweight And Obesity

Obesity can be defined as a disease in which excess body fat has accumulated such that health may be adversely affected. Conservative estimates of the economic costs of obesity in developed countries are between 2 and 7% of the total health costs, which represents a significant expenditure of national healthcare budgets (Seidell 1996). It is highly beneficial to be able to estimate the prevalence and secular trends in obesity to identify those at risk and assist policymakers and public health planners. The major health consequences of obesity are predictable from an understanding of the pathophysiology of increasing body fat. Obese individuals with excess fat in intra-abdominal depots are at particular risk of negative health consequences, with certain ethnic populations carrying different levels of risk (McKeigue *et al*. 1991). To make true comparisons of the burden of obesity between countries it is necessary to compare population-based data on measured height and weight that followed identical protocols for measurement and collection during the same period. The range of BMI of a population varies significantly according to the stage of economic transition and associated industrialization of a country (such as a shift from a dietary deficit to one of dietary excess). As the proportion of the population with a low BMI decreases there is an almost symmetrical increase in the population with a BMI above 25. This indicates the tendency for a population-wide shift as socio-economic conditions improve, with overweight replacing thinness. In the first stages of the transition, wealthier sections of society show an increase in the proportion of people with a high BMI, whereas thinness remains the main concern among the less wealthy. The distribution of BMI tends to change again in the later phases of transition with an increasing prevalence of high BMI among the poor. Importantly, changes in adult prevalence of obesity are reflected by a striking increase in childhood and adolescent weight in both industrialized and developing countries. The early onset of obesity leads to an increased likelihood of obesity in later life as well as an increased prevalence of obesity-related disorders (Kotani 1997; Dietz 1994).

Factors Influencing Obesity

Obesity is not a single disorder but a heterogeneous group of conditions with multiple causes. Body weight is determined by an interaction between genetic, environmental, and psychosocial factors acting through the physiological mediators of energy intake and expenditure. Although genetic differences are of undoubted importance, the marked rise in the prevalence of obesity is best explained by behavioral and environmental changes that have resulted from technological advances.

Genetics

Fatness runs in families but the influence of the genotype on the etiology of obesity may be attenuated or exacerbated by non-genetic factors. Apart from rare obesity-associated syndromes, the genetic influences seem to operate through susceptibility genes. Such genes increase the risk of developing a characteristic but are not essential for its expression or, by themselves, sufficient to explain the development of a disease. The susceptible-gene hypothesis is supported by findings from twin studies in which pairs of twins were exposed to periods of positive and negative energy balance (Bouchard 1990). The differences in the rate of weight gain, the proportion of weight gained and the site of fat deposition showed greater similarity within pairs than between pairs. This suggests differences in genetic susceptibility within a population determine those who are most likely to become obese in any given set of environmental circumstances. A candidate gene is defined as that part of the DNA molecule that directs the synthesis of a specific polypeptide chain closely associated with a particular disease. The search for obesity genes requires a multifaceted approach that involves studies of potential candidate genes derived from animal models, human obesity syndromes, and a genome-wide search using microsatellites covering the human genome. Candidate genes for obesity can be chosen for their possible effect on body fat composition, anatomical distribution of fat, food intake, and energy expenditure.

Environmental Factors

Implicit to the susceptible-gene hypothesis is the role of environmental factors that unmask latent tendencies to develop obesity. Predictions about possible interactions between genes and the environment are difficult because there may be a delay in an individual’s exposure to an obesogenic’ environment, and/or alteration in lifestyle related to living circumstances and uncertainty about the precise timing of the onset of weight gain.

Energy Expenditure

The most variable component of energy expenditure is physical activity, representing 20–50% of total energy expenditure. The analysis of the level of physical activity is similar in groups of subjects with a BMI of similar in groups of subjects with a BMI of <20, 20–25, and 25–35, which indicates similar levels of habitual activity. The measurement of energy expenditure within the home, using doubly labeled water, also shows comparable values between obese and lean subjects when corrected for different body sizes (Prentice *et al*. 1996).

