Applied Sciences Research Periodicals
September 2023, Vol. 1, No. 6, pp. 29-38

Evaluation of the Anti-Obesity Effect of Ethanolic Leaf Extract of Murraya Koenigii (Curry Leaf) on High Fat Diet Induced Obesity on Wistar Rat

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Abstract:
Obesity is a prevalent debilitating condition among individuals in both developed and developing nations. This investigation assessed the anti-obesity impact of ethanol extract of Murraya Koenigii (EEMC) on high fat diet obesity induced Wistar rats. Thirty (30) adults male Wistar rats with an average weight of 183g were divided into five experimental groups of six rats each Viz: Group A served as normal control and received standard animal feed. Group B was the control group and received high fat diet with no medications and treatment. Group C-D served as a treatment group administered with 200mg/kg and 400mg/kg of prepared EEMC respectively. While Group E received 150mg/kg of Xenical, which served as standard control. There was a statistically significant decrease ($P<0.05$) in the treatment and standard control groups (Groups C, D and E) and the decrease was in a dose-dependent manner. The total cholesterol (TC) increased in the normal and control group compared to other groups. There was a significant decrease ($P<0.05$) in the triglyceride (Tg) and low-density lipoprotein cholesterol (LDL-C) of all treatment groups. Their decrease was in a dose-dependent manner and there was a statistically significant decrease ($P<0.05$) between the groups compared to the normal and control. Moreover, the high-density lipoprotein cholesterol (HDL-C) had a significant inverse effect of the LDL-C as there was a significant increase ($P<0.05$) in HDL-C and the increase was more significant in the treatment groups (Groups C, D and E) respectively. The histopathology of the liver showed normal cellular architecture in the treatment group at the end of the experiment, thus posing no harmful effect. This investigation therefore suggests that EEMC has an anti-obesity property and elicited this through multiple mechanisms involving a decrease in body weight, body mass indices and alteration in lipid profile of obesity induced Wistar rats and could be used as a potential method of treating and managing patients with obesity disorder.

Keywords: Xenical, obesity, hypercholesteremia

INTRODUCTION

Since the 1980s, the global prevalence of obesity, a complex condition, has more than doubled. Research indicates that the majority of the world's population resides in countries where the rate of overweight individuals exceeds the rate of underweight individuals, and this imbalance leads to more deaths. (Anderson and Butcher et al., 2003).

Obesity is diagnosed as an abnormal disorder characterized by excessive weight gain and numerous health risk factors. Given its increasing prevalence and its association with chronic health conditions such as heart disease, high blood pressure, diabetes, joint problems, gout, and high cholesterol, obesity has become a significant public health concern in both developed and developing nations. (World Health Organization, 2022).

According to an article published by the World Health Organization, complications related to overweight or obesity result in at least 4 million deaths annually. (World Health Organization, 2022). It has been reported that globally, obesity has nearly tripled since 1975, with over 1.9 billion adults being overweight and 650 million being obese. (WHO, 2022). International research on obesity has identified various competing structural explanations for the increase in body mass index (BMI), including globalization, economic development, and changing gender roles, all of which likely influence underlying behavioural mechanisms. However, previous research has not systematically tested these different explanations for the global rise in obesity. Previous studies estimate that if the current rate of obesity continues in the foreseeable future, there will be a projected 38% increase in overweight individuals and a 20% increase in obesity among the adult population. (Kelly et al., 2008). Another study conducted in the United States projects an 85% increase in obesity by the year 2030. (Wang et al., 2008). Standard drug treatments for obesity often come with adverse side effects and result in weight gain once the medication is discontinued. As a result, ongoing research aims to develop natural, safe, and effective remedies for obesity that minimize side effects while maximizing efficacy. Certain plant extracts and their bioactive compounds have been found to promote weight loss and prevent diet-induced obesity and related ailments. (Wang et al., 2008). Frequently, the plants and herbs used in cooking possess underlying medicinal benefits, and one of these benefits may include reducing obesity and its associated risk factors. One such plant rumoured to have these medicinal effects is the Curry leaf, scientifically known as *Murraya koenigii*. *Murraya Koenigii* (M. koenigii) is a tropical plant in the R*utaceae* family and is naturally found in Asia. Its foliage, commonly referred to as curry leaves, are utilized as a local seasoning in Indian, Asian, and African regions and is a widely used spice among Asians. The leaf of the *Murraya Koenigii* is recognized as a primary source of carbazole alkaloids, which are fragrant heterocyclic nitrogenous organic compounds that can be found in various medicinal plants abundant with cytotoxic, antiviral, and antibacterial properties. (Wang et al., 2008).

