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INVESTIGATION OF ANTI-OBESITY ACTIVITY OF ETHANOLIC EXTRACT OF FOENICULUM VULGARE SEEDS, IN VIVO AND IN SILICO MODELS

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ABSTRACT

Background: Obesity is a chronic disease that triggers metabolic disorders due to excessive internal fat accumulation. *Foeniculum vulgare* (FV) is a very popular medicinal plant used to control obesity in the traditional medicine without any scientific evidence. Objectives: To evaluate the anti-obesity and anti-hyperlipidimic activity of ethanolic extract of *Foeniculum vulgare* seeds in vivo and identify the possible mechanism of action using molecular docking that is performed on plant constituent to target leptin receptor. **Material and Methods:** 16 albino rats were divided into four groups; group 1: none obese rats received (0.2 ml) normal saline daily, group 2: obesity control treated with modified High-fat hyper caloric diet, group 3: received plant extract (200 mg/kg) daily (I.P), and group 4: received Orlistat (2mg/kg) orally. Rats' body weight and Lipid profile were assayed. For Molecular docking, ligands were obtained from previous studies, screening was performed using structure-based drug design against phytochemical constituent of *Foeniculum vulgare*, molecular modelling was done using Auto Dock Vina; only best conformers ligands with highest and best score was selected. **Results:** Reduction in rat's body weights produced by *Foeniculum vulgare* ethanolic seed extract was12%, while Orlistat produced 6.4%. Triglycerides level wasn't affected by treatment with plant extract and Orlistat. Molecular docking shows that Stigmast-5-en-3-ol have stronger binding at the leptin receptor's binding site (-6.1 kcal/mol) when

analyzed using AutoDockVina. **Conclusion:** Finally, this study concluded that *Foeniculum* vulgare ethanolic seeds extract demonstrate promising action as anti-obesity remedy since it shows a significant reduction in rat body weight and correct lipid profile, which provide scientific rationale for its folkloric use. Molecular docking suggests that the Stigmast-5-en-3-ol is a good candidate as leading compound for management of obesity disorders.

KEYWORDS: Anti-Obesity, *Foeniculum vulgare*, leptin, stigmast-5-en-3-ol and Obesity Model.

INTRODUCTION

Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue, to the extent that health is impaired.[1] The incidence of obesity has doubled worldwide since 1980[2] with more than 1.5 billion adults being overweight [body mass index (BMI) over 25 kg/m ^{2}] and at least 500million of them being clinically obese [BMI over 30kg/m ^{2}].[3,4]

In 2010, overweight and obesity were estimated to cause 3·4 million deaths, worldwide, the proportion of adults with a body-mass index (BMI) of 25 kg/m (2) or greater increased between 1980 and 2013 from 28·8% to 36·9% in men, and from 29·8% to 38·0% in women[5] Prevalence has increased substantially in children and adolescents in developed countries; 23·8% of boys and 22·6% of girls were overweight or obese in 2013.

Obesity is a public health problem that has raised concern worldwide affecting both developed and developing countries and also all age groups. It is associated with increased incidence of cardiovascular disease, type 2 diabetes mellitus (DM), hypertension, stroke, dyslipidemia, osteoarthritis, and some cancers.[6]

Identification of factors associated with obesity is considered as a hot area of research, one of the important factors is a genetic contribution.

Leptin is secreted by adipocytes, transported into the brain and binds to its receptor in the hypothalamus,[7,8] and activates JAK-STAT3, leading to increase in "anorexigenic peptides" which normally inhibits food consumption and reduces weight.[9]

Foeniculum vulgare commonly known as Funnel (Apiaceae family), is one of the widespread annual or perennial plants with aromatic odor. It is a very popular medicinal and economic plant in Mediterranean region and Africa.[10]

The chemical constituents of ethanolic seed extract are 4 hydroxy-4-methyl-2 pentanone, Undecane, Cis-Anethole, trance anethole, Palmitic acid, Methyl oleat, Linoleic acid, Oleic acid, Stigmast-5-en-3-ol.[11] Fennel is a rich source of beta-carotene and vitamin C,[12] as well as calcium, magnesium iron, and lesser amounts of other metal cations.[13]

1. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental Animals and Ethical consideration

A total of 16 Albino rats, of both sexes, weighting 70 – 150g were obtained from the Animal House of International University of Africa (IUA), Faculty of Pharmacy, Khartoum, Sudan.