Energy Intake

Surprisingly, no direct correlation has been reported between the prevalence of obesity and increased energy intake in developed nations, given the ready availability of highly palatable foods. The understanding of the role of energy intake in the etiology of obesity is confounded by the failure to report food intake accurately. Under-reporting is widely recognized as a feature of obesity, with comparisons of energy intake and expenditure in obese subjects showing a consistent shortfall in self-reported food intake of approximately 30% of the energy requirements (Poppitt 1998; Lichtman 1993). There is good evidence that individual macronutrients (protein, fat, and carbohydrate) exert differing effects on eating behavior predominantly as a result of their effects on satiety. Fat has a weak satiating capacity, particularly when compared with protein and subjects in experimental situations readily overeat when presented with high-fat foods (Lawton *et al*. 1993). It seems likely that environmental influences act through increasing energy intake and/or decreasing energy expenditure. There is some evidence that high-fat diets are associated with an increased risk of obesity within populations, but cross-cultural dietary studies have failed to show any consistent relationship between nutritional factors and relative weights (Blundell & Macdiarmid 1997).

Obesity And Fat

Obesity is characterized by the accumulation of excess body fat. The body can adjust the mix of metabolic fuels it oxidizes so that alcohol, carbohydrate, and protein intakes are tightly regulated. In effect, the body can achieve carbohydrate and protein balances quickly. However, the body has a poor autoregulatory system for fat and an almost unlimited ability to store fat. Although positive energy balance results in obesity, fat intake is an important contributor to energy balance. There are several important ways in which fat intake and the consumption of high-fat diets play a significant role in the development of obesity. We begin with the presentation of experimental animal and clinical human research and then proceed to discuss controlled trials and epidemiologic issues.

A link between dietary fat content and obesity must be exerted through an effect of the ingested fat on energy balance, and a positive energy balance can be promoted by overconsumption of energy, e.g. owing to a lower satiating effect per joule of a high-fat versus a low-fat diet. Moreover, energy from fat may be more effectively absorbed from the intestine than carbohydrate and protein, and fat may also reduce energy expenditure, e.g. by a lower thermogenic effect of fat compared with carbohydrate and protein. All the evidence points to fat exerting its effect on energy balance by affecting spontaneous energy intake, and the relation between dietary fat and body fat should therefore be studied under ad libitum conditions, where the studied individuals have free access to food. There is a robust series of studies showing that most of the fattening effect of dietary fat is linked to the higher energy density of fatty foods than carbohydrate- and protein-rich foods (Rolls *et al*. 1995).

Consequences Of Obesity

Increasing body fatness is accompanied by profound changes in physiological function. These changes are, to a certain extent, dependent on the regional distribution of adipose tissue. Generalized obesity results in alterations in total blood volume and cardiac function, whereas the distribution of fat around the thoracic cage and abdomen restricts respiratory excursion and alters respiratory function. The intra-abdominal visceral deposition of adipose tissue, which characterizes upper body obesity, is a major contributor to the development of hypertension, elevated plasma insulin concentrations and insulin resistance, diabetes mellitus, and hyperlipidemia.

Cardiovascular Dysfunction In Obesity

The effects of increased body fatness on cardiovascular function are predictable. Total body oxygen consumption is increased as a result of an expanded lean tissue mass as well as the oxidative demands of metabolically active adipose tissue, and this is accompanied by an absolute increase in cardiac output. However, the values are within the normal range when they are normalized to body surface area (Masserli 1982)

The total blood volume in obesity is increased in proportion to body weight. This increase in blood volume contributes to an increase in the left ventricular preload and an increase in resting cardiac output (De-Divitiis 1981). The increased demand for cardiac output is achieved by an increase in stroke volume while the heart rate remains comparatively unchanged. The obesity-related increase in stroke volume results from an increase in diastolic filling of the left ventricle (Licata 1991). The volume expansion and increase in cardiac output lead to structural changes in the heart, and the increase in left ventricular filling results in an increase in the left ventricular cavity dimension and an increase in wall stress. As left ventricular dilatation is accompanied by myocardial hypertrophy, the ratio between ventricular cavity radius and wall thickness is preserved, and this thickening of the wall with dilatation results in eccentric hypertrophy.

Sleep-Breathing Abnormalities In Obesity

An increased amount of fat in the chest wall and abdomen has a predictable effect on the mechanical properties of the chest and the diaphragm and leads to an alteration of respiratory excursions during inspiration and expiration, reducing lung volume and altering the pattern of ventilation to each region. In addition, the increased mass of fat leads to a decrease in compliance of the respiratory system as a whole. All of these changes are significantly exaggerated when an obese person lies flat. The mass loading effect of fat requires an increased respiratory muscle force to overcome the excessive elastic recoil and an associated increase in the elastic work of breathing. The obesity-related changes in respiratory function are most important during sleep (Grunstein 1998; Kopelman 1992).

