Clinical Complementary Medicine and Pharmacology 2 (2022) 100035

Contents lists available at ScienceDirect

Clinical Complementary Medicine and Pharmacology

journal homepage: www.elsevier.com/locate/ccmp

The Anti-obesity Effect of *Allium cepa* L. leaves on High Fat Diet Induced Obesity in Male Wistar Rats

Babafemi J. Momoh, Shekins O. Okere, Gabriel O. Anyanwu*

Department of Biochemistry, Faculty of Science and Technology, Bingham University, P. M. B. 005, Karu 961105, Nigeria

ARTICLE INFO

Keywords:
Antiobesity
Allium cepa
High fat diet
Total phenolic content
Antioxidant
Gas chromatography-mass spectrometry

ABSTRACT

Background: Over the years, there has been research on the anti-obesity effect of the *Allium cepa* bulb, but a dearth of research was carried out on the leaves, which is consumed as vegetable salad and soup, hence this study was proposed.

Objective: This study aims at investigating the effect of *A. cepa* leaves on high fat diet (HFD) induced obesity in male Wistar rats.

Methods: Twenty-eight male Wistar rats were divided into four groups with seven rats each. Apart from Group 1 (normal control which received normal pelleted diet), obesity was induced in 21 rats of Group 2 to 4 with HFD. Group 2, the obese control was administered with 100% HFD, while the diet for group 3 and 4 was supplemented with 10% and 20% *A. cepa* powdered leaves, respectively, for 28 days.

Results: In the rats treated with 10% and 20% *A. cepa*, body weight, fat mass, blood glucose, total cholesterol, triglycerides, aspartate amino transferase, alanine amino transferase, creatinine and urea levels were reduced significantly ($P < 0.05$) in comparison with the obese control group. The liver of the rats treated with 10% and 20% *A. cepa* leaves revealed small and few amounts of fat deposits in comparison with the obese control group, which revealed numerous and large deposits of fat. The kidney of the rats treated with 10% and 20% *A. cepa* leaves showed moderate and mild inflammation, respectively, in comparison with the obese control group which showed acute inflammation. The leaves of *A. cepa* had antioxidant properties and the presence of volatile compounds with anti-obesity properties in *A. cepa* were identified using gas chromatography-mass spectrometry (GC-MS).

Conclusion: *A. cepa* leaves had weight-loss effect in that it decreased body weight, fat mass, glucose and lipid levels including fat deposits in the liver.

1. Introduction

Obesity, as a pathological condition, is often linked to increased risk of various diseases, such as type 2 diabetes, dyslipidaemia, hypertension, fatty liver disease, osteoarthritis, insomnia, cardiovascular diseases, gallstones and cancer (Heseker and Schmid, 2000; Meldrum et al., 2017). Obesity is referred to as an excess fat mass accumulated in the body as a result of the interaction of environmental, physiological and genetic factors (Heseker and Schmid, 2000). Specifically, some of these factors include over-nutrition, sedentary lifestyle, urbanization, and in few persons a physical condition or metabolic disturbance (Lokuruka, 2013).

Besides the various studies conducted on the treatment and management of obesity, its global incidence continues to rise resulting in huge socio-economic costs (Korner et al., 2003). The world has greater than 2.1 billion obese or overweight adults of which 640 million were obese in 2014 (WHO, 2015). Anti-obesity drugs which can reduce weight by regulating appetite, metabolism or caloric consumption are difficult to develop and possess negative side effects (Rodgers et al., 2012). Some existing antiobesity drugs are liraglutide, lorcaserin, phentermine/topiramate, orlistat, and naltrexone/bupropion (Anyanwu et al., 2020).

Plants are commonly used in the management or treatment of metabolic disorders including obesity because of the secondary metabolites present in them. Several biologically active components from medicinal plants have been reported to prevent obesity (Lokuruka, 2013; Srivastava et al., 2018). *Allium cepa* is a deeply investigated vegetable crop commonly known as onion, belonging to the genus *Allium* and the specie cepa (family *Amaryllidaceae*) as verified on (*http://www.theplantlist.org/tpl/record/kew-295261*). It is a biennial plant with distichous, glaucous leaves, adventitious and fibrous roots, and concentric bulb (Peruzzi et al., 2017).

* Corresponding author.
E-mail address: gabrielanyanwu@binghamuni.edu.ng (G.O. Anyanwu).

https://doi.org/10.1016/j.ccmp.2022.100035
Received 11 December 2021; Received in revised form 1 April 2022; Accepted 3 April 2022
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A. cepa is found to possess an array of compounds with abundant pharmacological properties including anticancer, antidiabetic, antimicrobial, antibacterial, analgesic, anti-inflammatory, hypolipideamic, antihypertensive, immune-protective effect, cardiovascular and antioxidant effects, as such justifying its possible use in the treatment of various human ailments (Kuete, 2017). Other pharmacological effect shown by *A. cepa* include anti-thrombotic, anti-arthritic and hypoglycemic effect (Ali, 2013). Diverse studies have reported the anti-obesity properties of the *A. cepa* bulb but this research focused on the *A. cepa* leaves. In the present study, we aimed to explore the effect of *A. cepa* leaves on HFD induced obesity in male Wistar rats. We performed antioxidant assays on *A. cepa* leaves and the presence of volatile compounds in the leaves were determined using GC-MS analysis.

2. Materials and Methods

2.1. Plant collection

Fresh leaves of the onions, *A. cepa,* was collected from Utako Market, Abuja, Nigeria. The plant was collected in March, 2020. The plant was authenticated by Dr. Jerome Ihuma in the Department of Biological Sciences, Bingham University Nigeria. The Collector's specimen number is BMT 153 and Herbarium Specimen number 27022 was allocated to the plant.

2.2. Experimental animals

Twenty-eight male Wistar rats weighing 200 ± 10 g were used for this study. The rats were acquired from the animal house, University of Jos, Nigeria and taken to the animal care unit of Bingham University, Nigeria. The animals were kept in cages of 7 rats per group for two weeks having free access to water and feed. The temperature (22 ± 2°C) and lighting (12:12 h light/dark cycle) of the room was regulated. The study was done in accordance with the prescribed guidelines for animal use in Bingham University, Nigeria.

2.3. Preparation of plant powder

The onion leaves were washed and sliced into tiny pieces and air-dried at temperature of 22 ± 2°C. The dried leaves were blended into powder, and were further dried to constant weight and kept in sealed plastic buckets until used.

2.4. Preparation of n-hexane and ethanol extract

50 g of the onion leaf powder was macerated in 500 mL of n-hexane in bottles for 72 h with intermittent shaking. Filtration was achieved through Whatman No.1 filter paper and the collected filtrate was evaporated under pressure using a rotary evaporator at 40°C. The yield was stored at 4°C in a refrigerator until required. The same procedure was repeated using ethanol as the solvent.