MATERIALS AND METHODS

Equipment Used for the Study

Rotary evaporator, (Model 349/2, Corning Ltd, England). Crestor high-speed milling machine, Whatman filter paper 1 (11µm), water bath (Chikpas Instrument, Enugu). Chemical balance (Gallenkamp, England), micro-pipettes (Perfect, USA), microscope slides, capillary tube. Handpicked leaves, Refrigerator (Haier thermocool, England).

Plant Collection, Identification, and Authentication

The Murraya Koenigii leaf was collected from University of Nsukka, Botanical Garden. The institution is in the Nsukka urban area, located in Enugu State, South-Eastern Nigeria. The plant was identified by Mr. A. Ozioko of the Bioresource Department and Conservation Program (BDCP), Research Centre Nsukka, and confirmed to belong to the Rutacaeae family, Murraya genus, and Murraya Koenigii species with a herbarium number of University of Nigeria Herbarium (UNH) No. 66.

Extraction of Ethanolic Extract of *Murraya Koenigi*

The collected leaves of *Murraya Koenigii* were dried to a constant weight at room temperature (29-35°C) for a period of one to two weeks. The dried leaves were pulverized into a coarse form with a Crestor high-speed milling machine (Chikpas Instrument, Enugu). The ethanol extraction of pulverized roots of *Murraya Koenigii* was carried out. One thousand grams (1000g) of the ground leaves of *Murraya Koenigii* were macerated in 1.5 liters of ethanol for 72 h, after which it was filtered with a mesh at 0.15 nm, followed by a Whatman filter paper. The filtrate was concentrated using a Rotary evaporator (Model 349/2, Corning Ltd, England) at a regulated temperature to separate the solvent from the extract.

Preparation of Ethanolic Extract of *Murraya Koenigii* for Administration

In previous investigation, it was discovered that the median deadly dosage (LD 50) of *Murraya Koenigii* for mice is 2500mg/kg/day (International Journal of PharmTech Research., n.d.). However, for the purpose of our trial, we utilized 8% (which is 200mg/day/kg) and 16% (400mg/day/kg).

Experimental Animal

Thirty (30) male Wistar rats (180 – 183g) obtained from the Animal House Unit of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka were utilized in the study. The animals were housed and acclimatized under standard conditions (25 ± 2°C) at 12h dark/light cycle. The rats were fed two times a day with high fat diet (Grand Cereals Ltd, Enugu Nigeria) and had access to clean drinking water *ad libitium*. The high fat diet contains 414.0 kcal/100g with 43% as carbohydrate, 17% as protein, and 40% as fat. The diet consists of a mixture of 68% normal rat chow pellet (Saintik Enterprise Malaysia), 20% instant milk powder (Dutch Lady), 6% corn oil (Krystal), and 6% ghee (Crispo). Normal rat diet contains 306.2 kcal/100g with 48.8% as carbohydrate, 21% as protein, and 3% as fat. (Levin and Dunn-Meynell, 2002) The high fat diet was given to the rats to induce obesity for duration of 6 weeks. Xenical was used as standard drug treatment in this study. The rats were acclimatized for duration of two weeks before the experiment commenced. The experimental animals were kept in 5 well-spaced cages with 6 rats per cage. The guide for the care and use of laboratory animals' procedures were followed in this study (Indian Council of Medical research, 2001).