Lipid Profile And Obesity

Lipid abnormalities in children and adolescents play an important role later in life in the development of coronary heart disease (Castelli *et al*.1986; Lauer *et al*. 1988), which is the major cause of morbidity and mortality in Western societies (Kannel *et al*. 1984). Therefore, detection and treatment of lipid abnormalities should begin during childhood. Currently, the American Academy of Pediatrics (AAP) recommends screening for high cholesterol levels only in children with a family history of hypercholesterolemia or premature coronary heart disease. However, several investigators have demonstrated that many children with "atherogenic" lipid profiles are missed when screening is limited to children with positive family history indicators (Garcia and Moodie 1989). The prevalence of childhood obesity has increased in epidemic proportions worldwide in the last two decades despite major efforts to promote weight reduction (Wong *et al*. 1991; Davidson *et al*. 1991). Long-term follow-up indicates that obese children and adolescents tend to become obese adults (Schonfeld-Warden and Warden 1997). Several studies have demonstrated that increased body fatness is associated with unfavorable lipid and lipoprotein profiles (Arsalanian and Suprasongsin 1996; Resnicow and Morabia 1990), and, therefore, it was suggested that obese children should also be screened for hypercholesterolemia". However, the exact prevalence of hypercholesterolemia among obese children and adolescents, and the effect of the degree of obesity on the lipid profile, are not known (Glassman and Schwarz 1993).

MATERIAL AND METHODS

Experimental Designs

A total number of forty-two male albino rats were acclimatized and grouped into six groups of seven rats each according to their dosing containing seven rats each. Below are the experimental groups

Table 1: Grouping of Animals

Category	Treatment
Group 1	Control
Group 2	High Cholesterol diet + 150 mg/ml of *Citrullus colocynthis*
Group 3	High Cholesterol diet + 75 mg/ml of *Citrullus colocynthis*
Group 4	High Cholesterol diet only
Group 5	150 mg/ml of *Citrullus colocynthis*
Group 6	75 mg/ml of *Citrullus colocynthis*

Materials

The materials used include; Beakers, a conical flask, a micropipette, a test tube, a centrifuge, a measuring cylinder, a mortar and pestle, a syringe and needle, an oral cannula, a dissecting set, filter papers, cotton wool, foil paper, sensitive weighing balance, water bath, spatula, disposable gloves, thermometer, plastic experimental cages, feeds.

Reagents

Kits and other chemicals used for assessment of oxidative enzyme parameters (SOD, MDA, NO, and CATALASE), were all of the analytical grades and were purchased from Sigma Chemicals Co. St. Lous, Mo, USA. Serum biochemical Kits were purchased from Randox Reagent. Distilled water was obtained from Ladoke Akintola University of Technology, Ogbomoso, Oyo State

Sample Collection

At the end of the experiment, the rats were weighed and euthanized on the 29th day of administration. Animals were anesthetized with 1ml of ketamine and were cut open. Blood was collected through cardiac puncture and used for biochemical assays and electrolytes antioxidant parameters. Organs were excised quickly as soon as the animals were sacrificed to keep the cells and enzymes intact, the excised Organs of the rats were weighed and washed in 0.1M phosphate buffer solution, pH 7.0, and stored in a clean, sterile plain bottle.

Biochemical Assay

Determination Of Total Cholesterol

Method

Total cholesterol was determined in the serum by the method described by Allian 1974 in Agape Kit

Principle

Enzymatic determination of total cholesterol was based on the following reaction

Cholesterol Ester + H20 CHE Cholesterol + Fatty acid

Cholesterol + O2 CHO 4 Cholesteri-3-one + H2O2

2H2O2 + O2 CHO 4 Cholesteri-3-one+ H20

2H2 + Phenol + 4 Aminoantipyrine POD Red quinone + 4H20

Where CHE : Cholesteroesterase

CHO: Cholesterol oxidase

POD: Peroxidase

Reagent Composition

Pipes buffer (pH 6.7)	50mmol/L
Phenol	24mmol/L
Sodium Cholate	0.5mmol/L
4-amino antipyrine	0.5mmol/L
Cholesterol esterase	180 U/L
Cholesterol oxidase	200 U/L
Peroxidase	1000 U/L
Cholesterol standard	1 x 5ml
Cholesterol standard concentration	200mg/dl

Procedure

10µL of serum was pipetted into a test tube

1000µL of working reagent was added

The mixture was incubated for 5 minutes at $37^{\circ}C$ and the absorbance of the sample and standard were measured at 505 against reagent blank.

Determination Of Triglyceride

Method:

Triglyceride was determined by the method of Schettler (G *et al*., 1975) described in Agappe kit

Principle :

Enzymatic determination of triglyceride was based on the following reaction.

