Uttar Pradesh Journal of Zoology

Volume 45, Issue 16, Page 454-463, 2024; Article no.UPJOZ.3856
ISSN: 0256-971X (P)

Effect of Polyherbal Formulation on High Fat Diet Induced Obesity in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i164328

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3856

Original Research Article

Received: 25/05/2024
Accepted: 30/07/2024
Published: 06/08/2024

ABSTRACT

Obesity is one of the major chronic diseases in which excess body fat accumulates in visceral organs. It is a chronic metabolic disease due to an imbalance between energy intake and expenditure. Various synthetic treatments are available in the market but have side effects such as Joint pain, chest tightness, tiredness, Back and stomach pain. The aim of study was to examine the anti-obesity potential of prepared polyherbal formulation (*Hugonia Mystax: Blumea Lacera*::1:1) in female Wistar rats. The obesity was induced by a high fat diet. Group 1 Placebo (normal control), Group 2-high fat diet (disease control), Group 3-high fat diet and orlistat (20 mg/kg for last 2 weeks, Group 4 treated with high fat diet and low dose elixir (200 mg/kg i.e. 4ml orally for last 2 weeks of study), Group 5- high fat diet and high dose elixir (400 mg/kg i.e. 8ml orally for last 2 weeks of study). At the end of study rats were subjected to evaluation of physical parameters, liver profile, serum blood glucose level and histopathology of liver and adipose tissue. The result states that formulation has the potential to work on various obesity mechanisms especially on energy intake. The result of the present study concluded that the prepared formulation has anti-obesity potential.

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Cite as: *Shirode, Devendra, Aishwarya Patil, Ashish Kulkarni, Shilpa Chaudhari, Smeeta Sadar, and Mayuri Tupe. 2024. "Effect of Polyherbal Formulation on High Fat Diet Induced Obesity in Rats". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (16):454-63. https://doi.org/10.56557/upjoz/2024/v45i164328.*

Keywords: High fat diet; polyherbal formulation; liver; adipose tissue; lipid profile; anti-obesity.

1. INTRODUCTION

Obesity is one of the major chronic metabolic and inflammatory diseases-according to the American Medical Association [1,2] that is the mainstay for many health issues and causes of disability and death [3]. It is now common epidemic problem not only in adults but also in children [3]. Obesity is a risk factor for atherosclerosis that leads to cardiovascular diseases, stroke, diabetes mellitus, many types of cancer, breathing disorders, mental health issues etc. [4,5]. Obesity occurs due to a massive imbalance between energy expenditure and energy intake and an imbalance of metabolic processes [2,3].

According to the WHO 2022 report, 2.5 million were overweight and 890 million people were found obese [6]. In 2013 it was observed that 42 million children under the age of 5 years were found obese and in 2014, 1.9 billion people of age (18 and more than 18) were found to be overweight and of them 600 million found obese. Progression of this by 2030 may result in 3.4 billion people being overweight and obese, which is over 60% of the world's population [7,8].

Numerous factors are involved in obesity which are complex interactions in genetics, hormones, environment, high-density diet, low physical activities, eating disorders, and adipokines secretions [2]. In-a literature survey, it was discovered that plants belongings to the family Asteraceae, Solanaceae and lineaceae have high ratings in anti-obesity. Earlier a synthetic drug such as sibutramine used as anorectic in the treatment of obesity but was later withdrawn from the market as a concern of safety [3]. Currently, orlistat is in use but has side effects such as insomnia, constipation etc. [5]. Hence there is a need to develop newer anti-obesity treatments based on the traditional ayurvedic system. Polyphenols present in plants have an anti-obesity effect [1,9]. The plants *Hugonia mystax* and *Blumea lacera* having anti-lipidemic activity [10] anti-inflammatory activity [11], and anti-oxidant activity [12], hypoglycaemic activity [13] so the combination of these two plants may be fruitful hence used in the formulation as an active ingredient for anti-obesity activity in rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Leaves of the plant *Hugonia mystax* were collected from Dindigul District, Tamilnadu, India. The whole plant of *Blumea lacera* was collected from the local area of pune region. Collected plant materials were authenticated from the Department of Botany, Sadguru Gadage Maharaj College, Shivaji University, Karad, Maharashtra.

2.2 Chemicals

The ingredients required for preparation of high fat diet were purchased from local market. Research grade chemicals and biochemical kits (BIOLAB diagnostics Pvt ltd) were used for estimation of various parameters like Triglycerides, total cholesterol, HDL- Cholesterol, SGOT, SGPT etc.