2.5. Total phenolic content and antioxidant activity determination

The quantity of total phenolics in the onion leaf extracts was estimated using Folin Ciocalteu's reagent by the method of Ferreira et al. (Ferreira et al., 2007). The crude and fractioned samples were prepared by liquefying 5 mg of the extract in 1 mL of 95% ethanol to yield a concentration of 5 mg/mL. 25 µL of extracts (5 mg/mL) was combined with 100 µL of Folin Ciocalteu reagent in 96-well microplates. The solution was mixed vigorously and incubated at 22°C in the dark for 3 min. Then 100 µL of Na_2CO_3 was included and gently mixed. After 60 min, the absorbance of the solution was read at 630 nm using a microplate reader (RT-2100C, *Rayto Life and Analytical Sciences Co. Ltd*, China). Changes in absorbance values were used. Total phenolic content was estimated as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the ethanol and hexane extracts of *A. cepa* was analyzed and expressed as percent scavenging activity using the method of Xu and Chang (Xu and Chang, 2007). 50 µL of ethanol and n-hexane solutions of plant extracts were added to 180 µL of 0.2 mM DPPH solution in a 96-well microtitre plate, and mixed thoroughly before incubation at 23°C for 60 min in the dark. The absorbance of the solution was read at 492 nm; ascorbic acid and ethanol were used as the positive and the percent scavenging activity was calculated from the change in absorbance: Scavenging effect (%) = [1- (Abs sample / Abs control)] × 100. The antioxidant activity of the extracts measured on the basis of β-carotene bleaching inhibition and expressed as a percentage was estimated by the method described by Amarowicz et al. (Amarowicz et al., 2008).

2.6. Gas chromatography-mass spectrometry (GC-MS) analysis of Allium cepa

The GC-MS equipment Agilent GC 7890B, MS detector MSD 5977A (Agilent Technologies, USA) coupled with a library software (MassHunter: NIST 14.L software) was used to analyze n-hexane fraction of *A. cepa* leaf. The GC-MS detection was performed with electron ionization system set at 70 eV ionization energy and Helium gas (carrier gas) at 1 mL/min constant flow rate. Other parameters were inlet temperature fixed at 250°C, oven temperature fixed at 100°C for one and half min and gradually elevated to 270°C at the rate of 5°C per min. An injection of 1 µL of diluted samples (1/100, v/v in appropriate solvents) and scan range of 40–600 was used for the analysis.

2.7. Obesity induction and treatment in the rats

A total of 28 rats were arbitrarily distributed into 4 groups. Group 1 (normal control) was fed with normal pelleted diet (NPD) which included 65% carbohydrate, 8% fat, 13% protein, 12.75% fiber, and 1.25% of vitamins and multi-minerals. Obesity induction was achieved in 21 rats by feeding with HFD (which contained 35% of carbohydrate, 50% of fat, 10% of protein, 3.75% fiber, and 1.25% of vitamins and multi-minerals) for 12 weeks. Rats fed on HFD with significantly increased ($P < 0.05$) body weight when compared to normal control group of rats were considered obese (Amin et al., 2009). [Treatment of all animals in group 3 and 4 with HFD supplemented with 10% and 20% of the grounded leaves of *A. cepa* respectively *ad libitum* started at the 13th week and lasted for another 4 weeks. While rats in Group 2 were fed with 100% high fat diet.] Experimental procedures for animal handling followed the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and ethical approval number (BHU-02/DE/M-3–3.18) was given by the Ethics Committee of Research, Bingham University, Karu, Nigeria.

2.8. Determination of food intake, body weight, organ weight and total fat mass

The daily food intake and weekly body weight was measured in grams (g) using a Tian Shan 2003B scale. After treatment period, rats were fasted for 12–14 hours. After being anesthetized with 1.9% inhaled diethyl ether that is, 0.08 mL/L of container volume (Aledani et al., 2020), organs such as the liver and kidney were harvested, rinsed in distilled water and weighed. Adipose depots were harvested from each rat after abdominal incision. The fats were weighed and the sum was regarded as the total fat mass.

2.9. Blood sample preparation

After overnight fast, cardiac puncture was employed to collect blood from the rats (Hoff and Rlatg, 2000). The blood which was collected in plain tubes was allowed to stand for 15 min to coagulate before centrifugation at 3500 rpm for 15 min at room temperature. The separated clear

and non-hemolyzed supernatant was pipetted using Pasteur pipette and preserved at -20°C.

2.10. Biochemical assays

Whole blood from the rat tail was used to measure glucose level using ACCU-CHECK glucometer at day 0, 7, 14, 21 and 28. Serum was used for subsequent lipid profile bioassay for the determination of total cholesterol level (Tietz, 1990), triglycerides level (Tietz, 1990), high density lipoprotein (HDL) level (Friedewald et al., 1972), low density lipoprotein (LDL) level (Okada et al., 1996), creatinine (Batton et al., 1977), urea level (Batton et al., 1977), uric acid (Barham and Trinder, 1972), alanine amino transferase (ALT) and aspartate amino transferase (AST) assays (Reitman et al., 1957).

2.11. Histopathological bioassay

Histopathological examinations of the liver were accomplished by paraffin embedding method. Sectioned tissues were fixed in 10% neutral buffered formalin to prevent autolysis. Dehydration and washing of organ tissues was performed by isopropyl alcohol grading and 50% ethanol respectively, and thereafter stained in heamatoxylin and eosin (Chavan et al., 2014). The stained sections were captured using a light microscope via 40× magnification. The histopathological test was carried out at the National Hospital in Abuja.

2.12. Statistical analysis

Data from the studies was analyzed using the *Graph Pad Prism 5* and *SPSS 16* software. While data from the *in vivo* studies were presented as mean ± S.E.M and were statistically analyzed by one way (ANOVA) and Tukey multiple range test. Differences at $P < 0.05$ were considered significant using *SPSS version 16* and *GraphPad Prism 5*.

3. Results

3.1. GC-MS analysis of the n-hexane extract of Allium cepa

Table 1a is the GC-MS result of the n-hexane extract of the *A. cepa*. The result reveals the presence of 32 compounds and all were identified. As for the ethanol extract of *A. cepa* leaves, thirteen compounds were identified which were different from those of the n-hexane extract. The total ion chromatogram of n-hexane and ethanol extracts of *A. cepa* are as seen in Fig. 1a and 1b, respectively.

3.2. Total phenolic content and antioxidant activity of the ethanol and n-hexane extracts of Allium cepa

Table 2 is the result of the total phenolic content determination in the ethanol and n-hexane extract of the *A. cepa* sample. The result clearly reveals that the phenolic content or gallic acid equivalent in the ethanol extract of the *A. cepa* sample is higher than that of the n-hexane extract. The ethanol extract contained higher levels of antioxidant capacity compared to the hexane extract with scavenging and inhibition effect of 78.65 ± 3.15% and 87.9 ± 8.22% against DPPH radical and in β-carotene-linoleate model system.