Triglyceride + H20 Lipoprotein, lipase Glycerol + fatty acid

Glycerol + ATP Glycerol kinase Glycerol -3-phosphate + ADP

Glycerol-3-phosphate + O_2 GPO Dihydroxyl acetone phosphate + H_2O_2

H_2O_2 + 4-AAP + P-chlorophenol POD Red quinone imine

Where GPO = Glycerol-3-Phosphate oxidase

4-AAP = 4-aminoantipyrine

Reagents Composition

Pipes buffer (pH 7.0)	50mmol/L
P-chlorophenol	5.3mmol/L
Potassium ferrocyanide	10µmol/L
Magnesium salt	17 mmol/L
4-Aminoantipyrine	0.7mmol/L
ATP	3.15mmol/L
Lipoprotein Lipase	1800U/L
Glycerol kinase	450U/L
Glycerol-3-phosphate oxidase	3500U/L
Peroxidase	450 U/L

Procedure

10µL of serum was pipetted into a test tube

1000µL of working reagent was added

The mixture was incubated at 37℃ for 5 minutes and the absorbance of the sample and standard was recorded at 505nm

Determination Of Hdl Cholesterol

Method:

HDL cholesterol was determined by the method of Assmann, G. 1979 described in the Agappe kit.

Principle:

The chylomicrons of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) of serum are precipitated by phosphotungstic acid and magnesium ions

After centrifugation, high-density lipoprotein (HDL) is contained in the supernatant. HDL content of the supernatant is measured by an enzymatic method

Reagent

HDL cholesterol reagent

Phosphotungstate	14mmol/L
Magnesium chloride	1mmol/L
HDL cholesterol concentration	50mmol/L

Procedure

50µL of the supernatant obtained after centrifugation was pipetted into the test tube

1000µL of the working reagent (CHOO-PAP) was added and the mixture was incubated at 37℃ for 5 minutes

The absorbance of both the standard and the sample was then recorded against a reagent blank at 505nm.

Results

Values were expressed as Mean ± SEM (n=5).a- statistically significant at ($p<0.05$) when compared with control group, b-statistically significant at ($p<0.05$) when compared with High Cholesterol Diet Only, c-statistically significant at ($p<0.05$) when compared with High Cholesterol Diet + 150mg/kg of *Citrullus colocynthis*, d- statistically significant at ($p<0.05$) when compared with 150mg/kg of *Citrullus colocynthis* Only group.

Table 2: Effect of *Citrullus colocynthis* on body mass index (BMI), Lee index, and specific rate of body mass gain (SRGM) in high cholesterol-induced obese rats.

Assays / Groups	BMI (mmol/l) Mean ± SEM	LEE INDEX (mmol/l) Mean ± SEM	SRBM (mmol/l) Mean ± SEM
High Cholesterol diet + 75mg/Kg AECC	0.5673 ± 0.01423[a,b,d]	298.2 ± 4.587[a,d]	0.8520 ± 0.01796[a,d]
High Cholesterol diet + 150mg/Kg AECC	0.5255 ± 0.004478[a,b,d]	282.0 ± 2.312[b,d]	0.7961 ± 0.02302[a,b,d]
75mg/Kg AECC only	0.4265 ± 0.006696[b]	262.2 ± 1.264[b]	0.6032 ± 0.007445[b]
150mg/Kg AECC only	0.4113 ± 0.005960[b]	256.6 ±1.406[b]	0.5937 ± 0.01154[b]
High Cholesterol diet only	0.6856 ± 0.02374[a]	309.7 ±7.592[a]	0.9388 ± 0.04383[a]
Control	0.4505 ± 0.01142	267.4 ± 2.823	0.6361 ± 0.01385

