ijlpr 2023; doi 10.22376/ijlpr.2023.13.5.L165-L175

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Chemistry for New drug discovery

Anti-Obesity Effect of Ethanolic Extract of *Citrus Paradisi and* Naringin in HFD-Induced Animal Model

Dr.R. Roghini [1*]

[1]*Research Associate, Central Research Lab, ACS Medical College and Hospital Poonamallee High Road, Velappanchavadi, Chennai- 600077, India*

Abstract: The study aimed to assess the effectiveness of *Citrus paradisi*, and Naringin on high fat diet rats.It must be clarified that the correlation between body fat and dietary fat levels, long-term energy intake and expenditure imbalances causes Obesity. Animal models have made a significant historical contribution to our understanding of the underlying variables that control the elements of human energy balance. studies have shown that genetics considerably affect how the body reacts to meals high in fat-derived energy. Five sets of six male Wistar rats (120–150g); each group had five animals. Group I (Control), Group II (High Fat Diet), Group III (HFD+Naringin (100mg/kg body weight)), Group IV (HFD + *Citrus paradis*e (500mg/kg body weight), and Group V (HFD +21.67mg/kg body weight) are the study groups (drug control). Physical factors such as body weight, organ weight, fat pad weight, and anthropometric parameters were assessed after introducing a high-fat diet. In addition, serum lipid profiles, glucose, insulin, leptin, and adiponectin levels, among other biochemical indicators, were analyzed. These outcomes would empower *Citrus Paradise* and Naringin to be utilized as anti-obesity agents. Haemotoxylin and eosin staining was used to detect histopathological alterations in adipose tissue. The effect of *Citrus paradisi* and Naringin treated with HFD-induced animals showed significantly higher ($p<0.001^{*}$). It was observed by its action in reducing organ weight, blood weight, and serum lipids. This current study evaluated the evidence for *Citrus* Paradise's effects on body weight, blood pressure, and lipid profile.

Keywords: Body weight, High Fat Diet, *Citrus paradisi*, organ weight, Histopathology, Obesity

***Corresponding Author**

Dr.R. Roghini , Research Associate, Central Research Lab, ACS Medical College and Hospital Poonamallee High Road, Velappanchavadi, Chennai- 600077, India

Received On 19 December, 2022

Revised On 3 April, 2023

Accepted On 17 April, 2023

Published On 1 September, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Dr.R. Roghini , Anti-Obesity Effect of Ethanolic Extract of Citrus Paradisi and Naringin in HFD-Induced Animal Model.(2023).Int. J. Life Sci. Pharma Res.13(5), L165-L175 http://dx.doi.org/10.22376/ijlpr.2023.13.5.L165-L175

1. INTRODUCTION

Obesity induces metabolic disorders like Heart disease and diabetes, distinguished by hyperlipidemia and hyperglycemia. Being overweight is defined as a body mass index (BMI) of 25, and Obesity is a BMI greater than 30. According to the global burden of illness, the problem has reached epidemic proportions, with over 4 million people dying annually from being overweight or obese in 2017. In children and adolescents aged 5 to 19, the prevalence of overweight or Obesity grew more than four times, from 4% to 18% worldwide. Children who are overweight or obese make up the majority of the population in developing nations, where the pace of increase has been more than 30% higher than in industrialized nations. Endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon cancers are all linked to Obesity. Even if a person is only somewhat overweight, their risk of developing one of these non-communicable illnesses rises, becoming more serious as their body mass index (BMI) rises. Childhood obesity increases the chance of related disorders developing prematurely and is linked to many major health issues. According to studies, children and teenagers who are obese will probably stay that way throughout adulthood if no action is taken. Many factors contributing to becoming overweight or obese can be avoided or reversed. However, no nation has yet managed to stop this epidemic's spread. Although additional contributing factors exist, an imbalance between calories consumed and expended is the primary cause of Obesity. Energy-dense meals high in fat and free sugars have become more popular as global diets have evolved in recent decades. A decrease in physical activity has also occurred due to growing urbanization, easier access to transportation, and the evolution of different job types. Reduced intake of calories from fats and carbohydrates, increased diet of fruit, vegetables, legumes, whole grains, nuts, and regular physical activity all help lower the risk of being overweight and obese. The majority of bodily systems are affected by Obesity. It impacts the reproductive system, joints, liver, kidneys, and heart. Numerous non-communicable diseases (NCDs), including type 2 diabetes, cardiovascular disease, hypertension, stroke, different types of cancer, and mental health problems, are caused by it. The prevalence of overweight and Obesity in both adults and children is rising. Obesity and its related metabolic difficulties now prevail among the world's population. Various methods of experiments have been introduced to examine these pathologies and to find a remedy for this metabolic disorder Marques, C [1]. This work is extended to HFD-induced animal models *in vivo* to investigate the anti-obesity efficiency of ethanolic extract of *Citrus paradisi* and Naringin to examine biochemical and physiological parameters. In the evaluating stage, the disease process plays an important role in diagnosing and treating the animal models as a unique experimental methodology Williams L [2]**.** The fundamental criteria in the energy balance are largely increased due to dietary fat, which contributes to more than 30% of total energy and plays a significant role in causing Obesity, as demonstrated in several papers on dietary models Hariri [3]. The reason for choosing the Wistar albino rats for *in vivo* studies, it shows similar weight gain and behavior, identical to human beings when fed with HFD Novelli E. L. B [4]**.**

2. MATERIALS AND METHODS

2.1. Plant material collection

The raw Grapefruit fruit was collected from the supermarket and was authenticated by Dr. P. Jayaraman, director of the National Institute of Herbal Science's Plant Anatomy Research Centre, Tambaram.

2.2. Plant extract preparation

The citrus paradise fruit's skin was peeled off, and the seeds were removed. Next, the fruit's pulp was extracted, diced up, dried, and ground into a powder. Later, using a Soxhlet device, the dried powder was extracted with water, ethanol, and ethyl acetate. Additionally, the extract was kept chilled in a glass bottle during the experiment.

2.3. Diet preparation

Pellet was bought from Kattupakkam, Chennai. The High Fat Diet was prepared by adding 30 percent of lard and 5 percent of sunflower oil to normal powder of pellets as mentioned by Kim *et al*.,[5] with few alterations**.** To stop Deterioration, HFD was prepared daily.

Table 1: Composition of Normal Diet

Normal Diet Composition	gm(%)	The energy acquired in Kilocalorie per kg of a normal diet
Fibre	50	0
Corn starch	253	1012
Sucrose	50	200
Cellulose	247	0
Mineral mix	35	0
L-cystine	2.5	0
Choline	2.5	0
Dextrinized starch	100	400
Oil	50	400
Vitamin mix	10	0
Casein	200	800
Total	**1000**	**2812**

Table 1 explains the composition of the Normal diet. In one kg of a normal diet, fat provides 14.22 percent of Kilo calories of energy, protein provides 28.44 percent of Kilo calories of energy, and carbohydrate provides 57.32 percent of Kilo calories of energy. The total Proportion of the pellet was 65 percent of Carbohydrates, 20 percent of crude protein, 5 percent of fiber, 5 percent of vitamins and minerals, and 5 percent of fat.