2.3 Animals

The experimental protocol was approved by IAEC DYPCOP/IAEC/2022/07. Thirty female Wistar rats (150-220 g) were procured from National Institutes of Biosciences, Bhor, Pune and were housed in standard conditions recommended by CPCSEA with 12:12 light-dark cycle followed for experimentation. The animals were supplied with adequate drinking water and a standard nutritional diet (Nutrivet Life Sciences, Pune) or HFD prepared in the laboratory as per protocol throughout the experiment.

2.4 Method for Preparation of Extracts and Elixir Polyherbal Formulation: [14]

Extract preparation: The plant material was cut into small pieces shade dried, and coarsely powdered extraction was done by the Soxhlet method. The extraction of plants is done separately.

Polyherbal Formulation preparation: the polyherbal formulation (elixir) is prepared by a simple solution method. Firstly, the active ingredients and vehicle were mixed, then preservatives and flavouring agents were added and finally volume was made. The formula for polyherbal elixir is as follows:

Table 1. Formula for elixir

Sr. no.	Material	Quantity
1	*Hugonia mystax* ethanolic extract *Blumea lacera* ethanolic extract (1:1)	2 gm
2	Sodium saccharin	0.5 gm
3	Alcohol	15 ml
4	Glycerine	2 ml
5	Methyl paraben	0.2 ml
6	Propyl paraben	0.02 ml
7	Orange syrup	2 ml
8	Water	Up to 100 ml

Table 2. Formula for high fat diet preparation

Sr. No	Ingredient	Quantity
1	Soyabean	65 gm
2	Casein	110 gm
3	Corn flour	6 gm
4	Coconut oil	57.5 gm
5	Vegetable ghee	172.5gm
6	Wheat flour	2 gm
7	Fructose	400 gm
8	Vitamin	10 gm
9	Calcium carbonate	157 gm
10	Sodium cholate	20 gm

The prepared polyherbal formulation was evaluated for viscosity, pH, refractive index, and stability.

2.5 High Fat Diet Induced Obesity in Rats:[15]

The animals were fed with a high-fat diet of composition (per 1000g) as follows for 7 weeks. Experimental obesity and other metabolic changes induced by dietary manipulation.

Female Wistar rats were divided into five groups and each group consisted of six animals. The treatment was given as follows [16].

Group 1 Normal control Group: Received standard pellet chow and placebo elixir treatment (1ml/100gm BW, p.o.) for 7 weeks.

Group 2 High fat diet control group: Received high fat diet and placebo elixir treatment (1ml/100gm BW, p.o.) for 7 weeks.

Group 3 Reference Standard group: Received high fat diet (throughout the study) and orlistat 20 mg/kg p.o. as a reference standard drug from 5^{th} week.

Group 4 Low dose treatment: Received high fat diet (throughout the study) and polyherbal formulation in low dose. (200mg/kg p.o.) from 5^{th} week.

Group 5 High dose treatment: Received high fat diet (throughout the study) and polyherbal formulation in high dose. (400mg/kg p.o.) from 5^{th} week.

Blood Sample collection for serum parameters and tissue antioxidant parameters.

At the end of the study blood was collected from retro orbital puncture. Animals were sacrificed for tissue isolation for histopathological purposes and tissue anti-oxidant estimation.

2.6 Physical Parameters

1. Body weight was monitored from 0^{th} day to 7^{th} week.
2. Abdominal circumference was measured at 0^{th} day, before starting of treatment and at the end of treatment.
3. Body mass index: BMI calculated at the start and end of the study.

$$BMI = (\text{body weight}) / (\text{naso-anal length})^2$$

2.7 Estimation of Serum Parameters (Lipid Profile) [15]

At the end of the study, the blood sample (1ml) was collected by puncturing retro orbital plexus under anesthesia using diethyl ether for estimation of serum parameters such as triglycerides, total cholesterol, HDL-Cholesterol, LDL- Cholesterol, glucose, VLDL, atherogenic index.

Atherogenic index: log (TG/HDL-C)

VLDL = TG/5.
LDL= (Total Cholesterol)- (HDL-C) -(TG/5).
Liver function tests:

Blood sample collected at the end of study after treatment day was used for estimation of SGPT and SGOT.

2.8 Tissue Antioxidant Parameters

After completion of treatment the animals were sacrificed by CO_2 inhalation for estimation of tissue parameters of the liver, lipid peroxidation and glutathione peroxidase.

2.9 Estimation of LPO

1 ml of liver tissue homogenate mixed with 2ml freshly prepared of TBA-TCA HCL (10%) solution and heated for 1 hour in boiling water bath and then cooled under tap water. Further sample was centrifuged at 1000 rpm and precipitate was removed, and absorbance was measured at 535 nm against blank.