3.3. The effect of Allium cepa on the feed intake of experimental rats

At day 28, the feed intake amongst the groups were not statistically different, that is there was no significant change ($P > 0.05$) in the groups treated with 10% and 20% *A. cepa* when compared with the obese and normal control groups (Table 3). The body weight of all the groups fed with HFD were elevated statistically ($P < 0.05$) above the normal control at the inception of treatment (day 0) due to obesity induction (Fig. 2). At day 28, the obese control group recorded significant increase ($P < 0.05$) in body weight compared to normal control and treated (10% *A. cepa* and 20% *A. cepa*) groups.

3.4. The effect of Allium cepa on the liver weight, kidney weight and fat mass of the rats

At the end of the experiment, the increase in the liver and kidney weight of the obese control group was significant ($P < 0.05$) compared to the normal control group (Table 4). Only the kidney weight of the rats treated with 20% *A. cepa* was significantly decreased ($P < 0.05$) when compared with the obese control group. Significant increase ($P < 0.05$) in the fat mass of rats in the obese control group was observed compared to the groups treated with 10% and 20% *A. cepa*. Table 4 also showed significant rise ($P < 0.05$) in ALT and AST activities among rats in the obese control group compared with the normal control. However, the ALT activity of the rats treated with 20% *A. cepa* when compared to obese control decreased ($P < 0.05$) significantly, but both 10% and 20% of *A. cepa* significantly reduced ($P < 0.05$) the AST activities of rats.

3.5. The Effect of Allium cepa on the blood glucose level of the rats

The glucose level of the normal control was fairly constant and stable over the time which the tests were taken (Fig. 3). At day 28, the blood glucose level of the obese control was significantly elevated ($P < 0.05$) than the normal control. Nevertheless, treatment with 10% and 20% *A. cepa* significantly decreased ($P < 0.05$) the blood glucose levels of the rats when compared with the obese control but not the normal control.

3.6. The effect of Allium cepa on the lipid profile of the rats

Table 5 showed the lipid profile of experimental animals. The total cholesterol, triglyceride, LDL-C and VLDL-C levels were found to have increased significantly ($P < 0.05$) in the obese control when compared with normal control. In contrast, only 20% *A. cepa* treated rats had significantly decreased ($P < 0.05$) total cholesterol, triglyceride, LDL-C and VLDL-C levels compared with the obese control. No significant change ($P > 0.05$) in the HDL-C levels was found amongst the normal control, obese control and the treated groups.

3.7. The effect of Allium cepa on the kidney function status of the rats

Table 6 showed the kidney function status of the animals, particularly the creatinine, urea and uric acid concentration of the rats. The creatinine and urea levels in the obese control were significantly increased ($P < 0.05$), while the uric acid showed no significant change ($P > 0.05$) when compared with normal control. Likewise, the creatinine and urea levels in the *A. cepa* treated groups were decreased significantly ($P < 0.05$) without the uric acid when compared with the obese control.

3.8. The effect of Allium cepa on the histopathology of the liver of the rats

Fig. 4 showed the histopathology results that were run on the liver samples from each group. The rats in the normal control group had small and few amounts of fat deposits on their livers (Fig. 4A). The rats in the obese control group were found to have numerous and large fat deposits (Fig. 4B). Consequently, the rats in the groups treated with 10% and 20% *A. cepa* had small and numerous amounts of fat deposits (Fig. 4C and D).

3.9. The effect of Allium cepa on the histopathology of the kidney of the rats

The histopathology results that were run on the kidney samples from each group are shown in Fig. 5. The rats in the normal control group showed mild inflammation as such indicated little or no lipotoxicity

Table 1a
GC-MS analysis of n-hexane extract of *Allium cepa*.

Peak	RT	Area (%)	Name of compounds in n-hexane extract of *A. cepa*
1	22.0854	0.7851	Heptadecane
2	23.4833	2.1600	1,1-Cyclopropanedicarboxamide
3	27.2509	1.1475	10-Methylnonadecane
4	28.2181	0.6885	Heptacosane
5	29.0601	0.8829	Octadecane,1-chloro-
6	29.6244	0.8383	Phytol, acetate
7	29.7228	1.1768	2-Pentadecanone, 6,10,14-trimethyl-
8	30.3640	0.6737	Cyclopentadecanone, 4-methyl-
9	30.6349	5.3659	Methoxyacetic acid, 2-tetradecyl ester
10	30.8860	0.4936	13-Octadecenal, (Z)-
11	31.0485	1.7848	Hentriacontane
12	31.3405	1.2347	Dodecyl nonyl ether
13	31.4699	23.4473	Squalene
14	31.6688	0.6173	Oxirane, tetradecyl-
15	31.8043	1.2809	Cyclopropaneoctanal, 2-octyl-
16	31.9249	4.9634	13-Hexyloxacyclotridec-10-en-2-one
17	32.1575	7.8546	6-Octadecenoic acid, methyl ester, (Z)-
18	32.2635	2.7012	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
19	32.3313	2.9579	16-Octadecenoic acid, methyl ester
20	32.4137	1.9020	Eicosane
21	32.4748	1.4171	3,8-Dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl-
22	32.6178	4.6256	Cyclopropaneoctanal, 2-octyl-
23	32.7246	3.8848	Cyclohexane, 1R-acetamido-2,3-cis-epoxy-4-cis-formyloxy-
24	32.7966	3.5544	Octadecane
25	33.4451	1.1967	Di-n-decylsulfone
26	33.8769	6.7864	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
27	33.9916	3.4850	Acetic acid, 13-methyl-3-phenylazo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl ester
28	34.1097	1.5992	Eicosyl isopropyl ether
29	34.8391	0.7856	1,2-Bis (trimethylsilyl)benzene
30	35.3366	0.9781	4H-1,2,4-triazole-3,5-diamine, N3-(4-fluorophenyl)-N5-methyl-
31	36.2961	6.5160	gamma.-Tocopherol
32	36.6722	2.2148	1H-Indole, 5-methyl-2-phenyl-

Table 1b
GC-MS analysis of defatted ethanol extract of *Allium cepa*.

Peak	RT	Area (%)	Name of compounds in Ethanol extract of *A. cepa*
1	5.2457	0.9986	Oleic Acid
2	18.6812	2.0495	Methyl tetradecanoate
3	20.1735	1.0050	Ethyl cyclohexanepropionate
4	22.9206	8.9758	Hexadecanoic acid, methyl ester
5	24.2781	33.6168	Hexadecanoic acid, ethyl ester
6	26.1800	0.9779	10,13-Octadecadienoic acid, methyl ester
7	26.3072	18.3772	11-Octadecenoic acid, methyl ester
8	26.8363	4.0542	Methyl stearate
9	27.4306	1.6766	Ethyl 9.cis.,11.trans.-octadecadienoate
10	27.5530	25.6993	Ethyl Oleate
11	28.0725	2.5951	Octadecanoic acid, ethyl ester
12	32.5941	1.3737	Acetic acid, 6-acetoxymethyl-2,2-dimethyltetrahydro[1,3]dioxolo[4,5-c]pyran-7-yl ester
13	37.8186	1.3997	n-Propyl 11-octadecenoate

Table 2
Total phenolic content and antioxidant capacity of ethanol and hexane extracts of *Allium cepa* leaves.