Table 2 : Composition of HFD		
HFD composition	**gm (%)**	**The energy acquired in Kilocalorie per kg of HFD**
Lard	245	2205
Casein	200	800
Soyabean oil	25	225
Sucrose	125	500
Maltodextrin	68	275
Vitamin Mix	10	40
L-Cysteine	3	12
Total	**676**	**4057**

Table 2 explains the composition of HFD. In one kg of High Fat Diet, fat supplies 55.11 percent of Kilo calories of energy, protein supplies 15.74 percent of Kilo calories of energy, and carbohydrates supply 29.1 percent Kilo calories of energy. HFD was 30 percent of crude protein and 35 percent of fat, including 30 percent of lard and 5 percent of sunflower oil, 5 percent of vitamins and minerals, and 40 percent of carbohydrates.

2.4. Animal model

In this experiment, 30 male Wistar albino rats of 120-150 grams of weight and ages of about six to eight weeks were used. These rats were bought and kept at Sathyabama University, Chennai. The current study was carried out by the regulations of the Institutional Animal Ethical Committee (IAEC) and (CPCSEA) approved by the committee with the IAEC approval number (IAEC NO: SU/CLATR/IAEC/X/083/2018) used for this study. The animals were fed a standard commercially available diet and water at the libitum.

2.5. Grouping of Animals Treatment Protocol

Group-I- (n=6, Control rats) Normal or regular diet for 8 weeks.
Group-II- (n=6, Obesity-induced rats) HFD for 8 weeks.
Group-III- (n=6, HFD fed +Naringin (100mg/kg body weight) simultaneously for 8 weeks.
Group-IV-(n=6, HFD fed + *Citrus paradis*e (500mg/kg body weight) simultaneously for 8 weeks.
Group-V-(n=6, HFD fed + Orlistat (21.67mg/kg body weight) simultaneously for 8 weeks.

2.6. Collection of Sample

After eight weeks, the animals were sacrificed (Fasted overnight). Animals were treated with anesthesia and the blood collection was done by cardiac puncture using heparinized capillary tubes. The serum was separated by centrifuging the blood samples at 3000 rpm for 10 minutes. This serum was used for biochemical assessment.

2.7. Assessment of fat pad and organ weight

Fat pads like perirenal, epididymal, and mesenteric fat and internal organs such as the heart, liver, kidney, spleen, and pancreas were used, cleaned with saline, and spotted using filter paper.

2.8. Body weight measurement

From the first week, the body weight of all the animals was noted for the next eight weeks. Then, using digital balance, the weight of the animals was taken every week.

2.9. Measurement of Anthropometrical parameters

On the day of sacrifice, anesthetized rats were taken to measure the thoracic and abdominal circumference. The measurement was done using anthropometrical parameters as described by Novelli *et al.* 2009 [6]

3. BIOCHEMICAL ASSESSMENT

3.1. Estimation of Blood glucose

The procedure described by Sasaki, T. (1972) 7 was used for the estimation of blood glucose.

3.2. Reagents required

Standard solution (10µg/ml), TCA (10%), and O-toluidine reagent.

3.3. Preparation of Standard solution

10 mg of glucose was added to 100 ml of distilled water with 0.2% benzoic acid.

3.4. Preparation of O-toluidine reagent

In 2000 ml of the volumetric flask, 90 ml of O-toluidine was taken with 5 grams of thiourea, and add added glacial acetic acid to make it up to one liter. This reagent was preserved in the refrigerator in a brown bottle.

3.5. Trichloroacetic acid (10%) Procedure

1.9 ml of TCA solution and 0.1 ml of blood were to be added to a test tube to form the precipitate of the proteins by centrifugation process. Then, I added four ml of O-toluidine reagent, added one ml of supernatant, and kept the mixture for fifteen minutes in boiling water. Simultaneously the standard solution was prepared and estimated it. Finally, the developed color intensity was read at 600nm, and results were expressed in mg/dl.

3.6. Determination of Adiponectin and Leptin

Estimation of Adiponectin and Leptin was done by using ELISA kit[8,9]. The test was done using the procedures given by the manufacturer, and values were represented in ng/ml.

3.7. Determination of Insulin

The serum insulin was estimated using the ELISA kit manufactured in Mercodia, Sweden. The test was done as per

the instructions given by the manufacturer, and the results were represented in µU/ml.

3.8. Estimation of Serum Total cholesterol

In the procedure mentioned by Zlatkis, A [10] was used to estimate Total cholesterol.

3.9. Reagents required

Con.H_2SO_4, Serum, 0.05% Ferric chloride – glacial acetic acid solution, normal cholesterol (In 100 ml of ferric chloride acetic acid solution, add four mg of cholesterol to it).

3.10. Procedure

About 9.9ml of ferric chloride–the glacial acetic acid solution was taken and added 0.1ml of serum was placed in a glass stopper centrifuge tube; centrifugation was done to mix the contents properly and incubate it for 30 minutes. Next, five ml of protein-free filtrate was added to three ml of concentrated sulphuric acid, and the tubes were rotated to facilitate proper mixing. Simultaneously standards were prepared, and the test tubes were allowed to hold for twenty minutes. The developed pink color was measured at 540nm, and the results of serum were represented in mg/dl.

3.11. Estimation of Triglycerides

As mentioned by Bucolo, G [11], the procedure was used to estimate Triglycerides.

3.12. Reagent required

Serum, Working reagent and Standard triglycerides(20mg/dl).

3.13. Working reagent composition

Glycerol-3-Phosphate oxidase(4U/ml), glycerol kinase(1.5U/L), Peroxidase(0.8U/ml), ATP (0.9mmol/l, pH-7), 4-Chlorophenol (6mmol/l),4-aminoantipyrine(0.75mmol/l), Magnesium chloride (5 mmol/l).

3.14. Procedure

Three test tubes were taken and marked as B, S, and T, and one ml of the working reagent was added to each test. 10µl of distilled water was added to tube B, 10µl of the standard was added to tube S and 10µl of serum was added to tube C. All the test tubes were incubated for ten minutes at room temperature, and absorbance was measured against the blank at 520 nm.

3.15. Determination of C-reactive Proteins

The procedure, as mentioned by Ledue, T. B [12], was used to estimate C-Reactive Proteins(CRP). Concentrations of CRP were evaluated using entirely frozen serum samples and estimated using the ELISA kit, which was manufactured in the USA. The test was done as per the instructions given by the manufacturer, and the results were represented in µg/mL.