The rats were maintained under standard condition (12: 12- hour's light/ dark cycle and at an ambient temperature of 25 ± 2°C, with 65 ± 5 % humidity) for 7 days as adaptation period.

All the studies and experimental protocols were approved by the Institutional Animal Ethical Committee (I.A.E.C) in the department of pharmacology, Faculty of pharmacy, IUA, (**Registration No:** IUA, IAEC/EXP.Ph.017/6).

2.1.2. Plant material collection and identification

Foeniculum vulgare seeds were purchased from herbalism store in Khartoum, Sudan, and identified at the Herbarium of Medicinal and Aromatic Plants & Traditional Medicine Research Institute (MAPTMRI), National Center for Research, Khartoum by Yahya Sulieman Mohamed.

2.1.3. Drugs and Chemicals

All chemicals, Solvent, and Reagents used throughout the study were purchased from (Biosystems - Spain), while Orlistat (Manufactured by Roche) is purchased from Local Market.

2.2. METHODS

2.2.1. Extraction method

The seeds were uniformly powdered using a milling machine (China). 150g powder of *Foeniculum vulgare* was mixed five times with 100 ml ethanol (SD fine chemicals, India). Extraction using maceration method continued until the extraction solvents became colorless (total solvent volume is 500 ml).

The obtained extracts were filtered over No.1 filter paper and the filtrate was collected, then ethanol was removed by a rotary evaporator until complete dryness (yield 4 %).

2.2.2. Preparation of Extract for Administration

0.1g of plant extract was suspended in 0.006 g of Carboxy methyl cellulose (CMC) dissolved in 10ml warm water to get uniform suspension.

2.2.3. Induction of obesity

Modified High-fat hyper caloric diet, which is composed of (25%) Fat, (25%) Protein, (50%) Carbohydrate and Cholesterol powder was used for obesity induction.

Then the suspension was administered in a dose of 5 ml/kg body weight of rats and was given to the rat by oral gavage.

"Chow" was used as a source of carbohydrate, Chickens meat and condensed milk were used as a source of protein.

2.2.4. Experimental design and treatment protocol

Animals were randomly divided into 4 groups each containing six rats. Group I was served as none obese group and received 0.2 ml normal saline daily, obesity was induced in group II, III and IV using modified hyper caloric diet for 14days (obese groups).

Rats body weight was measured then Group II received normal saline and served as obesity control, Groups III received plant extract (200 mg/kg p.o) daily, Groups IV (standard group) received Orlistat (2mg/kg p.o) rats were treated for another 14 days.

2.2.5. Biological evaluation

Blood was collected retro-orbitally from the inner canthus of the eye under diethyl ether anesthesia using capillary tubes. Every blood sample was divided into two tubes, one contains EDTA and the other is plain container.

The samples were separated in centrifuge (Germany) at 4000 rpm for 15 minutes to obtain serum and plasma that were used to measure (HDL, LDL, Triglycerides and total cholesterol) using UV spectrophotometer (China), and were determined at lab of hematology at IUA clinic.

2.2.6. Molecular docking

2.2.6.1. Ligands and Protein preparation and optimization

Ligands were the phytochemical constituents of *Foeniculum vulgare* from the study done by Muhammad Gulfraz[11] were selected and drawn using ChemDraw Professional 15.0 in SDF format, then converted to PDBQT format to be identified by AutoDockVina using Open babel.[14]

The crystallographic structure Leptin Receptor-antibody complex was retrieved from protein data bank as a PDB three dimensional structure file (PDB ID: 3V6O) with a resolution [Å]: 1.95, *R*-Value Free: 0.213, *R*-Value work: 0.171), deposited by Carpenter, B., Hemsworth, G.R., Ross, R.J., Artymiuk, P.J. on: 2011-12-20 and released: 2012-03-14.[15]

2.2.6.2. Geometry Optimization and Pre-docking procedure

In order to prepare the selected compounds for docking, hydrogens and Gasteiger charges were added.[16] and all the hetero-atoms and water molecules were removed from protein structure.