2.10 Estimation of GSH:[17]

Liver tissue was homogenised in ice cold TCA (10%) and centrifuge at 3000 rpm for 10 min. After centrifugation 0.5 ml of supernatant was added in 2 ml of 0.3 M disodium hydrogen phosphate and 0.2 ml of DTNB solution (0.4 mg/ml in 1% of sodium citrate) volume make up to 3ml with phosphate buffer. Absorbance was measured at 412 nm.

2.11 Histopathological Studies

A small portion of liver and adipose tissue was isolated from euthanized rats and fixed in 10% of formalin for 24 hours at room temperature. Both tissues were embedded in paraffin and 3-5 micrometre slides were stained with haematoxylin eosin and observed under the microscope.

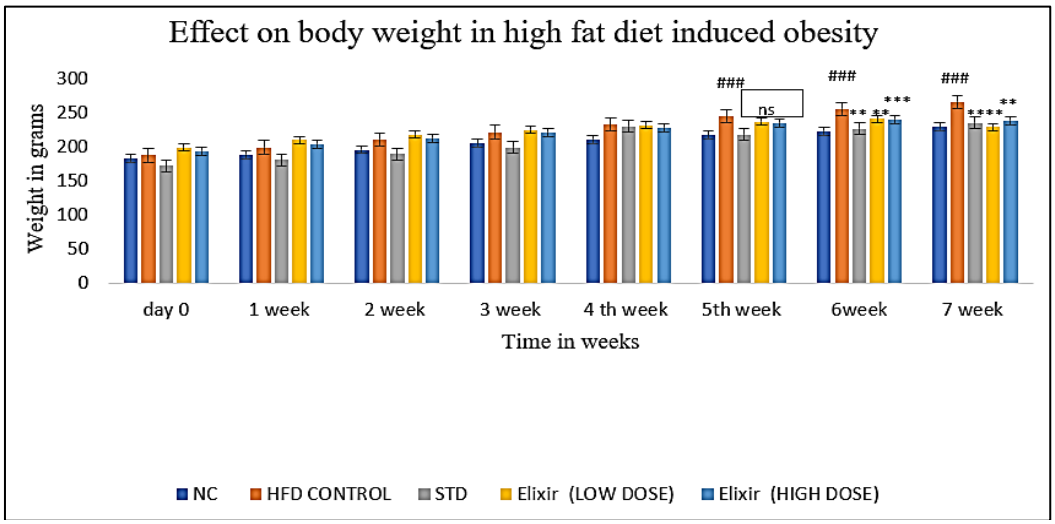
2.12 Statistical Analysis

Results were expressed as mean± SEM and statistical significance between groups by ANOVA followed by Tukey's multiple range test. INSTAT statistic software of version 3.01.

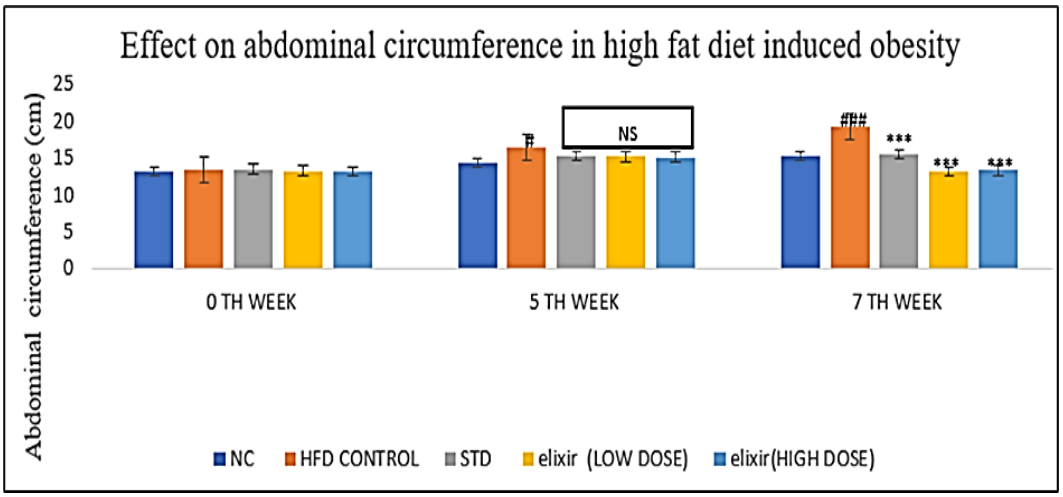
The probability value is $p<0.05$ was considered statistically significant. (# - comparison between normal control and high fat diet control group and * - comparison between high fat diet control and treatment groups).