Plant Sample	Total phenolic content (mg GAE/g extract)	DPPH(% scavenging effect)	β-carotene bleaching inhibition (%)
Ethanol extract	161.00 ± 22.50^{a}	78.65 ± 3.15^{a}	87.9 ± 8.22^{a}
n-hexane extract	32.30 ± 0.60^{b}	50.29 ± 5.51^{b}	9.23 ± 1.40^{b}

GAE = gallic acid equivalent, mean ± standard deviation values. Different superscript letters on mean values in the same column are significantly ($P < 0.05$) different.

Table 3
The effect of *A. cepa* on the weekly feed intake of experimental animals.

Groups	Feed Intake (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control	76.00 ± 2.31	83.00 ± 1.73	74.00 ± 3.06	76.33 ± 3.76	76.67 ± 1.76
Obese Control	77.33 ± 3.53	80.00 ± 1.73	75.00 ± 2.89	78.00 ± 3.21	80.33 ± 2.60
10% *A. cepa* Leaf	80.00 ± 3.46	79.00 ± 1.73	82.00 ± 2.31	80.00 ± 2.31	77.67 ± 1.86
20% *A. cepa* Leaf	80.00 ± 2.31	78.33 ± 5.49	77.00 ± 5.77	80.67 ± 2.40	77.00 ± 4.36

Values are presented as mean ± S.E.M. The means were not significantly different at $P > 0.05$.

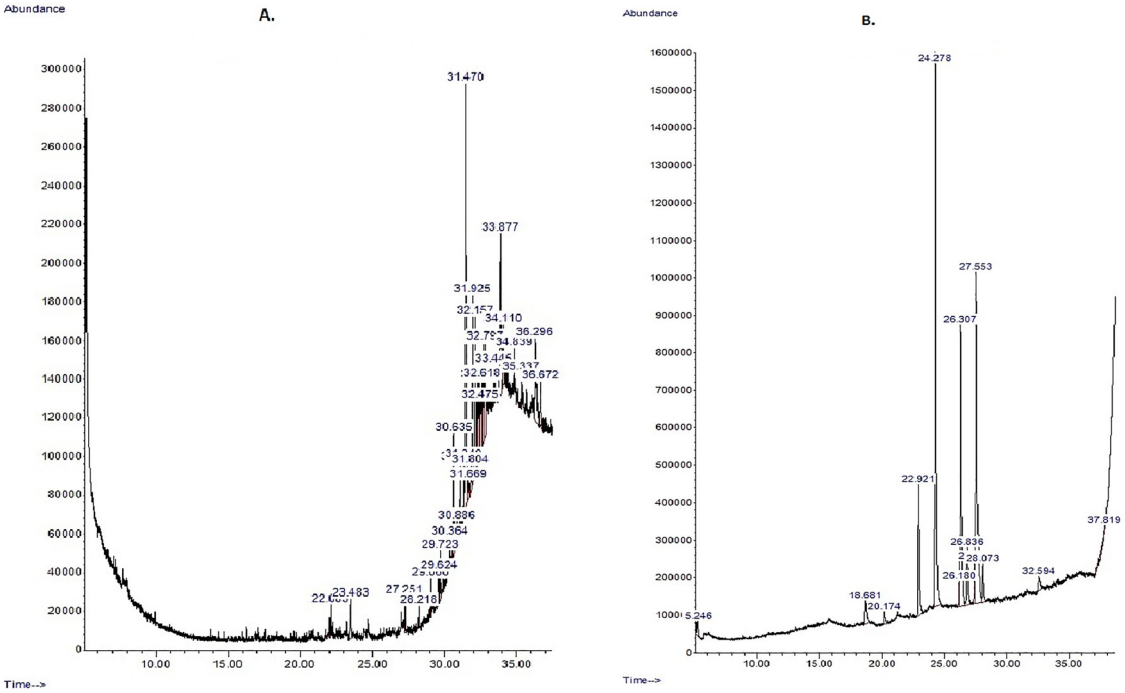

Fig. 1. **A.** Total ion chromatogram for the n-hexane extract of *Allium cepa* leaves. **B.** Total ion chromatogram for the ethanol extract of *Allium cepa* leaves.

Table 4
Effect of *Allium cepa* on organ weight and liver function parameters.

Groups	Liver weight (g)	Kidney weight (g)	Fat mass (g)	ALT (U/L)	AST (U/L)
Normal Control	7.68 ± 0.53^{b}	1.67 ± 0.05^{b}	4.11 ± 0.32^{c}	63.67 ± 1.86^{b}	64.67 ± 6.23^{b}
Obese Control	12.42 ± 1.38^{a}	2.20 ± 0.07^{a}	18.96 ± 1.70^{a}	72.67 ± 3.38^{a}	79.00 ± 4.93^{a}
10% *A. cepa* Leaf	11.83 ± 1.27^{a}	1.98 ± 0.20^{ab}	12.41 ± 0.40^{b}	68.33 ± 1.86^{ab}	47.33 ± 0.33^{c}
20% *A. cepa* Leaf	11.19 ± 0.29^{a}	1.78 ± 0.10^{b}	5.76 ± 1.21^{c}	64.33 ± 2.19^{b}	52.67 ± 3.71^{bc}

Values are presented as mean ± S.E.M. Means not sharing common letter(s) are significantly different ($P < 0.05$).

Table 5
The effect of *Allium cepa* on the lipid profile of male wistar rats.

Groups	TC(mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL(mg/dL)
Normal Control	102.11 ± 4.07^{b}	107.04 ± 3.10^{b}	23.41 ± 1.04^{a}	168.02 ± 6.27^{b}	21.41 ± 0.62^{b}
Obese Control	135.64 ± 7.29^{a}	136.71 ± 6.35^{a}	21.72 ± 1.64^{a}	315.01 ± 6.93^{a}	27.34 ± 1.27^{a}
10% *A. cepa* Leaf	120.93 ± 7.55^{ab}	128.26 ± 3.02^{a}	25.23 ± 2.18^{a}	203.25 ± 22.13^{b}	25.65 ± 0.61^{a}
20% *A. cepa* Leaf	106.13 ± 2.51^{b}	109.30 ± 3.63^{b}	26.09 ± 0.32^{a}	183.27 ± 8.30^{b}	21.86 ± 0.73^{b}

Values are presented as mean ± S.E.M. Means not sharing common letter(s) are significantly different ($P < 0.05$).