Experimental Design

A total of thirty male Wistar rats weighing between 180 and 183 kg were divided into 5 experimental groups, each consisting of 6 rats. The groups were as follows:

- Group A = Control group
- Group B = High fat diet, no intervention (positive control)
- Group C = High fat diet + 200mg/kg of *Murraya Koenigii* leaf extract (administered orally)
- Group D = High fat diet + 400mg/kg *Murraya Koenigii* leaf extract (administered orally)

- Group E = High fat diet + 150mg/kg Xenical (standard medication) [administered orally]

ETHICAL APPROVAL

Ethical clearance for this study was obtained from the Research Ethics Committee at the Directorate of Research and Publications, College of Medicine, University of Nigeria Enugu Campus.

STATISTICAL ANALYSIS

All data were presented as mean ±standard deviation from the norm. The variations among treatment groups were examined by one-way analysis of variance (ANOVA) followed by Tukey post hoc examination for multiple comparisons using SPSS Version 21. P ≤0.05 were regarded as statistically significant.

RESULTS

Table 4.2.1. Effects of Murraya Koenigii extract on mean body weight of Wistar rats induced with obesity.

Groups	Pre-Obesity induction body weights	Obese weights (g)	Post treatment body weight	Percentage change in body weight
A	185.00 ± 3.94	189.60 ± 3.05	194.40 ± 3.31	2.53
B	179.80 ± 8.50	477.20 ± 18.59*	502.80 ± 8.71*	5.36
c	185.80 ± 4.38	460.80 ± 41.76*	254.00 ± 8.71*	-44.89
D	185.80 ± 7.09	474.40 ± 7.89*	219.00 ± 8.80*	-53.84
E	181.00 ± 5.29	467.40 ± 15.99*	265.40 ± 5.64*	-43.20
P-value	**0.3809**	**5.8842e -15**	**P = 1.1102e -16**	

Values were expressed as mean ±standard deviation: *P<0.05 showed a statistically significant difference compared to the normal control group. Also values with negative signs indicate that the parameters decreased. At the end of the experiment group B had the highest increase in body weight followed by Group A. Meanwhile Group C, D & E decreased in their body weights. The decrease was highest in Group D. There was a statistically significant difference (P < 0.05) between groups B, C, D and E on the obese weight and post treatment weight compared to the normal control group.

Table 4.3.1: Effects of Murraya Koenigii on Body length of rats induced with obesity

Groups	Mean Initial body	Post obese body length	Post treatment body length	% Change in
A	0.18 ± 0.004	0.186 ± 0.05	0.185 ± 0.003	-0.54
B	0.178 ± 0.005	0.269 ± 0.007*	0.282 ± 0.006*	4.83
C	0.186 ± 0.006	0.277 ± 0.007	0.240 ± 0.008*β	-13.36
D	0.186 ± 0.006	0.277 ± 0.007	0.219 ± 0.006* βc	-20.9
E	0.182 ± 0.006	0.265 ± 0.006*cd	0.241 ± 0.007* βd	-9.06
P -VALUES	0.0520	P = 1.402e $^{-16}$	P = 1.1102e -16	

Values were expressed as mean ± standard deviation: *P<0.05 showed a statistically significant difference compared to the normal control group. Also values with negative signs indicate that the parameters decreased. At the end of the experiment, Group B had the highest increase in body length. Meanwhile Group C, D and E decreased significantly in their body lengths, with

Group D having the highest decrease. There was a statistical difference ($p < 0.05$) between groups B, C, D, E on the obese weight compared to the normal control. There was also a statistical difference in Group E as compared to Group C and D. In the post treatment, there was a statistical difference ($p <; 0.05$) between groups B, C, D, E compared to the normal control. Still under post treatment there is also another statistical difference in Group C compared to Group B, in Group D compared to Group B and C, and finally in Group E compared to Groups B and D.