3.16. Estimation of NEFA

As mentioned by Matthews, D. R [13], the procedure was used, and the estimation of NEFA was done using a ROCHE Modular P800 Automatic Biochemical Analyzer (Germany). The test was done as per the instructions given by the manufacturer, and the results were represented in mg/dl.

3.17. Study of Histopathology

The procedure mentioned by Sarawoot Palipoch., & Chochard Punsawad. (2013)[14] was used for the Study of Histopathology. A small portion of the organs like the pancreas, liver, Heart, Kidney, Spleen, and adipose tissue was used to study histopathology. About, 10 percent buffered formalin(pH-7.2) was used to fix the tissues; dehydration was done using a sequence of ethanol, and the tissues were cleaned and placed in paraffin wax. Then, the tissues were cut into pieces(4µm) and stained using a microtome. Finally, the pieces were placed in DPX and viewed under a light microscope.

4. STATISTICAL ANALYSIS

The values of six animals in each group were expressed as (Mean ± SD) one Way ANOVA was used to calculate the significant difference between control and treatment groups followed by the LSD test. Graphs were drawn by using a graph pad prism (version 6.0).

5. RESULTS AND DISCUSSION

Nowadays, the challenges that cause disorders among people are Obesity and being overweight. The cognitive and non-cognitive elements of Obesity exert an influence on the population. An individual's health changes variably due to the intake of a High Fat Diet (HFD) across the globe Stephen, C [15]. The consumption of HFD is an indicative feature due to the constituents of fatty substances. HFD induces some major changes in the body shape and weight of an individual linked with hyperphagia that is mainly due to the fat accumulation to a higher extent Coelho, D. F [16]. The effect of ethanolic extract of *citrus paradisi* and naringin on the experimental group was compared with High Fat Diet fed rats. The results are shown in Table 4.1. It was found that there was a gain in weight of group II animals (251.5 ± 1.80) which shows high significance in body weight compared to group I animals. Group IV animals showed high significance, whereas group III and V showed moderate significance in body weight, respectively, when compared with group II animals. Additionally, BMI and gain in body weight of normal diet-fed animals were correspondent to the report given by Haripriya, D., & Vijayalakshmi, K. (2014). [17]. There was a significant rise in abdominal circumference and a moderate increase in thoracic circumference of group II animals respectively on comparing with group1 animals. On the other hand, a moderate decrease in TC and AC values was noted in Group III, IV, and V animals compared to Group II. These results were related to the study of Shamsun Nahar [18], which demonstrates *moringa oleifera* leaf powder decrease TC and AC values more than HFD-fed groups.

Table 3: Effects of Ethanolic extract of *citrus paradisi* and Naringin on body weight anthropometric parameters in experimental animals

Experimental Groups	Initial weight (gm)	Final weight (gm)	Abdominal circumference (cm)	Thoracic circumference (cm)
Group - I	159.5 ± 1.8	176.3 ± 2.58	5.06 ± 0.02	12.21 ± 0.11
Group - II	157.5 ± 2.4a$^{\#}$	251.5 ± 1.80a*	7.16 ± 0.03a*	13.13 ± 0.03a$^{@}$
Group - III	154.9 ± 3.0b$^{\#}$	195.5 ±2.30b$^{@}$	6.06 ± 0.02b$^{@}$	12.12 ± 0.03b$^{@}$
Group - IV	154.8 ± 2.4b$^{@}$	174.5 ± 1.80b*	6.12 ± 0.04b$^{@}$	12.31 ± 0.15b$^{@}$
Group - V	156.3 ± 2.1b$^{@}$	210.5 ± 2.10b$^{@}$	6.09 ± 0.03b$^{@}$	12.58 ± 0.02b$^{@}$

All values are expressed as mean ± SD for each group that contains six animals. Statistical Significance p<0.001 *, p<0.01@, p<0.05\# a-Group II compared with Group I, b-Group III, IV, V were compared with Group II, Group I: Normal Diet (Control) Group II: High fat Diet (HFD) fed animals. Group III: Ethanolic extract of Citrus paradise (CP)(500mg/kg body weight) + HFD. Group IV: Naringin (100mg/kg body weight) + HFD. Group V: Orlistat - Standard drug (21.6 mg/kg body weight) + HFD

5.1. Effects of Ethanolic Extract of Citrus paradisi and Naringin on fat pad weight and organ weight

The organ's weight, such as Liver, Heart. kidney, spleen, and pancreas are given in Table 4.2. There was a noticeable increase in the organ's weight when group II animals were fed with HFD. It was relevant to the study of Mei-Yin Chien [19]. There was a significant rise in the weight of organs of group II animals compared to group I animals. Comparatively, the weight of the liver was higher then the other organs. There was a prominent decrease in the weight of the organs noticed in group III animals compared with group II animals. Even though the weight of the organs in Group IV and Group V was found to decrease, that was not significant. Sanaa R [20] reported that effect of Orlistat, herbal mixture, and HFD has significantly reduced the body weight of Orlistat.

5.2. Fat Pad

Fat pad distribution was studied in three regions: peri-renal, epididymal, and mesenteric. There was a significant rise in the fat pad weight in group II animals compared to group I. In addition, there was a reduction in the average weight in groups III, IV, and V, respectively, when compared with group II animals. Among these groups, group III animals show significant weight reduction. This investigation was related to the study by Woong Sun Jang and Se Young Choung, 2013, which demonstrate the reduction in fat pad weight by the ethanolic extract of *Laminaria japonica* Woong Sun Jan [21].

Table 4 Effects of the Ethanolic extract of *Citrus paradisi* and *Naringin* on a fat pad and organ weight in experimental animals.

Fat pads and organ weight(gm)	Group –I	Group –II	Group –III	Group –IV	Group –V
Liver	6.83 ± 0.06	7.17 ±0.02a$^{@}$	6.62 ±0.02b$^{@}$	7.68±0.03b^{NS}	6.98 ±0.41b$^{@}$
Heart	0.72 ±0.02	1.72 ±0.02a*	0.64 ±0.23b*	0.62 ±0.02b*	0.75 ±0.02b*
Kidney	1.31 ± 0.10	1.71 ±0.06a*	1.29 ±0.06b$^{@}$	1.44 ±0.03b$^{@}$	1.32 ±0.06b$^{@}$
Spleen	0.64 ± 0.03	0.83 ±0.04a*	0.55 ±0.03b*	0.75 ±0.30b$^{@}$	0.66 ±0.02b$^{@}$
Pancreas	0.25 ± 0.01	0.66 ±0.03a*	0.45±0.02b$^{@}$	0.55 ±0.26b$^{\#}$	0.47 ±0.03b$^{@}$
Epididymal fat	1.51 ± 0.97	3.58 ±0.16a*	2.12 ±0.05b*	2.56 ±0.77b$^{@}$	2.43 ±0.05b$^{@}$
Mesenteric fat	0.76 ± 0.01	3.29 ±0.15a*	1.39 ±0.07b*	1.65 ±0.66b$^{@}$	1.57 ±1.06b$^{@}$
Pre renal fat	0.46 ±0.02	0.83 ±0.05a*	0.59 ±0.04b*	0.69 ±0.04b$^{@}$	0.63 ± 0.03b$^{\#}$