Table 6
The effect of *Allium cepa* on the kidney function status of experimental rats.

Groups	Creatinine(mg/dL)	Urea(mg/dL)	Uric acid(mg/dL)
Normal Control	0.92 ± 0.04^{b}	116.21 ± 4.43^{b}	4.93 ± 0.09^{a}
Obese Control	1.12 ± 0.01^{a}	135.22 ± 6.48^{a}	5.36 ± 0.04^{a}
10% *A. cepa* Leaf	0.89 ± 0.03^{b}	85.77 ± 0.21^{c}	5.05 ± 0.40^{a}
20% *A. cepa* Leaf	0.89 ± 0.06^{b}	57.74 ± 3.59^{d}	5.20 ± 0.37^{a}

Values are presented as mean ± S.E.M. Means not sharing common letter(s) are significantly different ($P < 0.05$).

(Fig. 5A). The obese control group on the other hand showed acute inflammation as such indicated acute lipotoxicity (Fig. 5B). The treated groups showed moderate and mild inflammation for 10% and 20% *A. cepa* treatment respectively (Fig. 5C and D).

4. Discussion

4.1. Anti-obesity compounds in the n-hexane extract of Allium cepa

The purpose of conducting GC-MS analysis on the crude n-hexane extract was to identify some compounds particularly non polar compounds in the *A. cepa* leaves that might contribute to the anti-obesity effect of the plant. The n-hexane extract of the *A. cepa* leaf had 32 active compounds, of which Squalene (23%) was the dominant compound. Some of these compounds were already proven by previous studies to have anti-obesity properties (Ibrahim et al., 2020). For example, phytol which is present in the hexane extract is a chemically and biologically active compound and it possesses anti-obesity properties (Hiroko et al., 2020). Long term intake of phytol has beneficial effects on insulin resistance, obesity and diabetes and for improvement in lipid metabolism (Hiroko et al., 2018). Squalene was found in the n-hexane extract of *A.*

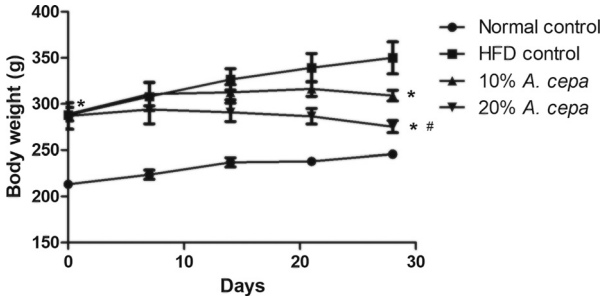

Fig. 2. Body weight of experimental rats post treatment against number of days. Values are presented as mean ± S.E.M. Means were significantly different ($P < 0.05$); (*) significantly different ($P < 0.05$) from normal control; (#) significantly different ($P < 0.05$) from HFD control.

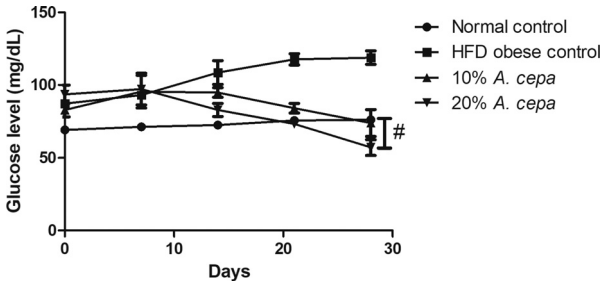

Fig. 3. Effect of treatment with *A. cepa* on the blood glucose level of experimental rats. Values are presented as mean ± S.E.M. (#) indicated means significantly different ($P < 0.05$) from HFD control.

cepa. It inhibits cholesterol biosynthesis and has been proven to possess anti-obesity properties (Ibrahim et al., 2020).

The major components of ethanol leaf extract of *A. cepa* were hexadecanoic acid, octadecenoic acid and ethyl oleate. Ethyl oleate has been reported to have antiobesity properties in rats via reduction in body weight and food intake (Kemp et al., 2008). Also, oleic acid consumed in meals reduced abdominal fat and central obesity in humans (Tutunchi et al., 2020). Hexadecanoic acid has been reported to have antioxidant activities (Sheela and Uthayakumari, 2013).

Some organic compounds which are characterized as essential oils have also been proven to possess antioxidant and anti-obesity properties (Giosue et al., 2016). Another class of compounds responsible for the anti-obesity effect of the *A. cepa* leaves are sulphur containing compounds (Mariangela et al., 2018). These compounds were present in the *A. cepa* leaves thus may account for the weight loss recorded in the rats fed with 10% and 20% *A. cepa* leaves. In this study acetic acid, which has been reported by Yamashita et al. to have anti-obesity effects in obesity-linked type 2 diabetic rats, might be also responsible for the anti-obesity effect of the *A. cepa* leaves (Yamashita et al., 2015).

Furthermore, tocopherol, otherwise known as Vitamin E which is also present in the *A. cepa* leaves as revealed by the GC-MS analysis, has been proven to possess anti-oxidative, anti-inflammatory, anti-obesity and anti-hyperglycemic properties (Wong et al., 2017). The tocopherol content of the *A. cepa* might have contributed to the weight loss of the treated rats over the treatment period. Also revealed through the GC-MS analysis is the presence of heptacosane which has been proven to possess anti-obesity properties in high fat diet obesity induced Wistar rats (Zoy et al., 2019). Heptacosane might have contributed to the weight loss recorded in the rats treated with *A. cepa* (Zoy et al., 2019).

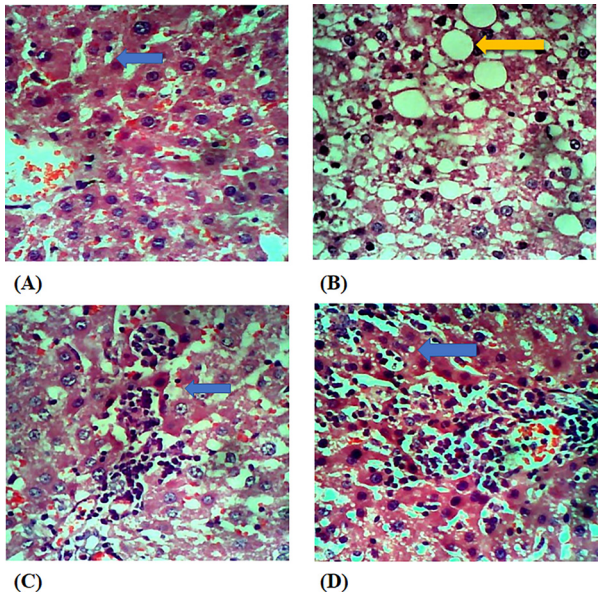

Fig. 4. Histopathology of the liver of experimental rats. A: Normal control – small and few amounts of fat deposits. × 40 [Blue arrow points to small fat deposits], **B:** Obese control – numerous and large deposits of fat deposits. × 40 [Yellow arrow points to large fat deposits], **C:** HFD + 10% of *Allium cepa* – small and numerous amounts of fat deposits. × 40 [Blue arrow points to small fat deposits], **D:** HFD + 20% of *Allium cepa* – small and numerous amounts of fat deposits. × 40 [Blue arrow points to small fat deposits].