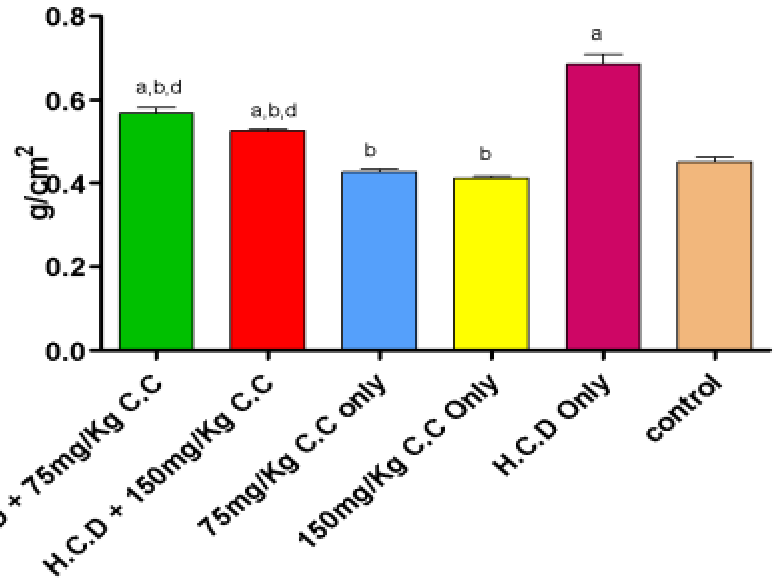

Figure 1: Effect of *Citrullus colocynthis* juice pulp extract on Body mass index (BMI) in high cholesterol diet-induced obese rats.

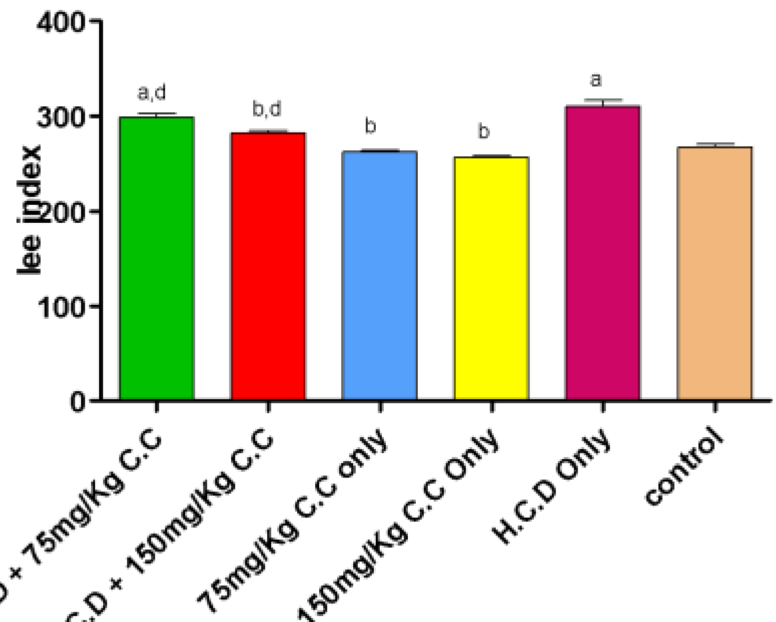

Figure 2: Effect of *Citrullus colocynthis* juice pulp extract on lee index in high cholesterol diet-induced obese rats.

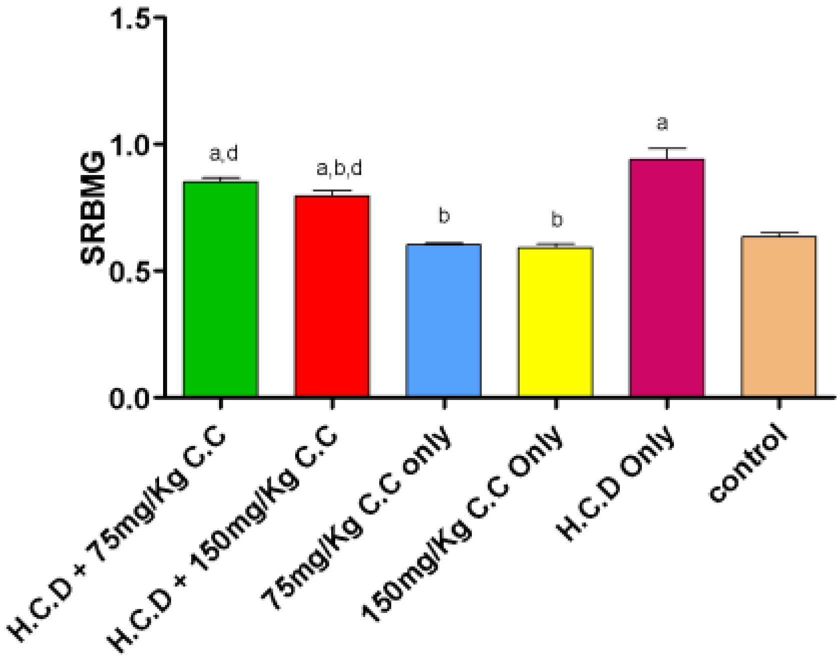

Figure 3: Effect of *Citrullus colocynthis* juice pulp extract on specific rate of body mass gain (SRGM) in high cholesterol diet-induced obese rats.