All values are expressed as mean ± SD for each group that contains six animals. Statistical Significance p<0.001 *, p<0.01@, p<0.05\# a-Group II compared with Group I b-Group III, IV, V were compared with Group II. Group I: Normal Diet (Control) Group II: High fat Diet (HFD) fed animals. Group III: Ethanolic extract of Citrus paradise (CP)(500mg/kg body weight) + HFD.Group IV: Naringin (100mg/kg body weight) + HFDGroup V: Orlistat - Standard drug(21.6 mg/kg body weight) + HFD

5.3. Effects of Ethanolic Extract of citrus paradisi and Naringin on blood glucose in experimental animals

Table 4.3 shows the levels of blood glucose and insulin values. Group II animals fed with high-fat diet showed changes in the biochemical factors. As the animals' body weight increased, the insulin and glucose levels also significantly increased compared to group I animals. On the other hand, there was a decrease in glucose and insulin levels of group III, IV, and V animals when compared with group II animals. Group IV animals show significant reductions in glucose and insulin levels among these groups. The increase in blood glucose levels of animals fed with HFD was given in the study by Sai Sruthi Kaveripakkam [22], which shows hyperlipidemia.

5.4. Effects of Ethanolic Extract of Citrus paradisi and Naringin on C- reactive Protein NEFA and lipid profile on experimental animals

Table 4.3 shows total cholesterol levels, triglycerides, and C-reactive protein values. Group II animals fed a high-fat diet show changes in the biochemical parameters. As the animals' body weight increased, the total cholesterol, triglycerides, and C- reactive protein levels were also significantly increased compared with group I animals. On the other hand, there was a decrease in total cholesterol, triglycerides, and C- reactive protein levels of group III, IV, and V animals when compared with group II animals. Among these groups group IV, animals show a significant reduction in total cholesterol, triglycerides, and C- reactive protein levels. Sai Sruthi Kaveripakkam [22] reports that by reducing appetite, lipid synthesis, lipid absorption, and inducing lipid catabolism, the osteospermum

suaveolens roots reduce lipid levels in serum on the induced obese rats. In NEFA, there was a decrease in group II animals compared to group I animals. Comparing group III and IV V with group II animals showed a reduction in the NEFA levels.

5.5. Effects of Ethanolic Extract of Citrus paradisi and Naringin on Leptin and Adiponectin Levels

5.5.1. Leptin

There was a significant rise in leptin levels on comparing group II animals with the group I animals. Also, there was a reduction in leptin levels in Group III, IV, and V animals compared to Group II. Still, there was a significant reduction in group III animals among these groups. This study was correlated with the report by Uygun A Iram Nazish [23,24]

5.5.2. Adiponectin

There was a moderate decrease in adiponectin levels in group II animals compared with group I animals. Comparing Group III, IV, and V with Group II animals showed a non-significant change in Group III animals, a moderate rise in Group IV animals, and a significant reduction in Group V animals. Similarly, this was demonstrated by Ramgopal Mopuri [25], which shows the evidence of ethanolic extraction of *Terminalia Paniculata.*

Table 5 Effects of Ethanolic extract of *citrus paradisi* and *Naringin* on lipid profile and blood glucose on experimental animals

Parameters	Group - I	Group - II	Group – III	Group - IV	Group – V
Glucose	94 ± 1.0	159 ± 3.0 a*	131 ± 1.0 b**	113 ±1.0 b*	130 ±1.0 b**
Cholesterol	106 ± 1.0	150 ± 1.0 a*	127 ±1.0 b**	87 ±1.0 b*	99 ±1.0 b*
Triglycerides	110± 1.0	160 ±1.0 a*	142 ±1.0 b@	120 ±1.0 b*	135 ±1.0 b**
C-reactive proteins	0.20 ±0.1	0.70 ±0.1 a*	0.40 ±0.1 b**	0.20 ±0.1 b*	0.30±0.1 b**
Insulin	10.54±0.01	20.54±0.01a*	15.50±0.10b**	12.51±0.01b*	13.51±0.01b**
Leptin	7.2 ±0.10	25.40±0.10a*	9.80 ± 0.10 b*	11.80±0.10b**	10.20±0.10b**
Adiponectin	8.40 ±0.10	5.60±0.10a**	5.10 ± 0.10 bNS	7.60 ±0.10 b**	4.40 ±0.10b*
NEFA	90.00±1.00	61.90±0.1a**	43.90 ±0.10 b@	33.50±0.10b**	21.50±0.10 b*

All values are expressed as mean ± SD for each group that contains six animals. Statistical Significance p<0.001 *, p<0.01**, p<0.05 @ , NS – Non significant. A-Group II was compared with Group I. Group III, IV, and V were compared with Group II. Group I: Normal Diet (Control). Group II: High fat Diet (HFD) fed animals. Group III: Ethanolic extract of Citrus paradise (CP) (500mg/kg body weight) + HFD. Group IV: Naringin (100mg/kg body weight) + HFD. Group V : Orlistat - Standard drug(21.6 mg/kg body weight) + HFD

5.5.3. Histopathological Studies

Group I to Group V animals were taken for histopathology examinations. The changes were noted by dissecting the internal organs and the tissue such as the kidney, liver, adipose tissue, spleen, pancreas, and heart.

5.5.4. Histology of Adipose tissue

Plates A to E shows the histological changes of fatty tissue from groups I to V animals (figure 4.1a.) Treating with ethanolic extract of *citrus paradisi* (Group II) and Orlistat (Group V) increased the size of adipose tissue fed with HFD animals compared to the normal animal. It has been identified that there will be a decrease in cell size when treated with ethanolic extract of zingiber *officinale* and *Terminalia paniculata*, as reported by Iram Nazish Ramgopal Mopuri [24, 25] In support of this, there was a reduction in the weights of the fat pad also identified earlier which was similar to our study.

5.5.5. Histology of Heart

No major changes in the morphology of heart sections were noticed (Plates A to E) shown by normal morphology in all the five groups shown in (figure 4.1b.)

5.5.6. Histology of Kidney

Figure 4.1c represents the morphological differences before and after the treatment (Plates A to E). Both the kidneys of the animals fed with HFD were covered with perirenal fat. After the treatment, the normal morphology was resumed and then correlated with the study of Iram Nazish [24], who demonstrated the decrease in fat mass by the effect of zingiber *officinale* extracts. In our study, the Ethanolic extract of *citrus paradisi* and Naringin has been capable enough to act as an antihyperlipidemic agent.