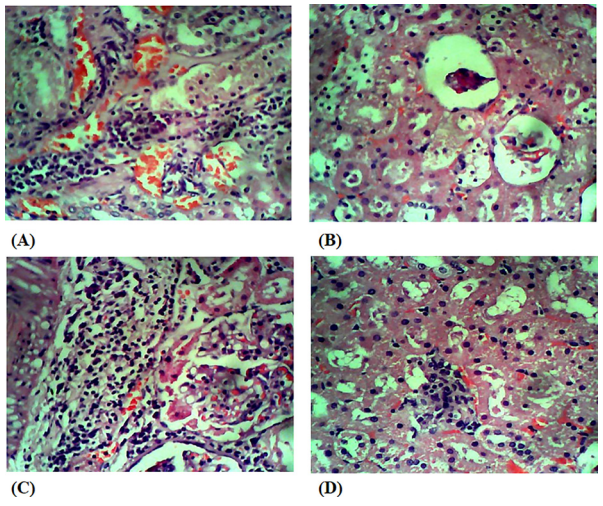

Fig. 5. Histopathology of the kidney of experimental rats. A: Normal control – mild inflammation of the kidney × 40, **B:** Obese control – acute inflammation of the kidney. × 40, **C:** HFD + 10% of *Allium cepa* – moderate inflammation of the kidney. × 40, **D:** HFD + 20% of *Allium cep a* – mild inflammation of the kidney. × 40.

4.2. The total phenolic content and antioxidant activity of the ethanol and n-hexane extract of Allium cepa

The ethanol and n-hexane extract of the *A. cepa* sample clearly reveals the presence of phenolic compounds in considerable proportion in the *A. cepa* sample. The ethanol and n-hexane extract of the *A. cepa* sample contained 161.0 ± 22.5 mg GAE/g and 32.3 ± 0.6 mg GAE/g, respectively. Phenolic compounds are proven by various studies to have anti-obesity properties and as such facilitate weight loss (Anyanwu et al., 2020). For instance, curcumin and epigallocatechin gallate have been shown to cause weight loss in rats fed with high fat diet

(Kao et al., 2000). Curcuminoids is also proven to prevent lipid accumulation in rats fed with high fat diet (Asai et al., 2001). Another phenolic compound proven to inhibit the pancreatic lipase is the licochalcone (Won et al., 2007). Soy isoflavone is another phenolic compound which has been proven to inhibit lipid accumulation in mice fed with high fat diet (Ahn et al., 2008). The high levels of antioxidant in the leaves of *A. cepa* may be useful against the progression of obesity and prevention cell or organ damage (Škrovánková et al., 2012).

4.3. *The effect of* Allium cepa *on body weight and feed intake of experimental rats*

Obesity has been described as significant increase in Body mass index (BMI) (Novelli et al., 2007) and/or body weight (Amin and Nagy, 2009) in laboratory animals. Within the four-week-period treatment with the *A. cepa* supplemented feed, there was a decrease in body weight of the test subjects using the obese control and normal controls as references. The group which was treated with a higher dosage (20% of *A. cepa*) of the plant sample was found to have reduced in body weight more than the group which was treated with just 10% of *A. cepa* sample. This suggests that *A. cepa* aids in body weight reduction and is dosage-dependent. This is in line with the studies of Yang et al. (Yang et al., 2018) who discovered that bulb oil decreased body weight and white adipose tissue weight in rats fed with high fat diet.

Consequently, there was an increase in feed intake for the group which was treated with 10% of *A. cepa* sample. The feed intake in the group treated with 10% of *A. cepa* was obviously higher than the group treated with 20% *A. cepa*. This implies that at a lower dosage, the plant possesses the ability to stimulate appetite but possibly suppress it at a higher dosage through certain biochemical mechanism such as the stimulation of satiety hormone such as leptin. The observed decrease in feed intake would be accompanied with lower exposure to calorie intake which would have affected the body weight of the group treated with 20% of *A. cepa*.

Although the group treated with 10% of *A. cepa* showed decrease in the total fat mass, the group treated with 20% of *A. cepa* showed a more significant reduction ($P < 0.05$) in fat mass in comparison with the obese control. *A. cepa* have been found to contain saponins and flavonoids which in turn possess pancreatic lipase inhibitory effect (Mariangela et al., 2016). Thus, it prevents lipase from hydrolyzing ingested dietary fat into fatty acids and monoglycerides leading to decreased fat absorption and consequently decreased caloric absorption and body weight.

4.4. *The effect of* Allium cepa *on lipid profile, ALT, AST and blood glucose*

The levels of triglyceride, total cholesterol, VLDL cholesterol and LDL cholesterol in the obese rats were significantly higher ($P < 0.05$) than those in the normal control. Also, the levels of triglyceride, total cholesterol, VLDL cholesterol, and LDL cholesterol in the rats treated with *A. cepa* decreased in comparison with the obese control. Aqueous extract of *A. cepa* was reported to decrease the levels of cholesterol, LDL cholesterol and HDL cholesterol without any significant effect on triglyceride level in rats fed with high fat diet (Jamshid et al., 2012).

This implies that the reduction in the body weight of the rats treated with 10% and 20% *A. cepa* is as a result of the decrease in levels of triglyceride, total cholesterol, VLDL cholesterol, and LDL cholesterol and also through some certain biochemical mechanisms and pathways or due to the effect of several compounds proven to possess anti-obesity properties such as squalene which inhibits cholesterol biosynthesis via inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the liver (Ibrahim et al., 2020).

ALT and AST enzymes are reliable markers for hepatotoxicity evaluation (Hayes, 1989). The elevation of ALT and AST activities have been associated with hepatic fat deposition linked obesity (Wallace et al., 2007). The group which ingested 20% of *A. cepa* decreased ALT and AST activities thereby enhancing the metabolism of fat, it also implies that an added dosage of the plant shows significant decrease ($P < 0.05$) on ALT and AST activities. Also, a reduction in fat deposition was revealed in the histopathology test carried out on the liver of the rats treated with 20% of *A. cepa* leaves. The blood glucose level of the treated rats was shown to have reduced over the treatment period. This implies that glucose metabolism was enhanced thus explaining the increase in triglyceride and other lipid profiles. This suggests that *A. cepa* enhances glucose tolerance and its metabolism to fatty acids.