3. RESULTS

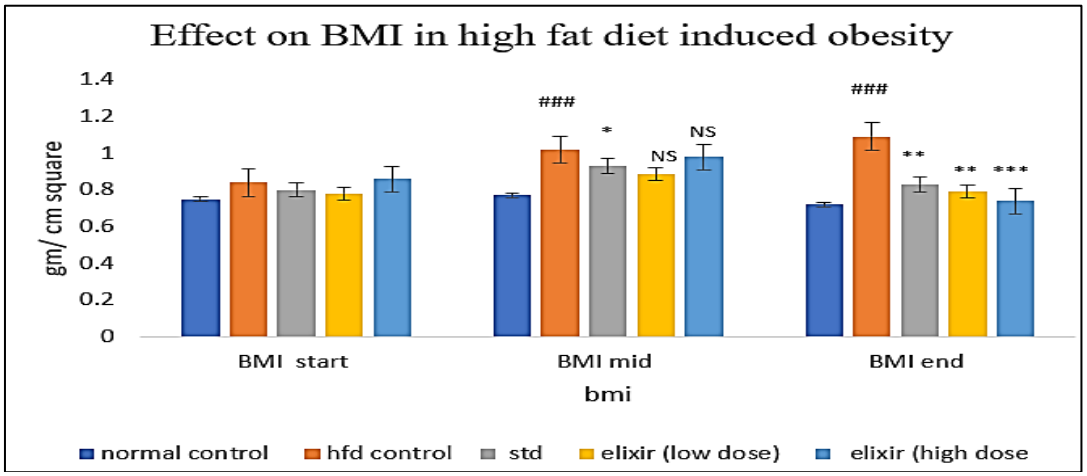
The viscosity of formulation found 59 cps, refractive index of formulation found 1.437, pH of formulation 6.8 while after stability viscosity found 58 cps and pH found 6.4.



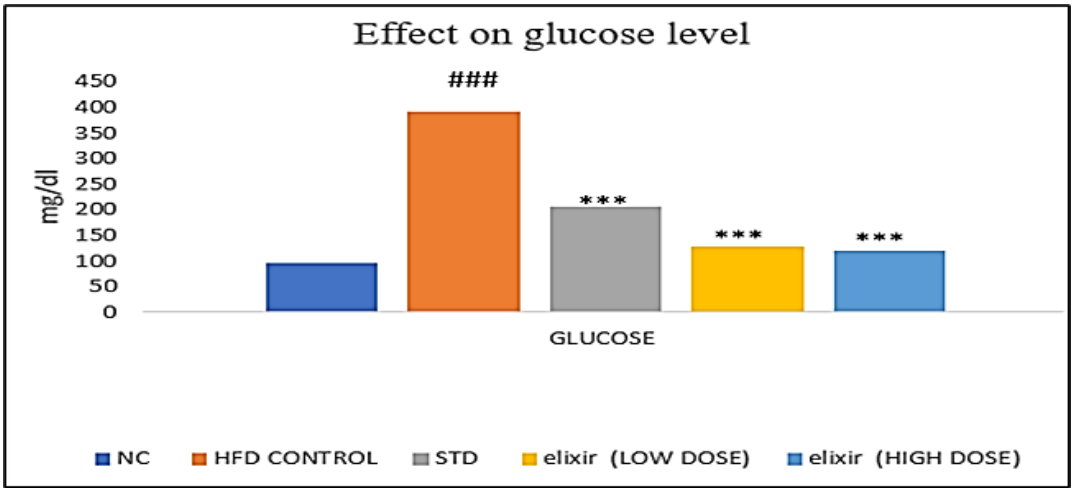
Graph 1. Effect of polyherbal elixir formulation on body weight in high fat diet induced obesity