Table 3: Effect of Citrullu colocynthis on cholesterol (CHOL), triglyceride (TAG), High-density lipoprotein (HDL), and Low-density lipoprotein (LDL) level in serum of high cholesterol diet-induced obese rats.

Assays Groups	CHOL (mg/dl) Mean ± SEM	TAG (mg/dl) Mean ± SEM	HDL (mg/dl) Mean ± SEM	LDL (mg/dl) Mean ± SEM
High Cholesterol diet + 75mg/Kg C.C	155.4 ± 3.969[a,b,d]	154.1 ± 3.294[a,b,d]	17.55 ± 1.392 [a,d]	95.66 ± 3.458 [a,c,d]
High Cholesterol diet + 150mg/Kg C.C	141.1 ± 2.253[a,b,d]	130.5 ± 3.580[b,d]	22.05 ± 1.150 [d]	59.76 ± 3.325 [b,d]
75mg/Kg C.C only	78.35 ± 3.843[a,b]	98.74 ± 4.006[b]	27.91 ± 1.697 [b,d]	34.97 ± 3.839 [b]
150mg/Kg C.C only	62.45 ± 3.053[a,b]	83.48 ± 2.398[a,b]	36.39 ± 1.856 [a,b]	34.76 ± 2.301 [b]
High Cholesterol diet only	194.7 ± 6.570[a]	197.2 ± 11.88[a]	16.14 ± 2.142 [a]	110.3 ± 7.076 [a]
Control	115.3 ± 3.904	110.4 ± 3.895	27.28 ± 0.8177	46.67 ± 3.564

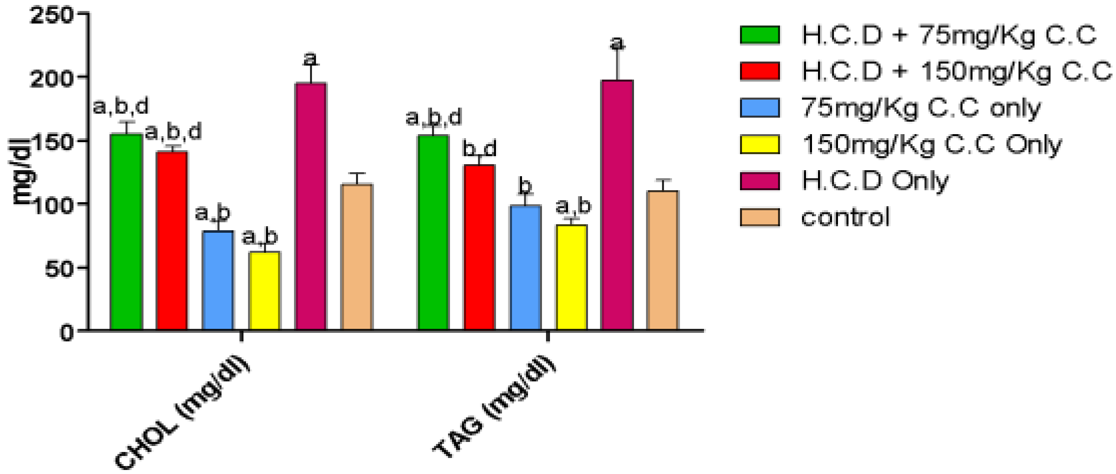

Fig 4: Effect of *Citrullus colocynthis* on cholesterol (CHOL), triglyceride (TAG) level in serum of high cholesterol diet-induced obese rats.