Table 4.3.1: Effects of Murraya Koenigii on Body mass index of rats induced with obesity

Groups	Pre-induction (kg/m^2)	Post induction (kg/m^2)	Post treatment (kg/m^2)
A	5.64	5.46	5.58
B	5.65	6.59	6.31
C	5.35	5.99	10.40
D	5.35	6.18	4.56
E	5.46	6.65	4.56

Table 4.4.1: Effects of *Murraya Koenigii* on Total Cholesterol

Groups	Pre-obese	Obese	Post treatment	% Change in TC
A	125.40±3.71	125.40±3.71	138.80±4.15	10.69
B	90.80±9.96*	153.20±4.55*	155.00±6.16*	1.17
C	86.80±5.22*	150.80±7.05*	$85.80±4.15^{\beta}$	-43.10
D	83.80±3.03*	153.60±5.32*	$88.40±8.08^{\beta}$	-42.45
E	86.40±4.56*	149.20±10.06*	$94.20±3.77^{\beta}$	-36.86

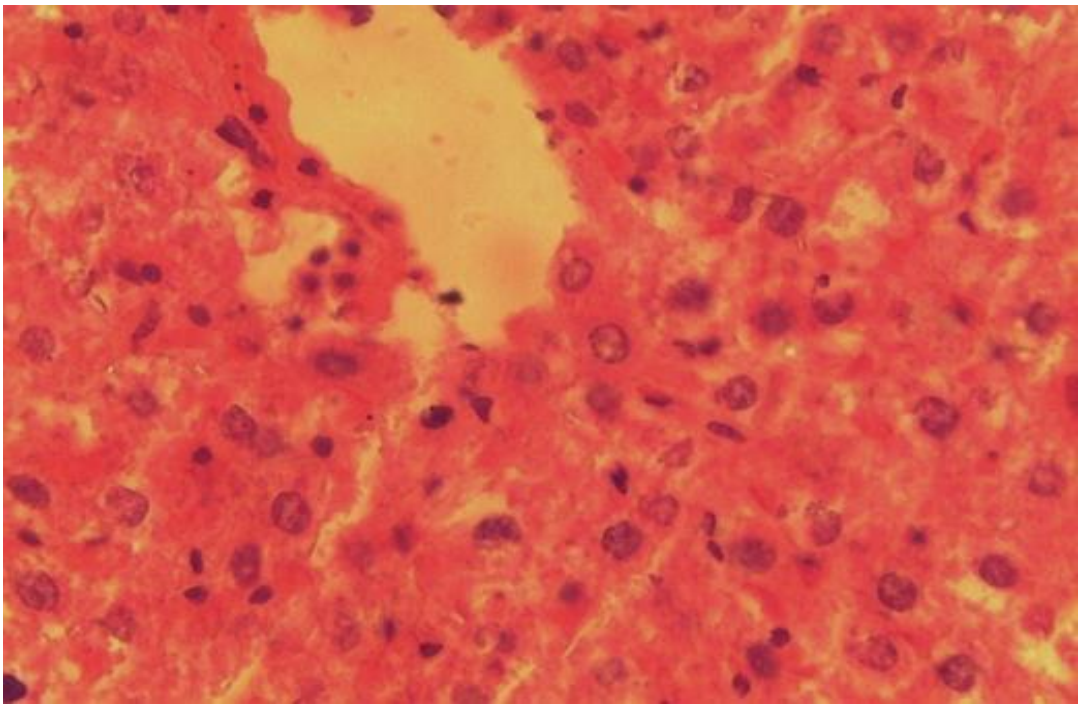

Figure 1: Photomicrograph of section of the liver from experimental rats of groups 1. Showed the normal hepatic histology architecture and degeneration and necrosis of hepatocytes in (black arrows). Note the veinous/sinusoidal congestion in (black arrows). H and E x 400

At the end of this experiment, group A had the highest increase in total cholesterol, followed by group B. Meanwhile group C, D & E decreased in their total cholesterol. The decrease was highest

in group E. There was a statistically significant difference (P < 0.05) between groups B, C, D and E on the obese weight compared to the normal control group. There was also a statistically significant difference (P < 0.05) between groups C, D and E on the post treatment compared to the positive control group