The protonated protein initially optimized in order to remove all the bad steric clashes using UCSF Chimera software[17] for 100 steepest descent steps at root-mean-square gradient of 0.02 with an update interval of 10 and using AMBER ff12SB force field,[18] while Ligands where minimized for 200 steepest descent steps at root-mean-square gradient of 0.02 with an update interval of 1 and using MMFF94 force field[19] using Open babel.

2.2.6.3. Docking Strategy and setup

All computational docking studies were carried out using AutoDockVina[20] (Scripps Research Institute, La Jolla, CA, USA) installed in a single machine running on MSI (Core-i7 - 6700HQ processor, 12GBs of DDR4 RAM, Nvidia Geforce GTX 960m Graphic Card, 1TBs HDD Memory) with Windows as an operating system.

Docking studies were performed for each ligand (other parameters were kept default) as follow:

(1) The unnecessary chain was deleted; N-Acetyl-D-Glucosamine, Cysteine, Ethylene Glycol, Acetate Ion and Sodium Ion of co-crystallization were deleted.

(2) The docking was done with the default settings as following:

a) 10 conformers of the ligand were retained with highest and best score by default.

b) A grid box centered covering the Leptin receptor with a dimensions (Angstrom) of (**X:** 74.95 **Y:** 74.97 **Z:** 66.58).

(3) The scoring configuration of the ligand–Target complexes was selected on energetic grounds ((kcal/mol)); best poses with the lowest binding energy was chosen for each compound.

2.2.7. Statistical analysis method

Statistical evaluation was performed with Prism 5.0 computer program. The data were expressed as Mean ± S.E.M.

The statistical analysis was carried out by means of the two-tailed unpaired t-test. The result was considered to be significant at ($p \leq 0.05$).

3. RESULTS

3.1 Effect of modified obesity model on Induction of obesity

In order to evaluate the modified obesity model, rat weight were compared before (pre-induction) and after (post- induction) feeding with modified HFHD, and the results were presented in figure (1) below.

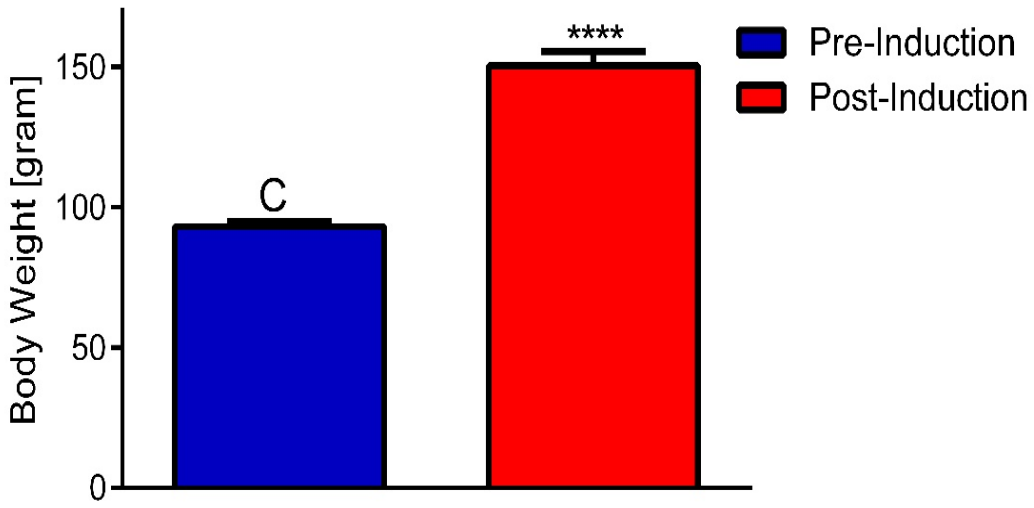

Figure 1: Rats body weight before and after induction of obesity, ****$p \leq 0.0001$.

3.2 Effect of *Foeniculum vulgare* on rats body weight

Effect of *Foeniculum vulgare* extract and on Orlistat on rats' body weight fed with HFHD for 14 days.

Table No 1: Percent reduction in rat's body weight produced by *Foeniculum vulgare* (200mg/kg) (G3) and Orlistat (2mg/kg) (G4). Both Control and obesity control showed no reduction on mean body weight.