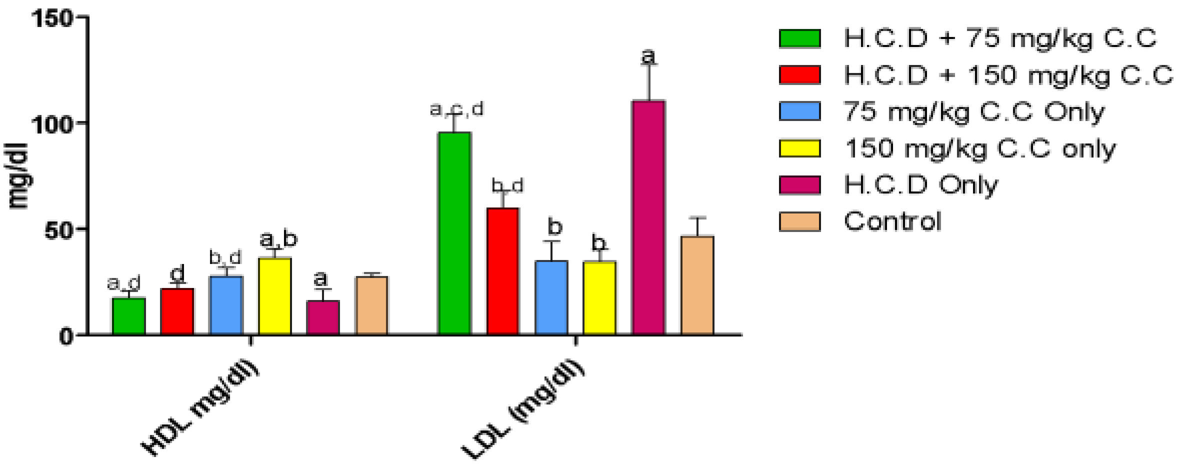

Fig 5: Effect of *Citrullus colocynthis* on High-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels in serum of high cholesterol diet-induced obese rats.

The level of significance was taken at $P < 0.05$

Where

A – represents significant change when compared to Control

B - represents significant change when compared to a high-cholesterol diet Only

C - represents a significant change when compared to a High Cholesterol Diet + 150mg/kg of *Citrullus colocynthis*

D - represents a significant change when compared to 150mg/kg of *Citrullus colocynthis*

H.C.D - High Cholesterol diet

C.C - *Citrullus colocynthis*

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

Obesity is a global health concern that is associated with a multitude of adverse health effects, including dyslipidemia, insulin resistance, and an increased risk of cardiovascular diseases (CVDs) (World Health Organization, 2020). Dyslipidemia, characterized by elevated levels of total cholesterol (CHOL) and triglycerides (TAG), and imbalances in high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, is a common lipid disorder in obesity (Grundy et al., 2018).

The prevalence of obesity has been steadily increasing, with sedentary lifestyles and high-calorie diets being major contributing factors (World Health Organization, 2020). The excessive consumption of high-fat diets has been linked to disturbances in lipid metabolism, leading to dyslipidemia and an increased risk of CVDs (Grundy et al., 2018).

Natural products derived from medicinal plants have gained significant attention as potential therapeutic agents for managing various diseases, including dyslipidemia associated with obesity (Ali-Shtayeh et al., 2019). Citrus colocynthis, commonly known as bitter apple, is a medicinal plant belonging to the Citrus family. It has been traditionally used for its pharmacological properties, including anti-inflammatory, antioxidant, and lipid-lowering effects (El-Beshbishy et al., 2014; Ali-Shtayeh et al., 2019). Understanding the potential lipid-modulating effects of Citrus colocynthis is of great importance in developing novel strategies for the management of dyslipidemia and reducing the risk of CVDs in obese individuals. Therefore, this research aims to evaluate the effect of Citrus colocynthis on the lipid profile of high-fat diet-induced obese rats by assessing the changes in CHOL, TAG, HDL, and LDL levels in the serum of obese rats following *Citrullus colocynthis* administration.

Body mass index(BMI), Lee index, and specific rate of body mass gain (SRGM) are used as indicators of obesity. BMI is a measure that relates a person's weight to their height, commonly used as an indicator of body fatness or obesity. (WHO, 2000) while the Lee index, which is the cube root of body weight in grams by the nasoanal length in millimeters multiplied by 1000 is also used to confirm obesity (Hoiki *et al*., 2010). The Specific Rate of Body mass gain (SRGM) is the measure of the rate at which an organism's body mass increases over a specific period (Dey *et al.*, 2014). The assessment of BMI and Lee index which were represented in Table 2, Figure 1, and Figure 2 revealed that the BMI and Lee index in the high cholesterol diet group was significantly higher than in the control group, indicating that the rats in the group are obsessed. However the BMI and Lee index were significantly reduced in high cholesterol diet treated with 75mg/kg C.C and the high cholesterol diet treated with 150mg/kg C.C groups compared to the control group. This shows a potential beneficial effect of *Citrullus colocynthis* in reducing the elevation of BMI and Lee index caused by a high cholesterol diet. This is similar to the findings of Novelli (Novelli et. al.2002). Similarly, Table 2 and Fig 3. revealed that the SRGM in the high cholesterol diet-only group was significantly higher than the control group, showing that the rats in the group are obsessed. However, the SRGM was significantly reduced in a high-cholesterol diet treated with 75mg/kg and 150mg/kg of C.C. compared to the control group.