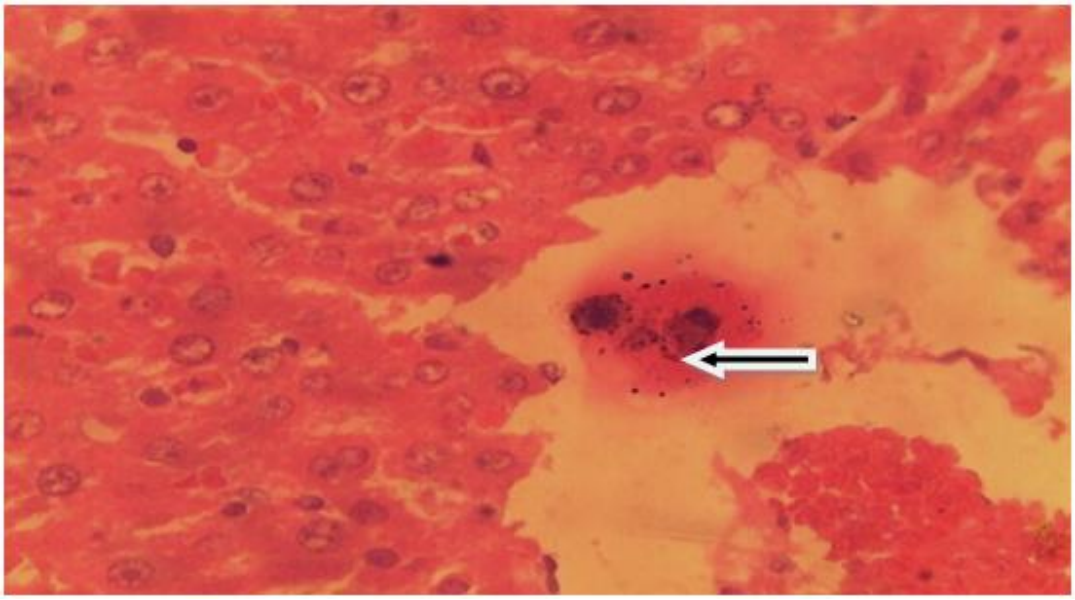

Figure 2: Photomicrograph of sections of the liver from experimental rats of groups 2. Showed mild dilatation of sinusoids in (black arrows) degeneration and necrosis of hepatocytes with mild to moderate infiltration of inflammatory leucocytes (Arrow CV). Note the veinous/sinusoidal congestion in (black arrows). H and E x 400