Treatment groups	Mean Body Weight		
Starting weight (day 0)		Final weight (day7)	Percentage %
V	108.7	97.22	12%
F. Orlistat	103.8	97.55	6.4

3.3 Plasma Lipid Profile

The effects of F.V extract on plasma cholesterol, triglyceride, HDL and LDL are presented in figures 3, 4, 5 and 6, respectively.

3.4.1 Plasma cholesterol level

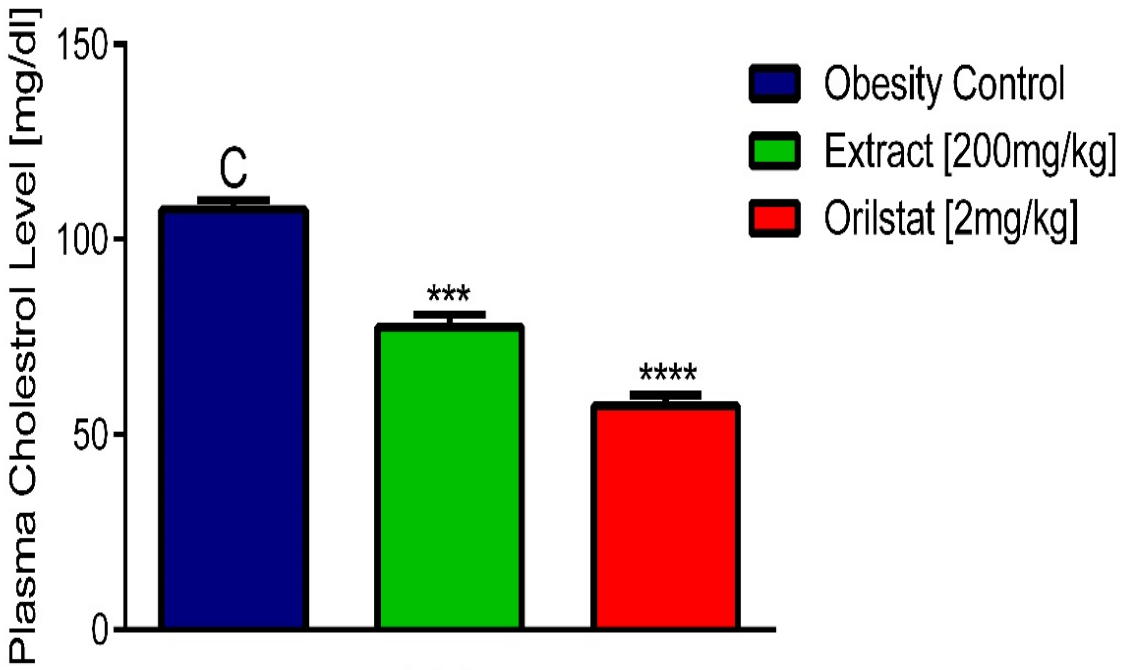

Figure 3: Plasma cholesterol level of extract (200mg/kg), Orlistat (2mg/kg) and Obesity control ***$p \leq 0.001$, ****$p \leq 0.0001$.