Total cholesterol (CHOL) represents the overall amount of cholesterol present in the bloodstream. It includes both HDL and LDL cholesterol. Elevated levels of total cholesterol, especially when accompanied by high LDL cholesterol, are considered a risk factor for cardiovascular diseases such as atherosclerosis and coronary artery disease (Grundy et al., 2018).

Triglycerides (Trig) are the most abundant form of fats in the body. They are derived from dietary fats and are also synthesized in the liver. Elevated triglyceride levels are commonly observed in individuals with obesity, insulin resistance, and metabolic syndrome. High triglyceride levels have been associated with an increased risk of cardiovascular diseases, especially when combined with low HDL cholesterol and high LDL cholesterol levels (Grundy et al., 2018).

High-density lipoprotein (HDL) is commonly referred to as the "good" cholesterol because it plays a crucial role in cardiovascular health. HDL removes excess cholesterol from the bloodstream and transports it to the liver for excretion, thereby reducing the risk of plaque formation in arteries. Studies have consistently shown an inverse relationship between HDL levels and the risk of cardiovascular diseases (Gordon et al., 2016).

On the other hand, low-density lipoprotein (LDL) is often termed the "bad" cholesterol due to its association with increased cardiovascular risk. LDL carries cholesterol from the liver to various tissues, including arteries. If LDL levels are elevated, it can lead to the accumulation of cholesterol in artery walls, contributing to the development of atherosclerosis and an increased risk of cardiovascular diseases (Gordon et al., 2016).

From Table 3 Fig 4 and Fig 5, Comparing the results of the High Cholesterol diet group to the Control group, it is evident that the high-cholesterol diet-induced unfavorable changes in lipid parameters, including increased CHOL and TAG levels and decreased HDL levels. These changes are associated with an increased risk of cardiovascular diseases. However groups of animals who received a high-cholesterol diet along with a dose of 75mg/Kg of *Citrullus colocynthis* (High Cholesterol diet + 75mg/Kg C.C), significant reductions were observed in CHOL, TAG, and LDL levels compared to the High Cholesterol diet only group. This indicates that *Citrullus colocynthis* at this dose has a potential lipid-lowering effect, as evidenced by the decrease in the levels of these lipid parameters. Similarly, in the group receiving a high-cholesterol diet along with a dose of 150mg/Kg of *Citrullus colocynthis* (High Cholesterol diet + 150mg/Kg C.C), significant reductions were observed in CHOL and TAG levels compared to the High Cholesterol diet only group. Additionally, there was a significant decrease in LDL levels, indicating the potential of *Citrullus colocynthis* at this dose to lower LDL cholesterol.

When *Citrullus colocynthis* was administered alone at a dose of 75mg/Kg (75mg/Kg C.C only), significant reductions were observed in CHOL and TAG levels compared to the Control group. Moreover, there was a significant increase in HDL levels, which is indicative of the potential of *Citrullus colocynthis* to improve the protective HDL cholesterol. In the group receiving *Citrullus colocynthis* alone at a dose of 150mg/Kg (150mg/Kg C.C only), there were significant reductions in CHOL and TAG levels compared to the Control group. Additionally, there was a significant increase in HDL levels, indicating the potential of *Citrullus colocynthis* to positively modulate the lipid profile.

The significant reductions in CHOL and TAG levels observed in the groups receiving *Citrullus colocynthis* suggest its potential as a natural intervention for lowering cholesterol and triglyceride levels. Moreover, the significant increase in HDL levels observed in the groups receiving *Citrullus colocynthis* indicates its potential to enhance the levels of "good" cholesterol, which has a protective effect against cardiovascular diseases. This suggests that *Citrullus colocynthis* may have cardioprotective properties and could be a promising agent for reducing the risk of cardiovascular complications associated with dyslipidemia.

CONCLUSION

In conclusion, the findings of this study demonstrate the potential beneficial effects of Citrus colocynthis on the lipid profile of high-fat diet-induced obese rats. The administration of Citrus colocynthis resulted in significant improvements in lipid parameters, including reductions in total cholesterol (CHOL), triglycerides (TAG), and low-density lipoprotein (LDL) levels, as well as an increase in high-density lipoprotein (HDL) levels. These changes indicate a positive modulation of the lipid profile, suggesting that Citrus colocynthis may have a lipid-lowering effect and could potentially reduce the risk of cardiovascular diseases associated with dyslipidemia.

RECOMMENDATION

It is recommended that investigation is made on different doses of Citrus colocynthis to determine the optimal dosage for achieving the maximum lipid-lowering effect. It is essential to establish the dose-response relationship to ensure the safety and efficacy of Citrus colocynthis as a potential therapeutic intervention.

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