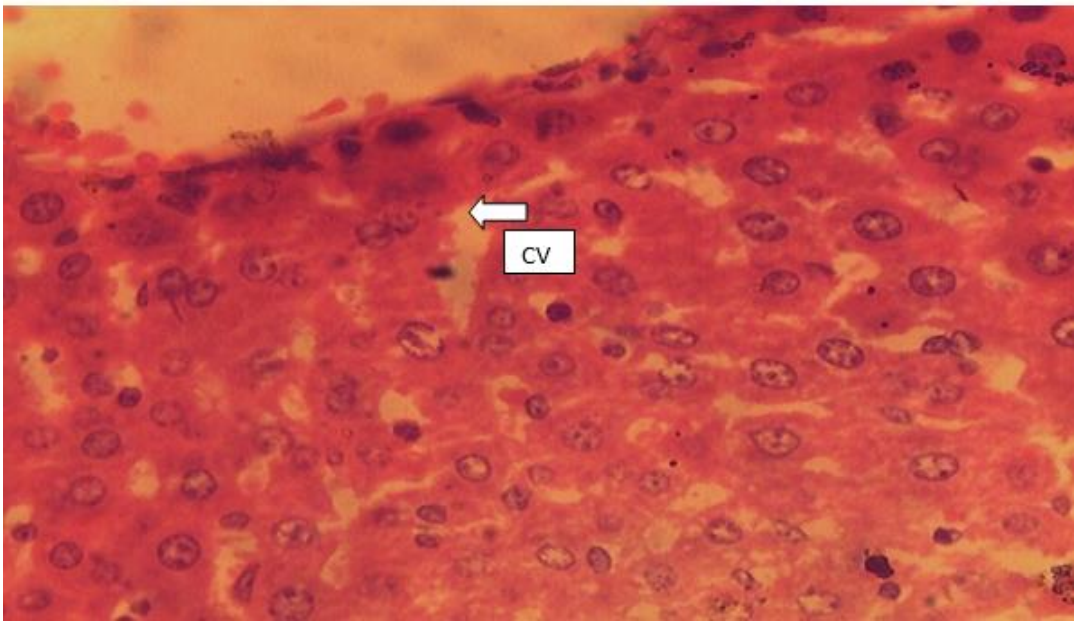

Figure 3: Liver sections from groups 3 showing apparently normal hepatocytes distributed in a radial pattern away from the central veins with no significant change except mild congestion of the central veins (black arrows). H and E x 400

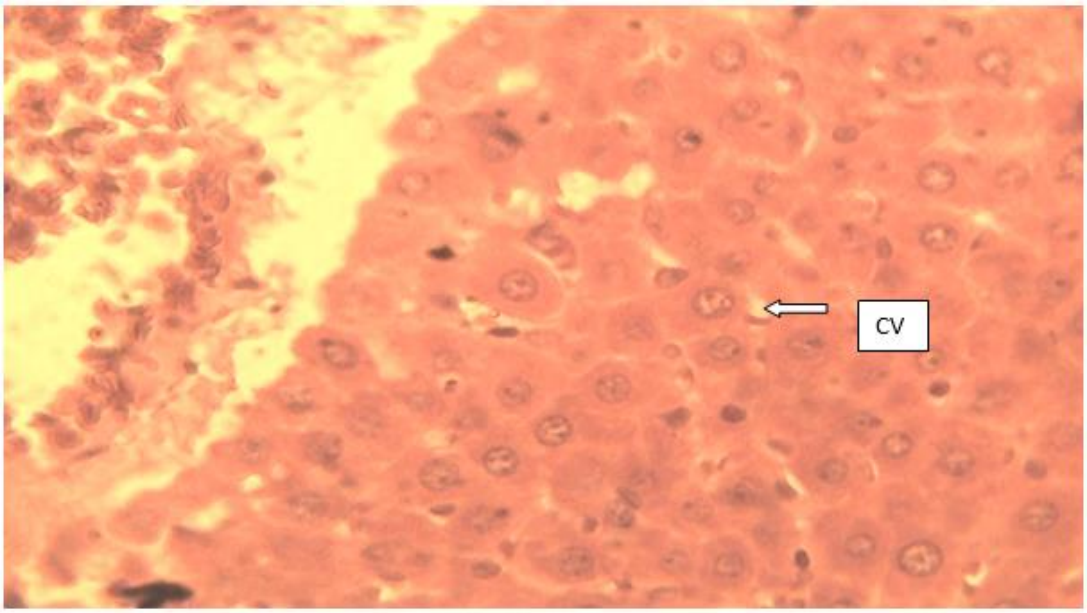

Figure 4: Photomicrograph of sections of the liver from experimental rats of groups 4. Showed normal hepatocytes arranged in interconnecting cords around the central vein (CV). Note the dilatation of sinusoids in (black arrows) and degeneration and necrosis of hepatocytes. H and E x 400