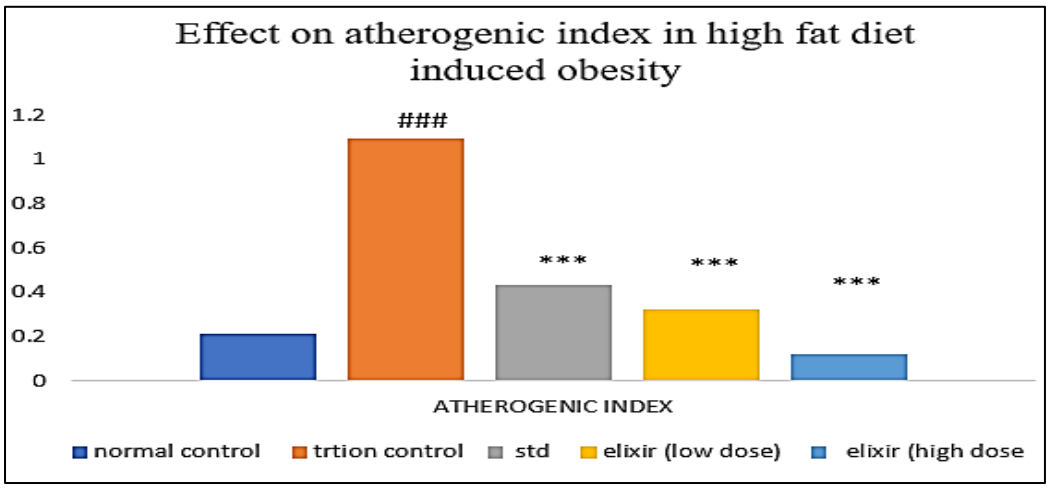
Graph 2. Abdominal circumference



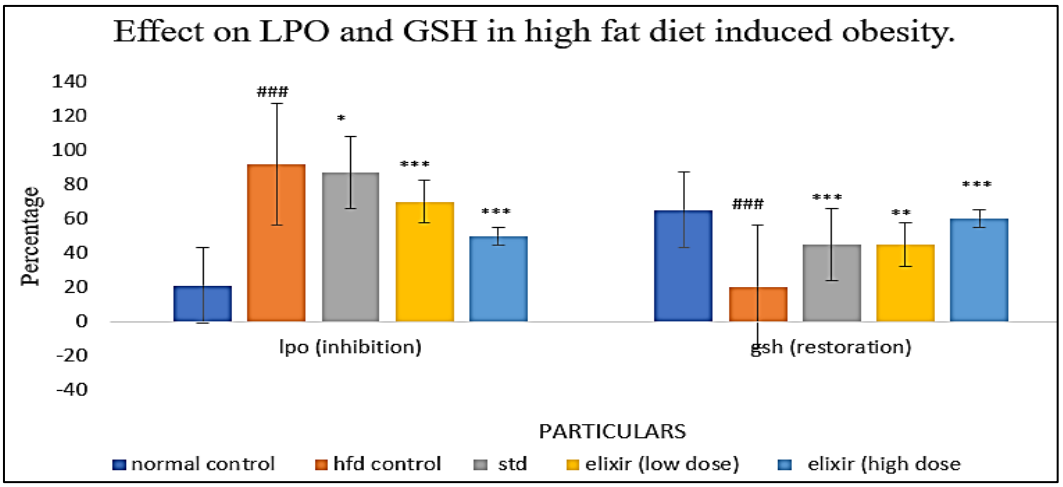
Graph 3. Effect on BMI



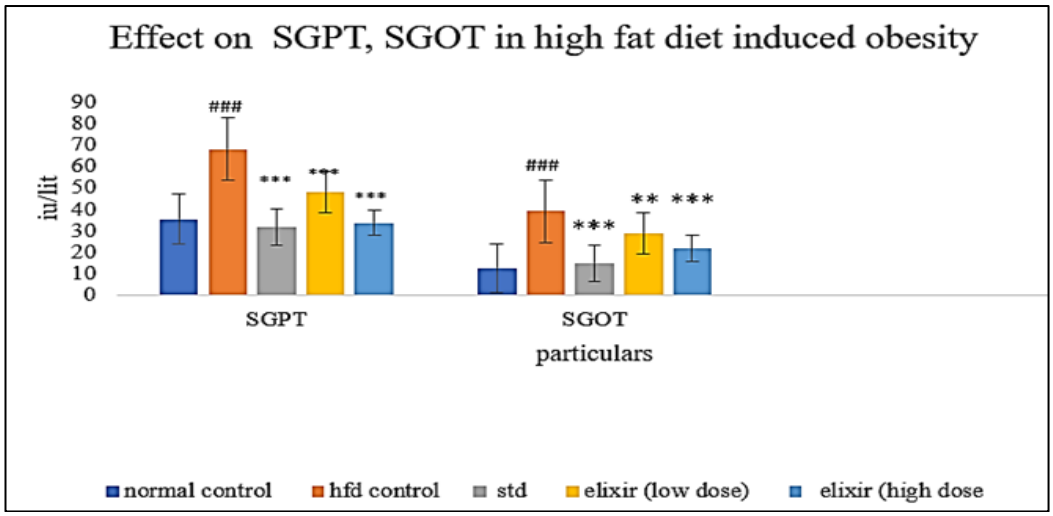
Graph 4. Effect on blood glucose level



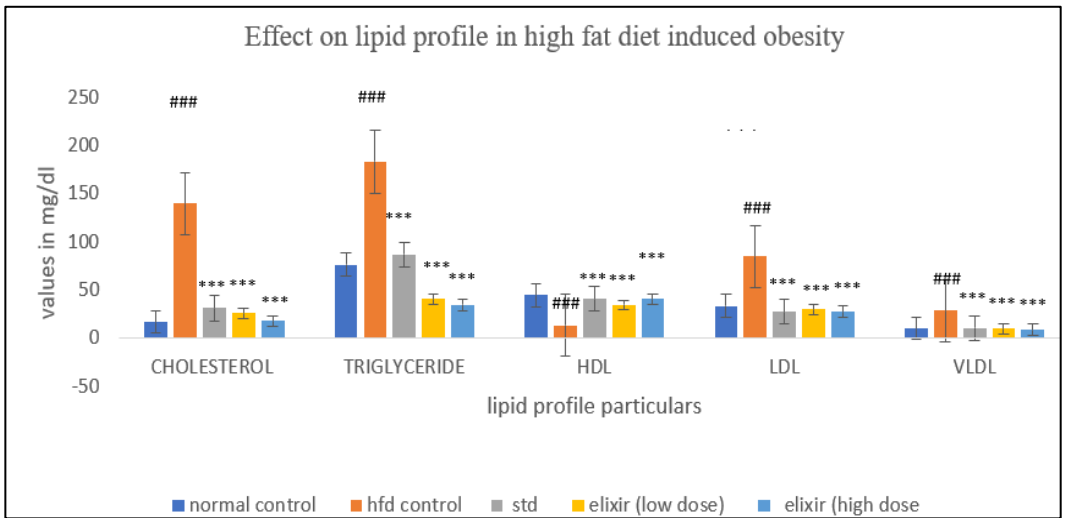
Graph 5. Effect on atherogenic index



Graph 6. Effect on LPO and GSH



Graph 7. Effect on SGPT and SGOT



Graph 8. Effect on lipid profile

1. Graph 1: The mean body weight of the HFD control group was found to significantly (###p<0.001) increased from the 5th week to the 7th week onward when compared to the normal control group. PHF elixir treatment at low dose (200mg/kg,p.o) and high dose (400mg/kg,p.o.) showed significant (***p<0.001) and equipotent reduction in mean body weight at 06th week and (**p<0.01) at 7th week when compared HFD control group. The reference standard orlistat was more potent in this regard and showed significant body weight reduction from the 5th week to 7th week.
2. The body weight found to significantly (p<0.001) reduced at 7 th week in elixir treated group and significantly increase (p<0.001) found in the body weight of high fat diet treated group when it compared to the normal control
3. Graph 2: Abdominal circumference significantly increase(p<0.001) in 5 th week and at 7 th week found significantly decrease(p<0.001) in standard group and elixir treated group while significantly increased in high-fat-diet treated group.
4. Graph 3: the BMI found (at end of the study) is significantly reduced (p<0.001) in elixir treated groups, while significantly increase (p<0.001) in BMI found at 7 th week in high fat diet treated group.