3.4.2 Plasma triglyceride level

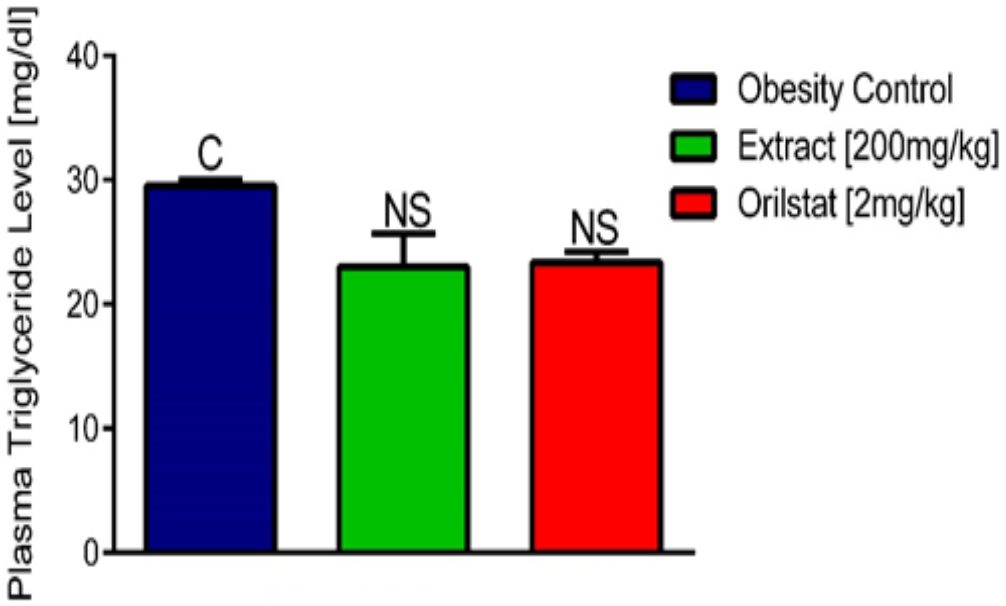

Figure 4: Plasma triglyceride level of extract (200mg/kg), Orlistat (2mg/kg) and Obesity control, NS: non-significant.

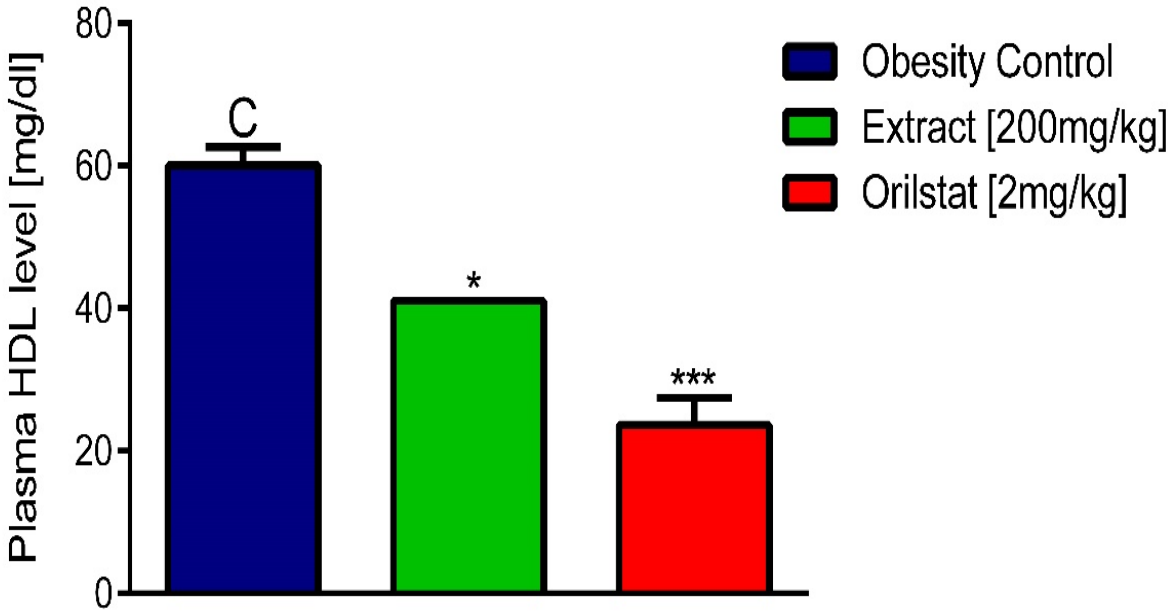

Figure 5: Plasma HDL level of extract (200mg/kg), Orlistat (2mg/kg) and Obesity control, NS: non-significant * $p \leq 0.05$, *** $p \leq 0.0001$.