DISCUSSION

This study aimed to assess the anti-obesity impact of the alcoholic extract of Murraya Koenigii on obesity induced by a high-fat diet in Wistar rats. The need for safer and more natural methods to combat obesity, as alternatives to manufactured drugs, is growing. In Nigeria, the use of Murraya Koenigii as a spice or herb for cooking purposes is not heavily regulated, and its potential as a medicinal herb has not yet been thoroughly explored. While food herbs are popular in Nigeria, little is known about the potential benefits and risks associated with isolated consumption. Currently, there is insufficient empirical data on the anti-obesity effects of Murraya Koenigii. This study aimed to evaluate the impact of oral and repeated administration of Murraya Koenigii on physiological parameters. In this study, oral administration of Murraya Koenigii had a significant effect on the average body weight of Wistar rats with induced obesity. There were significant changes in values across all groups compared to the control group. The treatment groups that received Murraya Koenigii (Groups C & D) experienced a significant reduction in body weight after four weeks of treatment. This is attributed to the presence of Mahanimbine, a major class of carbazole alkaloids, which primarily prevents hyperlipidemia and fat accumulation in adipose tissue (Jagtap et al., 2016). Treatment with 120mg of Xenical (Standard Drug) also resulted in a significant decrease in body weight at the end of the treatment period. This is because Xenical belongs to a group of medications known as lipase inhibitors, which work by preventing the absorption of dietary fat in the body rather than suppressing appetite (Consumer Medicine Information XENICAL ® Orlistat 120 mg capsules, n.d.). There were also notable significant changes in the body length and body mass index of the Wistar rats with induced obesity, with the experimental groups (Groups C & D) showing the highest percentage decrease in body length. The assessment of rat lipid profile revealed that oral administration of Murraya Koenigii significantly decreased the overall cholesterol in all treatment groups (Groups C & D) and this is possible due to the phytochemical properties of the Murraya Koenigii (Reshma, Prabhachandh and Babychan, 2017). The treatment groups were dose dependent and overall cholesterol (TC) significantly reduced in comparison to the control group. There was also a change

in values in the triglycerides (TAG) across groups, with a significant decrease in the treatment groups in comparison to the control groups orally exposed to the Murraya Koenigii. Conversely, there was a significant increase in the High-Density Lipoprotein (HDL) in the treatment groups in comparison to the control group while the alteration of values in the Low-Density Lipoprotein (LDL) showed significant reduction in comparison to the control groups whose effects were also dose-dependent. Previous research involving evaluation of the anti-obesity properties of the Murraya Koenigii in high fat diet induced rats shows a decrease in the overall cholesterol and triglycerides. In experimental research involving investigating the anti-obesity and anti-hypoglycemic, high fat diet induced Wistar rats had reduced Triglyceride, Cholesterol and Low Density Lipoprotein levels while conversely having elevated levels of High Density Lipoprotein (Tembhurne and Sakarkar, 2012) and findings from this study show the same result when compared to the control group.The lipid profile indices are useful in monitoring the health status of the cardiovascular system which are associated risks related to obesity. Elevated levels of TAG and LDL may predispose to cardiovascular related disorders like heart failure and coronary heart disease. Elevated levels of TC will likely lead to atherosclerosis. Previous study shows that extremely low levels of HDL are associated with a higher risk of death due to cardiovascular disease (MPH, 2016). Furthermore, in order to evaluate whether the oral and repeated exposure to Murraya Koenigii was capable of having an effect on the food and fluid intake of the Wistar rats during the treatment stage, close attention was paid to the rate at which the rats consumed the high fat diet. It was discovered that their rate of feeding, slowly reduced and was more prominent in the higher dose treatment groups. The light microscopic examinations of rat liver.

CONCLUSION

The research findings revealed that there were significant alternative alterations in the body weight and body mass indices, lipid profile of rats induced with obesity and treated with the Murraya Koenigii in favor of combating obesity. Notable changes were observed in the body weight and lipid profile compared to the control group. Therefore, the Murraya Koenigii can be considered as a potentially beneficial herb in improving the rate at which the obesity disorder is addressed and managed.

ACKNOWLEDGEMENT

The authors are thankful to God, for His sustenance and guidance throughout the duration of this research and also the efforts and support of my supervisors (Mr. Nweke Maduka Luke) of University of Nigeria Enugu Campus and Dr Celestine Ani of Enugu state University of Science and Technology, Parklane, Enugu State for their Supervision and guidance throughout the course of the study.

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