5. Graph 4: The glucose level was significantly reduced (p<0.001) in elixir treated and standard treated group and in high fat diet treated group found significantly increased (p<0.001) in comparison to normal control group.
6. Graph 5: The atherogenic index of high fat diet group found significantly increased (p<0.001) when it compared to normal control, and significantly decreased (p<0.001) in the standard and elixir treated group when compared with the high-fat-diet treated group.
7. Graph 6, The lipid peroxidation percentage found significantly increased (p<0.001) when it compared to normal control and in the treated group found significantly reduced (p<0.001) and in the standard treatment group it found reduced (p<0.05), and GSH restoration percentage found significantly reduced(p<0.001) in high fat diet treated group and in standard and elixir treated group found significantly decreased(p<0.001).
8. Graph 7, the SGOT and SGPT values found increased(p<0.001) in high fat diet treated group while significantly reduction (p<0.001) in the treatment groups found when it compared to high fat diet treated group.
9. Graph 8, the all-lipid profile parameters such as, triglyceride, cholesterol, LDL and VLDL were found significantly decrease (p<0.001) in treatment groups while in only high-fat-diet treated group is found significantly increase (p<0.001) in these parameters. HDL value found significantly decreased(p<0.001) in high fat diet treated group and while a significant increase (p<0.001) was found in other treatment groups.
10. Histopathology of the liver:
11. Histopathology of adipose tissue:

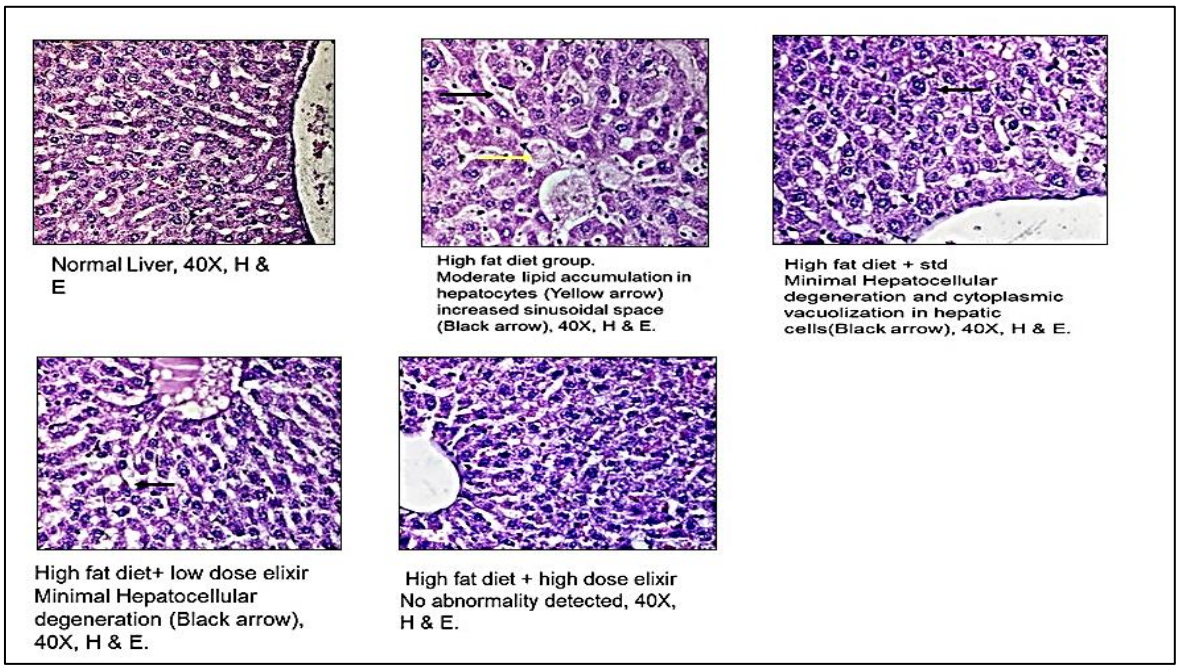


Fig. 1. Histopathological evaluation of liver

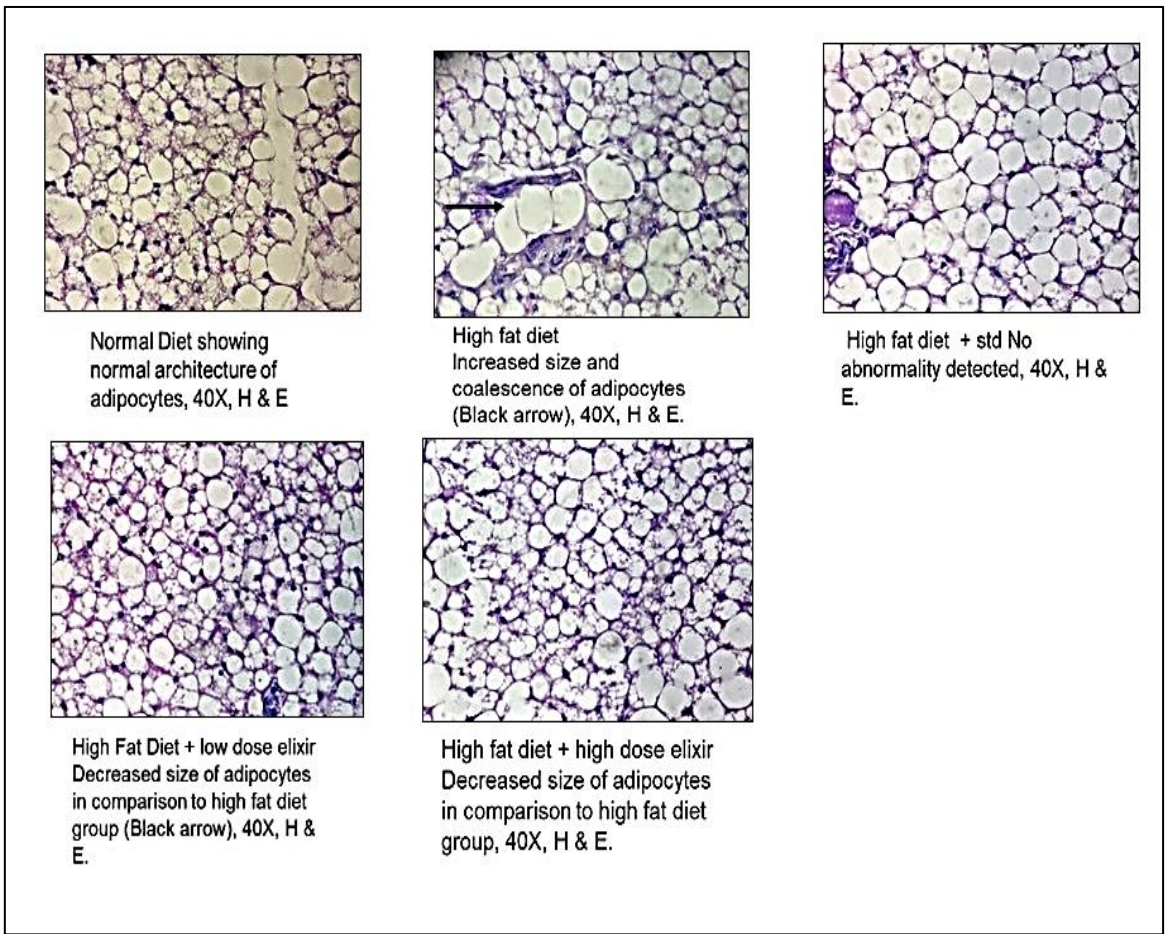


Fig. 2. Histopathological evaluation of adipose tissue

4. DISCUSSION

Obesity is a metabolic disorder, and more than 60 % populations are affected by it [1]. There is a major concern to treat complex disease with Obesity. Regulation of Daily lifestyle including diet, exercise and habits are essential to fight in severe case of obesity [3]. Available treatment for obesity is not sufficient and effective to treat obesity. Available allopathic systems also cause adverse effects [3]. Even some synthetic drugs were also withdrawn from the market, hence there is a great demand to develop a formulation which have anti-obesity effect [3]. In such cases, ayurvedic preparations are an alternative and safer option than the allopathic system [8]. In this view, we searched out traditionally claimed plant and modern literature report and accordingly prepared herbal formulations to get synergistic, multiple, and potential effect to treat and overcome obesity complexity. In high-fat-diet induced model, there are various mechanism which includes an increase in body weight, physical parameters, and lipid profile(hyperlipidaemia). and it alters the normal functioning of the liver and adipose tissue [18-20] In the present study, the high fat diet showed an increase in physical parameters, serum parameters and liver parameters and all these parameters were found normalized into treatment groups. The formulation may lead to utilising energy, significantly regulate lipid profile as hypolipidemic, reduces the fat accumulation as it reduces abdominal circumference, may maintain the normal function of the liver by normalizing the SGOT and SGPT level and tissue oxidative stress reduction as it restores GSH level of liver, and histopathological study of liver and adipose tissue reveals that tissue architecture is