5.5.7. Histology of Liver

The Histopathological study of the liver emphasized the accumulated fat mass in the hepatocytes preceding steatosis in the HFD-induced animals. It is represented in Figure 4.1d. (Plates A to E). However, these unusual conditions returned to normal when the rats were induced with MEMOL extract, as reported by Souravh Bais [26].

5.5.8. Histology of Pancreas

The pancreatic tissue of experimental rats is shown in Figure 4.1e. (Plates A to E). The histopathological study of the pancreas in group I rats represents the normal morphology. At the same time, in group II animals, the pancreas decreased the number of islets of Langerhans. This decrement in the number of islets of Langerhans showed a reduction in the survival of Beta cells. Our examination related to the observation shown by Alexis panny Chung [27].

5.5.9. Histology of Spleen

There were no major changes in the morphology of Spleen sections, as noticed by (Plates A to E) which shows normal morphology in all five groups (figure 4.1f.)

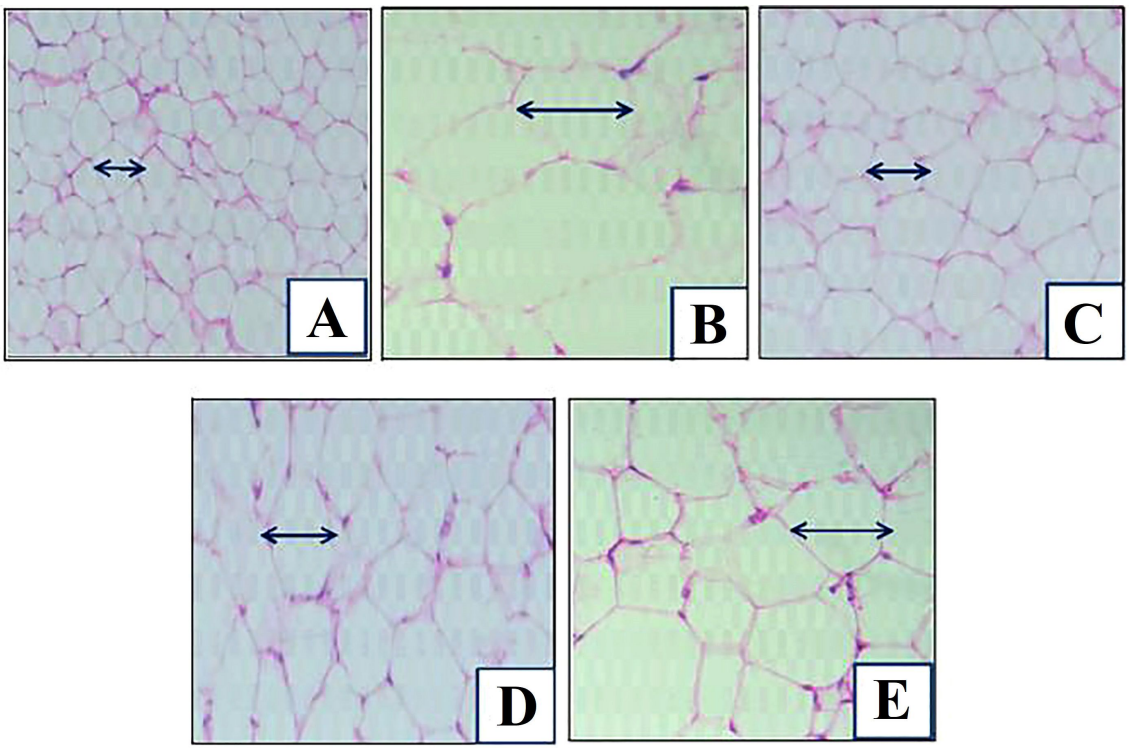

Fig: 4.1a: Histopathology of Adipose tissue in experimental animals

Plate A – Group I – Normal morphology
Plate B – Group II – Enlargement of adipocytes
Plate C – Group III – Normal morphology
Plate D – Group IV – Normal morphology
Plate E – Group V – Slight Enlargement of adipocytes
The images are shown at 400x magnification. Plates A to E display the histological alterations in adipose tissue from animals in groups I to V. (figure 4.1a.) Compared to normal animals, the size of adipose tissue supplied to HFD animals was enhanced when treated with ethanolic extract of citrus paradisi (Group II) and Orlistat (Group V). Group I: Normal Diet (Control) Group II: High fat Diet (HFD) fed animals.Group III: Ethanolic extract of Citrus paradise (CP)(500mg/kg body weight) + HFD. Group IV: Naringin (100mg/kg body weight) + HFDGroup V: Orlistat – Standard drug (21.6 mg/kg body weight) + HFD

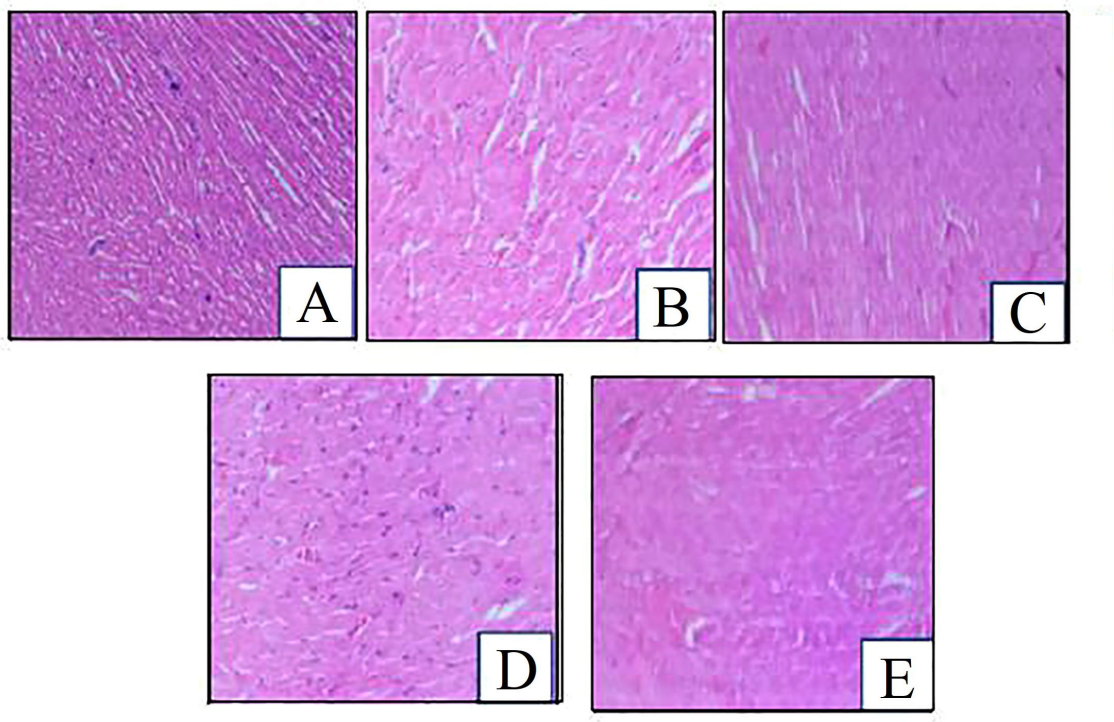

Fig: 4.1b: Histopathology of Heart in experimental animals

Plate A – Group I – Normal morphology
Plate B – Group II – Normal morphology
Plate C – Group III – Normal morphology
Plate D – Group IV – Normal morphology
Plate E – Group V – Normal morphology