3.4.4 Serum LDL level

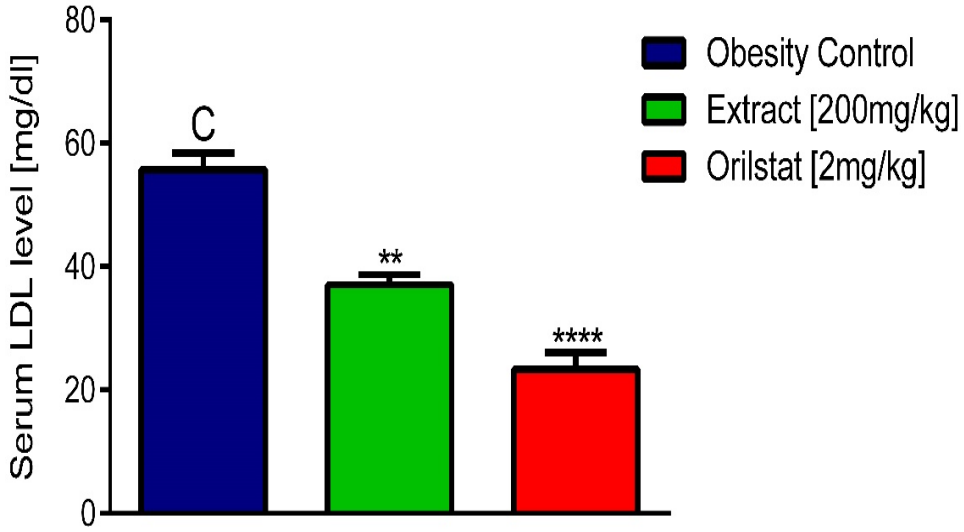

Figure 6: Serum LDL level of extract (200mg/kg), Orlistat (2mg/kg) and Obesity control, NS: non-significant * $p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$.

3.5 Molecular Docking

In current study chemical constituent of ethanolic seed extract which are 4 hydroxy-4-methyl-2 pentanone, Undecane, Cis-Anethole, trance anethole, Palmitic acid, Methyl oleat, Linoleic acid, Oleic acid, Stigmast-5-en-3-ol were analyzed.

Stigmast-5-en-3-ol have shown stronger binding at the receptor's binding site (-6.1 kcal/mol) when analyzed using AutoDockVina.

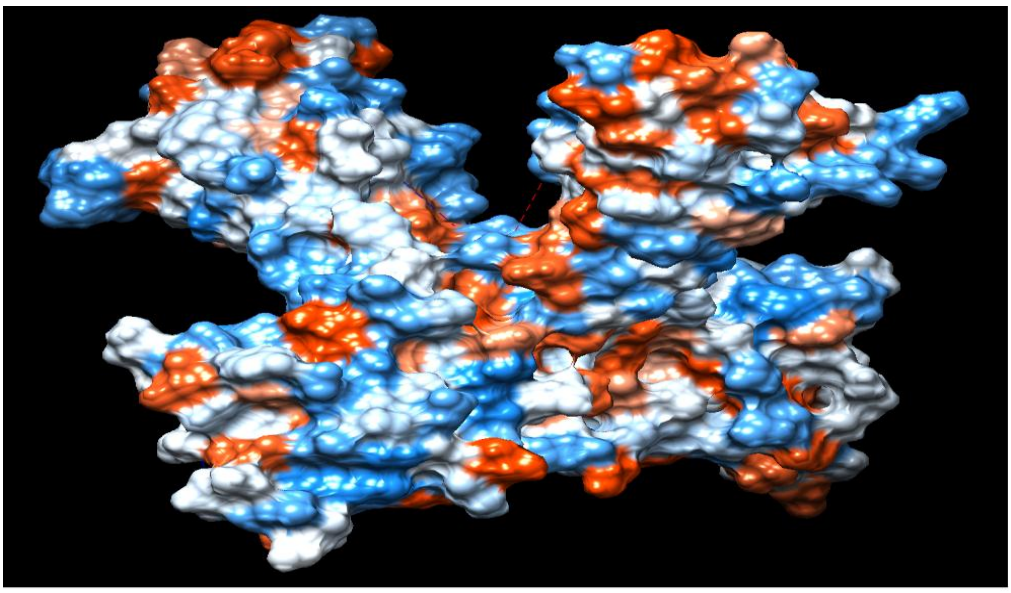

Figure 8: Molecular surface representation of Leptin receptor.

Table 1: The docking binding energy of selected compound (ranked by the kcal/mol).