4.5. *The effect of* Allium cepa *on the kidney function status and on the histopathology of the kidney*

Other laboratory studies have proven that creatinine, urea and uric acid levels are biochemical markers for the kidney function status (Gowda et al., 2010). Increased concentration of creatinine and urea beyond normal levels could portend possible risk of a renal failure. Clearly the creatinine and urea level in the obese control group increased considerably in comparison with the normal control group, thus suggesting possible osmotic diuresis and a reduced glomerular filtration in an obese condition. Therefore, deposition of the biochemical markers, that is creatinine and urea, into the serum could present renal failure if aggravated (Musso et al., 2005). On the other hand, the creatinine and urea level in the test group decreased in comparison with the obese control group, thus suggesting increased glomerular filtration and adequate reabsorption of the biochemical markers by the kidney glomerulus for further biodegradation.

The histopathology of the kidney in the obese control group reveals acute inflammation in comparison with the normal control. This suggest possible lipotoxicity, a condition characterized with the storage of non-esterified fatty acids and their products such as ceramides and diacylglycerol in organs such as kidney thus inducing chronic inflammation and this outcome is in consonance (Ayse-Basak, 2017). Consequently, for the groups treated with 10% and 20% of *A. cepa* there were moderate and mild inflammation respectively which do not pose any level of toxicity to the organ. Although some studies reveal that at an extremely high level of *A. cepa* may be toxic to organs such as kidney and to the entire system with symptoms such as heartburn, abdominal bloating, bad breath and hypoglycemia.

4.6. *The effect of* Allium cepa *on the histopathology of the liver*

The histopathology of the obese control group clearly reveals numerous and large deposits of fat in comparison with the normal control group which only had small and few amount of fats. This clearly reveals that the high fat diet in the obese control led to the deposition of excess triglycerides in the adipose tissue and in the liver. On the other hand, the rats treated with 10% and 20% of *A. cepa* only had small and numerous amounts of fat deposits, thus indicating the reversal of obesity in the treated groups. The reversal of obesity in the treated groups, a lack of deposition of numerous and large deposits of fat in the liver is due to many reasons, amongst which the presence of various phytochemical compounds in the *A. cepa* sample may be responsible, such as flavonoids, polyphenols, terpenes, amines and different organic compounds as revealed by the GC-MS analysis.

5. Conclusion

In conclusion, *A. cepa* leaves has weight loss potential as revealed by decreased body weight, glucose level, fat mass, fat deposits in the liver and improved lipid profile in animals. Though the presence of compounds with anti-obesity properties in *A. cepa* levels suggests the reason for weight loss; however, the mechanism through which weight loss is achieved is a subject for further studies.

Ethical Approval

The Ethics Committee of Research in Bingham University had approved the study protocol and assigned the ethical (Approval Number: BHU-02/DE/M-3–3.18).

Data Availability

Nil.

Funding

Nil.

Declaration of Competing Interest

All authors have no conflict of interest to declare.

CRediT authorship contribution statement

Dr. Gabriel Anyanwu conceptualized and supervised this study with assistance from Mr. Shekins Okere who served as co-supervisor. The laboratory experiments were performed by Mr. Babafemi Momoh and Dr. Gabriel Anyanwu, likewise data analysis and manuscript writing, while all authors reviewed and edited the manuscript. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Acknowledgement

Nil.

ORCID

Gabriel O. Anyanwu, http://orcid.org/0000-0003-3110-2627.

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccmp.2022.100035.

References

Aledani, A.H.E., Khudhair, N.E., Alrafas, H.R., 2020. Effect of different methods of anesthesia on physiobiochemical parameters in laboratory male rats. Basrah Journal of Veterinary Research 19 (1), 206–214.

Ali, E., 2013. Pharmacological effects of Allium species grown in Iraq. An overview. International Journal of Pharmaceuticals and Health Care Research 1 (4), 132–147.

Amarowicz, R, Estrella, I, Hernández, T, Troszyńska, A., 2008. Antioxidant activity of extract of adzuki bean and its fractions. Journal of Food Lipids 15, 119–136.

Anyanwu, G.O., Andreas, F.K., Giovanna, B., 2020. Anti-obesity functional leads and targets for drug development. Phytochemicals as Lead Compounds for New Drug Discovery. Science Direct Journal 143–160.

Ahn, J., Lee, H., Kim, S., Park, J., Taeyou, H., 2008. The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochemical and Biophysical Research communications 373 (4), 545–549.

Amin, K.A, Nagy, M.A., 2009. Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. Diabetology and Metabolic Syndrome Journal 1, 17–24.

Asai, A., Miyazawa, T., 2001. Dietary curcuminoids prevent high-fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. Journal of Nutrition 131 (11), 2932–2935.

Ayse-Basak, E., 2017. What is Lipotoxicity? Journal of Advances in Experimental Medicine and Biology 960, 197–220.

Barham, D., Trinder, P., 1972. Measuring Uric Acid level chemically. Analyst Journal 97, 142–145.

Batton, C.J., Crouch, S.R., 1977. Urea can be chemically measured. Annals of Clinical Chemistry 49, 464–469.

Chavan, V.R., Muley, D.V., 2014. Effect of heavy metals on liver and gill of fish *Cirrhinus mrigala*. International Journal of Current Microbiology and Applied Sciences 3 (5), 277–288.

Ferreira, I.C.F.R, Baptista, P., Vilas-Boas, M., Barros, L., 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chemistry. Science Direct Journal 100 (4), 1511–1516.

Friedewald, W.T, Levy, R.I, Fredrickson, D.S., 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without the use of preparative ultra centrifuge. Journal of Clinical Chemistry 18, 499–502.

Giosue, C., Maria, C.G., Daniel, V., Claudiu, T.S., Stefano, A., 2016. Active components of essential oils as anti-obesity potential drugs investigated by in silico techniques. Journal of Agriculture and Food Chemistry 64 (26), 5295–5300.

Gowda, S., Prakash, B.D., Shruthi, S.K., Vinayak, V.H., Avinash, A.K.M., Sonal, N.V., 2010. Markers of renal function tests. North American Journal of Medical Sciences 2 (4), 170–173.

Hayes, M.L., 1989. Guidelines for acute oral toxicity testing in animals: Principles and methods of toxicology. Raven Press, Ltd, N.Y., p. 248.

Heseker and Schmid, 2000. doi:10.1024/0040-5930.57.8.478, https://europepmc.org/article/med/11026082.

Hiroko et al., 2018. doi:10.1128/genomeA.00342-18.

Hiroko, M., Daisuke, S., Masaya, A., Shinpei, O., Ritsuki, S., Ryo, O., Takaaki, S., 2020. Effects of dietary phytol on glucose uptake and insulin secretion in vitro and in vivo. Food and Nutrition Current Research Journal 1 (1), 1–9.