The images are shown at 400x magnification. Normal morphology in all five groups was displayed in Plates A to E, showing no significant alterations in the morphology of heart sections (figure 4.1b.)Group I: Normal Diet (Control). Group II: High fat Diet (HFD) fed animals. Group III: Ethanolic extract of Citrus paradise (CP)(500mg/kg body weight) + HFD. Group IV: Naringin (100mg/kg body weight) + HFD.Group V : Orlistat - Standard drug(21.6 mg/kg body weight) + HFD

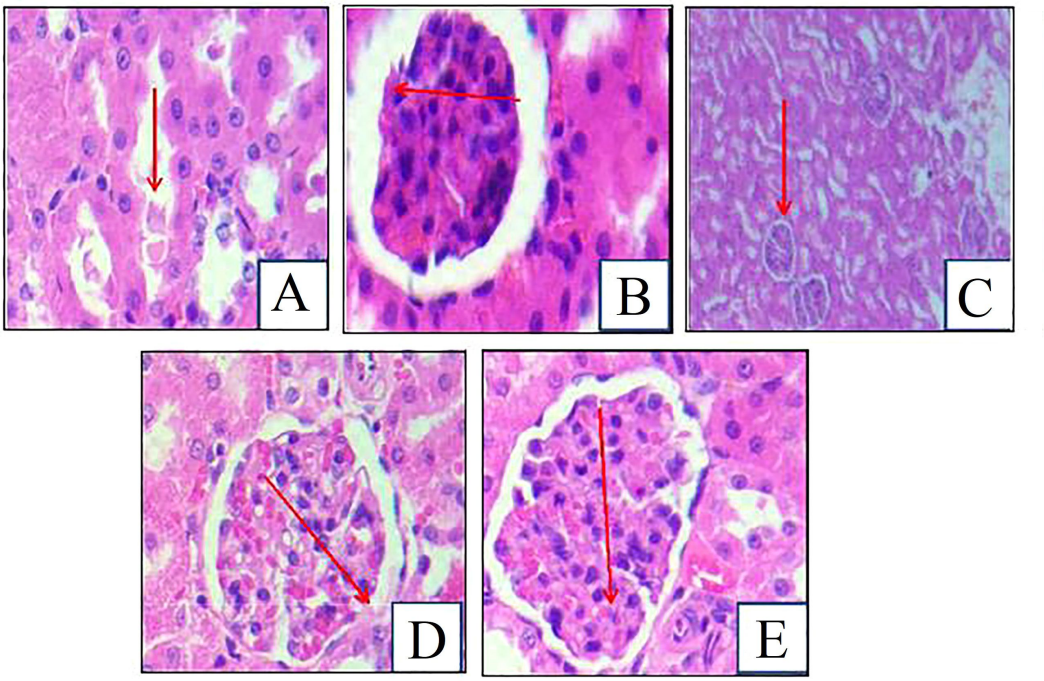

Fig: 4.1c: Histopathology of Kidney in experimental animal

Plate A – Group I – Normal morphology
Plate B – Group II – Development of perirenal fat
Plate C – Group III – Normal morphology
Plate D – Group IV – Normal morphology
Plate E – Group V – slight enlargement

The images are shown at 400x magnification. The morphological variations before and after the treatment are shown in Figure 4.1c (Plates A to E). After the treatment, the normal morphology of the animals' kidneys returned to both of their HFD-fed kidneys. Group I: Normal Diet (Control).Group II: High fat Diet (HFD) fed animals.Group III: Ethanolic extract of Citrus paradise (CP)(500mg/kg body weight) + HFD.Group IV: Naringin (100mg/kg body weight) + HFDGroup V: Orlistat - Standard drug(21.6 mg/kg body weight) + HFD

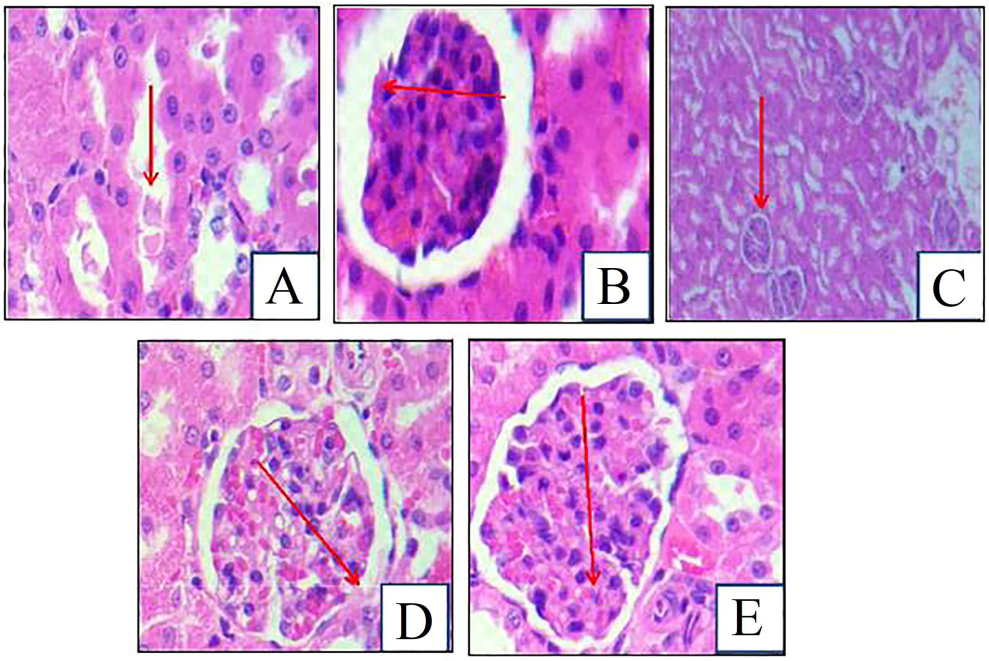

Fig: 4.1d: Histopathology of Liver in Experimental Animal

Plate A – Group I – Normal morphology
Plate B – Group II – Steatosis of liver
Plate C – Group III – Normal morphology
Plate D – Group IV – Normal morphology
Plate E – Group V – slight enlargement