maintained in the treatment group. It stops or clear out the lipid accumulation in hepatocytes and degeneration of hepatocytes as well it reduces the size of coalescences in adipose tissue. Mainly saponin, phenolic acids, alkaloids and flavonoids, which are present in formulation responsible for activity. From this we conclude that polyherbal formulation have anti-obesity activity in dose dependent manner.

5. CONCLUSION

The present study was carried out to assess the anti-obesity effect of the combination of both extracts. The formulation showed a marked anti-obesity effect, in a group fed with a high fat diet in dose dependent manner. Formulation effects may be inhibition in lipid absorption i.e., hypolipidemic activity and inhibit fat deposition in tissues. The present study confirms anti-obesity effect of the formulation is due to a decrease in body weight, BMI, abdominal circumference, fat deposition in tissue, normalization of lipid parameters etc. From histopathology moderate lipid accumulation in hepatocytes, minimal focal necrotic foci and increased sinusoidal space indicating and in the polyherbal formulation comparable regenerative effect found in hepatocytes and in adipose tissue of high fat diet induced group revealed increased size and coalescence of adipocytes, mild perivascular Infiltration of polymorphonuclear cells whereas, size of adipocytes was reduced in the polyherbal formulation and also found ability to suppress the development of adipose tissue by high-fat-diet induced obesity. It is concluded that in future, this work can be extended for the identification of responsible constituent in formulation for its potent therapeutic effect.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENTS

We are thankful to Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune for their constant support and providing all the facilities to carry out this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3856