Hoff, J., Rlatg, L., 2000. Methods of blood collection in the mouse. J. Lab. Anim. 29, 45–47.

Ibrahim, N.I., Fairus, S., Zulfarina, M.S., Naina Mohamed, I., 2020. The efficacy of squalene in cardiovascular disease risk-a systematic review. Nutrients 12 (2), 414.

Jamshid, G.G., Ahmad, A., Ramin, S., Doust, N., Abdolreza, Y., 2012. Hypolipidemic effects of aqueous extract of onion (*Allium cepa L.*) on serum level of cholesterol, triglyceride, LDL and HDL compared with Zn sulfate supplementation in the rats. European Journal of Experimental Biology 2 (5), 1745–1749.

Kao, Y.H., Hiipakka, R.A., Liao, S., 2000. Modulation of obesity by green tea catechin. The American Journal of Clinical Nutrition 72 (5), 1232–1233.

Kemp, C.J., D'Alessio, D.A., Scott, R.O., Kelm, G.R., Meller, S.T., Barrera, J.G., Seeley, R.J., Clegg, D.J., Benoit, S.C., 2008. Voluntary consumption of ethyl oleate reduces food intake and body weight in rats. Physiol Behav 93 (4-5), 912–918.

Korner, J., Aronne, L.J., 2003. The emerging science of body weight regulation and its impact on obesity treatment. The Journal of Clinical Investigation 131 (11), 565–570.

Kuete, V., 2017. Allium cepa. In: Medicinal Spices and Vegetables from Africa. Therapeutic potentials against Metabolic, Inflammatory. Infectious and Systemic Diseases, pp. 353–361. doi:10.1016/8978-0-12-809286-6.00014-5.

Lokuruka, M.N.I., 2013. A Literature review of role of obesity in Adult Health with reference to Africa. African Journal of Food Agriculture, Nutrition and Development 13 (1), 1–17.

Mariangela, M., Filomena, C., Giancarlo, S., 2016. Effects of saponins on lipid metabolism: A review of potential health benefits in the treatment of obesity. Journal of Molecules 21 (10), 1404.

Mariangela, M., Valentina, A., Giancarlo, S., Filomena, C., 2018. Biological Properties and Bioactive Components of *Allium cepa L*: Focus on potential benefits in the treatment of obesity and related comorbidities. Journal of Molecules 24 (1), 119.

Meldrum, D.R., Morris, M.A., Gambone, J.C., 2017. Obesity Pandemic: Causes, Consequences And Solutions. Fertility and Sterility. Science Direct Journal 107 (4), 833–839.

Musso, C.G., Macias-Nunez, J.F., 2005. Renal physiology in the oldest old: the Sphinx remakes her question. International Journal of Urology and Nephrology 37 (3), 653–654.

Novelli, E.L.B., Diniz, Y.S., Galhardi, C.M., Ebaid, G.M.X., Rodrigues, H.G., Mani, F., ernandes, A.A.H., Cicogna, A.C., Novelli, J.L.V.B., 2007. Anthropometrical parameters and markers of obesity in rats. Journal of Laboratory Animals 41 (1), 111–119.

Okada, M., Matsui, H., Ito, Y., Fujiwara, A., Inano, K., 1996. Low density lipoprotein can be chemically measured. Journal of Laboratory and Clinical Medicine 132 (3), 195–201.

Peruzzi, L., Carta, A., Altinordu, F., 2017. Chromosome Diversity and Evolution In *Allium* (Allioideae, Amaryllidaceae). Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 151 (2), 212–220.

Reitman, S., Frankel, S., 1957. Measuring Alanine amino transferase chemically. American American Journal of Clinical Pathology 28 (1), 56–63.

Rodgers, R.J., Tschöp, M.H, Wilding, J.P., 2012. Anti-obesity drugs: Past, Present and Future. Disease Models and Mechanism Journal 5 (5), 621–626.

Sheela, D., Uthayakumari, F., 2013. GC-MS analysis of bioactive constituents from coastal sand Dune taxon-Sesuvium portulacastrum. Bioscience Discov 4, 47–53.

Škrovánková, S., Mišurcová, L., Machů, L., 2012. In: Chapter Three - Antioxidant Activity and Protecting Health Effects of Common Medicinal Plants, Editor(s): Jeyakumar Henry, Advances in Food and Nutrition Research, 67. Academic Press, pp. 75–139.

Srivastava, G., Apovian, C., 2018. Future Pharmacotherapy for Obesity: New Anti-obesity Drugs On the Horizon. Current Obesity Report Journal 7, 147–161.

Tietz, N.W., 1990. Clinical Guide to Laboratory Tests. W. B. Saunders Company, Philadephia, USA, pp. 554–556.

Tutunchi, H., Ostadrahimi, A., Saghafi-Asl, M., 2020. The Effects of Diets Enriched in Monounsaturated Oleic Acid on the Management and Prevention of Obesity: a Systematic Review of Human Intervention Studies. Adv Nutr 11 (4), 864–877.

Wallace, T.M., Utzschneider, K.M., Tong, J., Carr, D.B., Zraika, S., Bankson, D.D., Knopp, R.H., Kahn, S.E., 2007. Relationship of liver enzymes to insulin sensitivity and intra-abdominal fat. Diabetes Care Journal 30 (10), 2673–2678.

WHO, 2015. Obesity and Overweight. World Health Organization, Geneva 2015.

Wong, S.K., Chin, K.Y., Suhaimi, F.H., Ahmad, F., Ima-Nirwana, S., 2017. Vitamin E as a Potential Interventional Treatment for Metabolic Syndrome: Evidence from Animal and Human Studies. Frontiers in Pharmacology Journal 8 (444), 1–12.

Won, S.K., Kim, Y.M., Kim, P.H., Lee, J.H., Ryu, J.W., Kim, A., 2007. Licochalcone: lipase inhibitor from the roots of Glycyrrhiza uralensis. Food Research International Journal 40 (8), 1046–1050.

Xu, B.J., Chang, S.K.C., 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Science 72, S159–S166.

Yamashita, H., 2015. Biological function of acetic acid-improvement in obesity and glucose tolerance by acetic acid in type 2 diabetic rats. Critical Reviews in Food Science and Nutrition Journals 56 (1), 171–175.

Yang, C., Li, L., Yang, L., Lu, H., Wang, S., Sun, G., 2018. Anti-obesity and hypolipidemic effects of garlic oil and onion oil in rats fed with high fat diet. Nutrition and Metabolism Journal 15 (43), 1–8.

Zoy, I.N, Dildar, A., Hafiz, M.R., Muhammad, T.Q., Matheus, F., Sarfraz, A., Muhammad, U.M., 2019. *In vitro* Antidiabetic, anti-obesity and anti-oxidant analysis of *Ocimum basilicum* aerial biomass and *in silico* molecular docking simulations with alpha-amylase and lipase enzymes. Journal of Biology 8 (4), 92.