The images are shown at 400x magnification. The histopathological analysis of the liver highlighted fat accumulation in the hepatocytes before steatosis in HFD-induced rats. Figure 4.1d depicts this in more detail (Plates A to E).Group I: Normal Diet (Control).Group II: High fat Diet (HFD) fed animals.Group III: Ethanolic extract of Citrus paradise (CP)(500mg/kg body weight) + HFD.Group IV: Naringin (100mg/kg body weight) + HFDGroup V: Orlistat - Standard drug(21.6 mg/kg body weight) + HFD

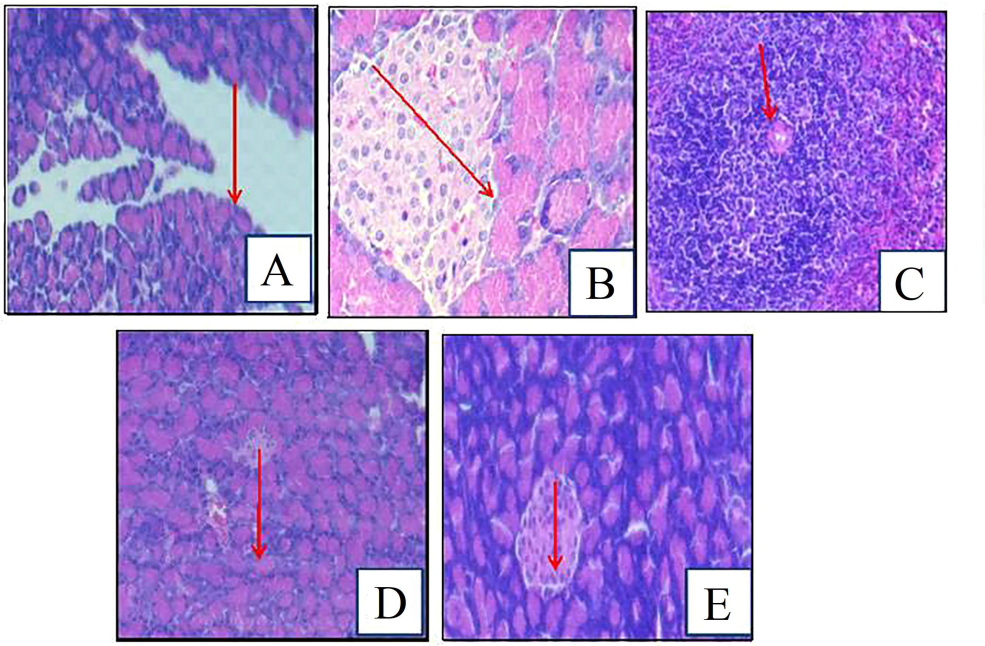

Fig: 4.1e: Histopathology of the pancreas in experimental animals

Plate A – Group I – Normal morphology
Plate B – Group II –Even distribution of Islet of Langerhans
Plate C – Group III – Normal morphology
Plate D – Group IV – Normal morphology
Plate E – Group V – Normal morphology

The images are shown at 400x magnification. Figure 4.1e displays the pancreatic tissue from experimental animals (Plates A to E). The normal shape of the pancreas was studied histopathologically in group I rat rats. The number of islets of Langerhans has reduced in the pancreas of group II animals at the same time. Group I: Normal Diet (Control).Group II: High fat Diet (HFD) fed animals. Group III: Ethanolic extract of Citrus paradise (CP)(500mg/kg body weight) + HFD. Group IV: Naringin (100mg/kg body weight) + HFD. Group V : Orlistat - Standard drug(21.6 mg/kg body weight) + HFD

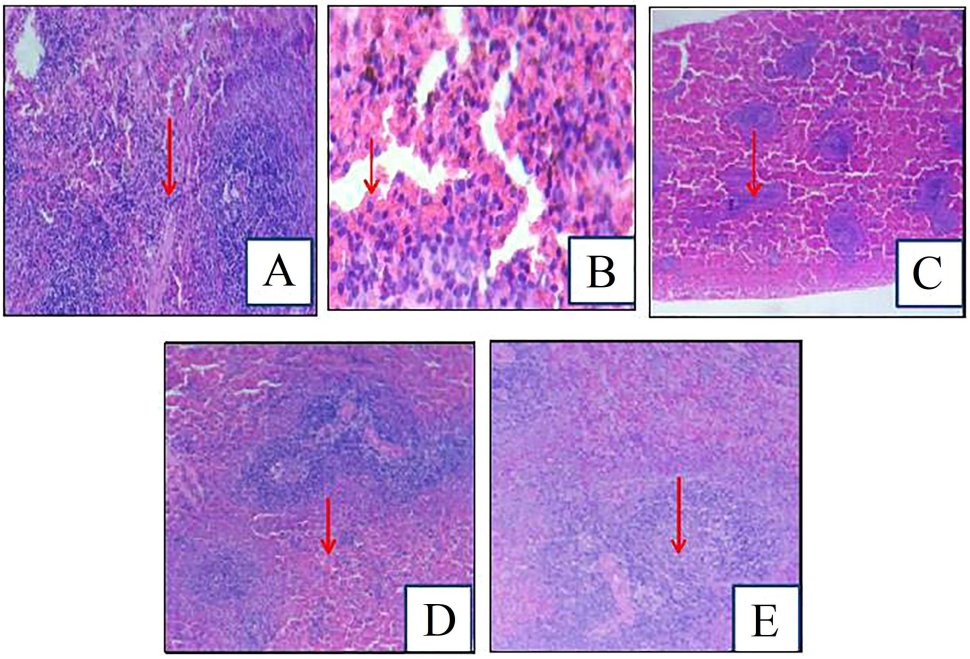

Fig: 4.1 f: Histopathology of Spleen in experimental animal

Plate A – Group I – Normal morphology
Plate B – Group II –Slight Normal morphology
Plate C – Group III – Normal morphology
Plate D – Group IV – Normal morphology
Plate E – Group V – Normal morphology

The images are shown at 400x magnification. Plates A to E, which depict normal morphology in all five groups, show no significant alterations in the morphology of the spleen sections. Group I: Normal Diet (Control). Group II: High fat Diet (HFD) fed animals. Group III: Ethanolic extract of Citrus paradise (CP)(500mg/kg body weight) + HFD. Group IV: Naringin (100mg/kg body weight) + HFD. Group V : Orlistat - Standard drug(21.6 mg/kg body weight) + HFD

6. CONCLUSION

From the above study, ethanolic extract of *Citrus paradisi* and Naringin has played a role as an antiobesity and antihyperlipidemic agent. These findings would be a milestone in highlighting the effects of ethanolic extract of *Citrus paradisi* and Naringin treated with HFD-induced animals. It was observed from its action by reducing organ weight, blood glucose, body weight, and serum lipids. Further to this, the signifying effect of the fruit extract would be assessed on the extension of this study to investigate the gene expression, which may throw light on the mechanism of its action.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES

1. Marques C, Meireles M, Norberto S, Leite J, Freitas J, Pestana D et al. High-fat diet-induced obesity rat model: a comparison between Wistar and Sprague-Dawley Rat. Adipocyte. 2016;5(1):11-21. doi: 10.1080/21623945.2015.1061723, PMID 27144092.
2. Williams L, Seki Y, Vuguin PM, Charron MJ. A review of animal models of in utero exposure to a high-fat diet. Biochim Biophys Acta. 2014;1842(3):507-19. doi: 10.1016/j.bbadis.2013.07.006, PMID 23872578.
3. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. Nutr Res Rev. 2010;23(2):270-99. doi: 10.1017/S0954422410000168, PMID 20977819.
4. Novelli ELB, Diniz YS, Galhardi CM, Ebaid GMX, Rodrigues HG, Mani F et al. Anthropometrical parameters and markers of Obesity in rats. Lab Anim. 2007;41(1):111-9. doi: 10.1258/002367707779399518, PMID 17234057.
5. Kim MS, Kim JK, Kwon DY, Park R. Anti-adipogenic effects of Garcinia extract on lipid droplet accumulation and transcription factor expression. BioFactors. 2004;22(1-4):193-6. doi 10.1002/biof.5520220140, PMID 15630282.
6. Novelli ELB, Diniz YS, Galhardi CM, Ebaid GMX, Rodrigues HG, Mani F et al. Anthropometrical parameters and markers of Obesity in rats. Lab Anim. 2007;41(1):111-9. doi: 10.1258/002367707779399518, PMID 17234057.
7. Sasaki T. Effect of acetic acid concentration on the color reaction in the O-toluidine boric acid method for blood glucose determination. Rinsho Kagaku. 1972;1(3):346-53.
8. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun. 1996;221(2):286-9. doi 10.1006/bbrc.1996.0587, PMID 8619847.
9. Mizuno TM, Kelley KA, Pasinetti GM, Roberts JL, Mobbs CV. Transgenic neuronal expression of proopiomelanocortin attenuates the hyperphagic response to fasting and reverses metabolic impairments in leptin-deficient obese mice. Diabetes. 2003;52(11):2675-83. doi 10.2337/diabetes.52.11.2675, PMID 14578285.
10. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med. 1953;41(3):486-92. PMID 13035283.
11. Bucolo G, David H. Quantitative determination of serum triglycerides by using enzymes. Clin Chem. 1973;19(5):476-82. doi: 10.1093/clinchem/19.5.476, PMID 4703655.
12. Ledue TB, Weiner DL, Sipe JD, Poulin SE, Collins MF, Rifai N. Analytical evaluation of particle-enhanced immunonephelometric assays for C-reactive protein, serum amyloid A and mannose-binding protein in human serum. Ann Clin Biochem. 1998;35(6):745-53. doi: 10.1177/000456329803500607, PMID 9838988.
13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9. doi: 10.1007/BF00280883, PMID 3899825.
14. Palipoch S, Punsawad C. Biochemical and histological study of rat liver and kidney injury induced by cisplatin. J Toxicol Pathol. 2013;26(3):293-9. doi: 10.1293/tox.26.293, PMID 24155562.
15. Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P. A controlled high-fat diet induces an obese syndrome in rats. J Nutr. 2003;133(4):1081-7. doi: 10.1093/jn/133.4.1081, PMID 12672923.
16. Coelho DF, Pereira-Lancha LO, Chaves DS, Diwan D, Ferraz R, Campos-Ferraz PL et al. Effect of high-fat diets on body composition, lipid metabolism, and insulin sensitivity, and the role of exercise on these parameters. Braz J Med Biol Res. 2011;44(10):966-72. doi: 10.1590/s0100-879x2011007500107, PMID 21876873.
17. Haripriya D, Vijayalakshmi K. The effect of cinnamaldehyde on high-fat diet-induced Wistar rats-a preliminary study. Int J Pharm Sci Res. 2014;5(12):5398.
18. Nahar S, Faisal F, Iqbal J, Rahman M, Yusuf M. Antiobesity activity of Moringa oleifera leaves against high fat diet-induced Obesity in rats. Int J Basic Clin Pharmacol. 2016;5:1263-8. doi: 10.18203/2319-2003.ijbcp20162427.
19. Chien M-Y, Ku Y-H, Chang J-M, Yang C-M, Chen C-H. Effects of herbal mixture extracts on Obesity in rats fed a high-fat diet. J Food Drug Anal. 2016;24(3):594-601. doi: 10.1016/j.jfda.2016.01.012, PMID 28911566.
20. Galaly SR, Hozayen WG, Amin KA, Ramadan SM. Effects of orlistat and herbal mixture extract on brain, testes functions, and oxidative stress biomarkers in a rat model of a high-fat diet. Beni Suef Univ J Basic Appl Sci. 2014;3(2):93-105. doi: 10.1016/j.bjbas.2014.05.002.
21. Jang WS, Choung SY. Antiobesity Effects of the Ethanol Extract of Laminaria japonica Areshoung in High-Fat-Diet-Induced Obese Rat. Evid Based Complement Alternat Med. 2013;2013:492807. doi: 10.1155/2013/492807. PMID 23365609.
22. Kaveripakam SS, Adikay S, Retnasamy G. Anti-Obesity efficacy of roots of Stereospermum suaveolens in high

fat-induced obese rats. J Young Pharm. 2017;9(2):234-8. doi: 10.5530/jyp.2017.9.46.
23. Uygun A, Kadayifci A, Yesilova Z, Erdil A, Yaman H, Saka M et al. Serum Leptin Levels in Patients With Nonalcoholic Steatohepatitis. American Journal of Gastroenterology. 2000;95(12):3584-9. doi: 10.1111/j.1572-0241.2000.03297.x.
24. Nazish I, Ansari SH, Arora P, Ahmad A. Antiobesity activity of Zingiber officinale. Phcogj. 2016;8(5):440-6. doi: 10.5530/pj.2016.5.5.
25. Mopuri R, Ganjayi M, Banavathy KS, Parim BN, Meriga B. Evaluation of anti-obesity activities of ethanolic extract of Terminalia paniculata bark on high fat diet-induced obese rats. BMC Complement Altern Med. 2015;15(1):76. doi: 10.1186/s12906-015-0598-3, PMID 25887331.
26. Souravh Bais S, GS, Sharma R. Antiobesity and hypolipidemic activity of Moringa oleifera leaves against high fat diet-induced Obesity in rats. Adv Biol. 2014;2014.
27. Panny Chung A, Gurtu S, Chakravarthi S, Moorthy M, Palanisamy UD. Geraniin protects against high-fat diet-induced oxidative stress in Sprague Dawley Rats. Front Nutr. 2018;5(17):1-14.