Ligand name	Binding energy (kcal/mol)
Stigmast-5-en-3-ol	-6.1
Trance anthenole	-5.3
Linoleic acid4	-5.2
Cis anthenole	-5.1
Linoleic acid1	-4.9
Oleic acid2	-4.9
Linoleic acid3	-4.8
Palmitic acid3	-4.8
Undecane	-4.5
Oleic acid3	-4.2
Linoleic acid3	-4.1

4. DISCUSSION

Obesity, is chronic disease that triggers metabolic disorders due to excessive internal fat accumulation, is known to cause a variety of complications, such as hypertension, hyperglycemia, diabetes, and cardiovascular diseases.[21,22]

With the increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs, interest in the use of plants have revived throughout the world.[23]

Therefore searching for effective and safe anti-obesity agents is ongoing and World Health Organization has also recommended the development of herbal medicine in this concern.[24] *Foeniculum vulgare* is a very popular medicinal plant in the temperate and tropical regions of the world. This herb has many traditional medicinal uses; in the treatment of a variety of symptoms of the gastrointestinal and respiratory tract[25] and it is used by herbalism as obesity controlling agent. This study aimed to investigate about the rationale of this practice and to include *Foeniculum vulgare* as anti-obesity agent based on a scientific evidence.

Studies have shown that, cholesterol accumulation in adipose tissue that used in vivo models.[26] and excess TG and cholesterol accumulation leads to hypertrophied adipocytes which lead to obesity[27] and since that most of obesity models use carbohydrates, fats and proteins to induce obesity, such as "High fat hypercloric diet",[28] thus we modified this model by addition of cholesterol to promote faster obesity induction.

Selection of cholesterol dose based on pilot study carried for one week on four rats, 40% cholesterol concentration, reveals the highest weight gain (21.1%), thus it was used throughout the study.

The body weight gains in the groups treated with modified HFHD for 14 days was (40 %), while the average weekly body weight gain in the group treated with high fat hyper caloric diet for 28 days was twice as much as that of the control group[28] which illustrated that modified HFHD can successfully use to induce obesity in short period.

Foeniculum vulgare ethanolic seed extract show reduction of 12% in rat's body weights in group received plant extract which indicate anti-obesity activity of *Foeniculum vulgare* when compared with G4 that treated with Orlistat which showed 6.4% reduction on body weight after two weeks.

Regarding the effect of *Foeniculum vulgare* on lipid profile (total cholesterol, Triglycerides, HDL, and LDL); rats treated with *Foeniculum vulgare* ethanolic seed extract for two weeks significantly decreased total cholesterol ($p \leq 0.001$), HDL ($p \leq 0.05$) and LDL ($p \leq 0.01$), with no significant effect on triglycerides level, in comparison to control group, this finding go parallel with the studies of anti-obesity effect *Foeniculum vulgare* (Fruits) done by Dr. Chanchal Garg in2011[29] were *Foeniculum vulgare* fruit extract present significant effect on Triglycerides level.

On the other hand, Orlistat significantly decreased (plasma cholesterol ($p \leq 0.0001$), HDL ($p \leq 0.001$) and LDL ($p \leq 0.0001$), with no significant effect on triglycerides level which agree with result done by Brian Hutton and Dean Fergusson in 2004 on human.[30]

In the current investigationmolecular docking was used which enables scientist to virtually screen a number of candidate compounds based on their binding ability and binding orientation with a target molecule of known three-dimensional structure. It also allows one to select compounds with strong affinity for the target site.

Eleven phytochemical constituents were screened and analyzed for their stimulatory action against human Leptin receptor, then compounds that shown stronger binding at the receptor's binding site is selected.

Biswajit Satapathy[31] in 2004 analyze the protein Leptin, to establish its relation in obesity. Their results showed that Fluoxetine Hydrochloride is more potent Leptin receptor stimulator analogs based on their binding energy values.

When using Autodock-Vina Fluoxetine Hydrochloride and Stigmast-5-en-3-ol give binding energy values (-7 and -6.1 kcal/mol respectively), which indicate that Stigmast-5-en-3-ol could have stability on binding with liptin receptor.

5. CONCLUSION AND RECOMMENDATIONS

Finally, the study concluded that *Foeniculum vulgare* ethanolic seeds extract demonstrate promising action as anti-obesity plant since it shows a significant reduction on rat body weight and correct lipid profile, which provide scientific rationale for its folklore use.

In conclusion, this analysis suggests that the stigmast-5-en-3-ol could be efficacious in the treatment of obesity induced disorders.

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