Advances in Bioresearch
Adv. Biores., Vol 16 (3) May 2025: 430-436

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.3.430436

Advances in Bioresearch

ORIGINAL ARTICLE

Therapeutic Effects of Polyherbal Formulation on Triton X-100 Induced Obesity in Rats

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ABSTRACT

Obesity is an escalating worldwide health issue marked by the excessive buildup of body fat, which heightens the risk of numerous metabolic disorders such as type 2 diabetes, certain types of cancer, and cardiovascular diseases. Research into the underlying mechanisms of obesity and potential therapeutic interventions remains a critical area of study. One widely used method to induce obesity in experimental models is the administration of Triton X-100 (non-ionic surfactant). Triton X-100 is known for its ability to induce hyperlipidemia by disrupting lipid metabolism, resulting in increased levels of circulating lipids and promoting fat deposition. This method has been extensively employed to study the effects of various pharmacological agents and natural products on lipid metabolism and obesity-related parameters. In this research, our goal was to examine the anti-obesity effect of a polyherbal formulation (PHF) of Allium sativum, Gymnema sylvestre, Glycyrrhiza glabra, and Zingiber officinale, using the Triton X-100-induced obesity model in rats. The PHF was composed of herbal extracts known for their potential lipid-lowering and metabolic regulatory properties. The objective was to evaluate the efficacy of the PHF in reducing body weight, serum lipid levels, liver function profile and improving overall metabolic health in obese rats. This study could provide important insights into the development of natural alternatives for the treatment of obesity and its associated complications.

Keywords: Obesity, Triton X-100, Hyperlipidemia, Polyherbal Formulation (PHF), Lipid Metabolism, Anti-obesity Activity.

Received 29.03.2025 Revised 26.04.2025 Accepted 27.05.2025

How to cite this article:
Gunjansing R, Prasad B, Avinash G, Vaishnavi P, Manaswi D, Devendra S. Therapeutic Effects of Polyherbal Formulation on Triton X-100 Induced Obesity in Rats. Adv. Biores., Vol 16 (3) May 2025: 430-436.

INTRODUCTION

Obesity is a health condition characterized by a substantial buildup of body fat, which poses major health hazards. It is generally measured using the Body Mass Index (BMI), and a BMI of 30 or greater is considered obese [1]. The reasons for obesity are multifaceted, involving genetic, environmental, and behavioural factors. Genetically, some individuals may have predispositions affecting metabolism, fat storage, and appetite control, often influenced by family history. Environmentally, diets high in calories but low in nutrients, physical inactivity, and limited access to healthy foods and recreational facilities are major contributors. Behaviourally, overeating, frequent snacking, a preference for high-fat and high-sugar foods, and a lack of regular exercise play significant roles. Psychological factors, such as stress, emotional eating, and mental health disorders like depression and anxiety, also contribute [2]. Additionally, medical conditions like hypothyroidism [3] and medications such as antidepressants can lead to weight gain [4].

Obesity is linked to numerous health complications, significantly impacting quality of life and longevity [5]. Cardiovascular diseases, including hypertension[6], coronary artery disease [7], and stroke [8], are common among obese individuals. Metabolic disorders, such as type 2 diabetes and dyslipidemia, occur due to insulin resistance and unhealthy cholesterol levels [9]. Respiratory problems, like sleep apnea and asthma, are more frequent due to fat deposits obstructing breathing and increased inflammation [10].

Musculoskeletal disorders, including osteoarthritis and chronic back pain, result from excessive pressure on joints and the spine. Gastrointestinal issues, like gastroesophageal reflux disease (GERD) and gallstones, are also more prevalent[11]. Moreover, obesity increases the risk of certain cancers, such as breast, colon, endometrial, and kidney cancers [12]. Increased rates of anxiety, depression, low self-esteem, social isolation, and discrimination are examples of psychosocial impacts.

Globally, Obesity is a serious public health issue that has reached epidemic proportions. According to the World Health Organization (WHO), global obesity has nearly tripled since 1975. In 2016, there were more than The World Health Organization (WHO) estimates that worldwide obesity has nearly tripled since 1975. In 2016, there were more than 1.9 billion overweight adults and 650 million obese adults. Childhood obesity is also on the rise, with 39 million children under the age of five classified as overweight or obese by 2020.1.9 billion overweight adults and over 650 million obese adults. Childhood obesity is also rising, with 39 million children under the age of 5 being overweight or obese in 2020 [13]. Between 2010 and 2040, the prevalence of overweight among Indian adults aged 20–69 years is expected to more than double, while the prevalence of obesity will triple. By 2040, it is projected that 30.5% of men and 27.4% of women will be overweight, and 9.5% of men and 13.9% of women will be obese [13]. Obesity (defined as a BMI of ≥30 kg/m^2) is projected to increase globally from 14% in 2020 to 24% by 2035, according to the World Obesity Atlas 2023. Over 800 million persons are predicted to suffer from obesity [14].

Available treatment is not satisfactory to cure obesity and has side effects. In this regards, ayurvedic preparation is an alternative option that has fewer side effects in the treatment of obesity. *Allium sativum, Gymnema sylvestre, Glycyrrhiza glabra, and Zingiber officinale were* selected for PHF preparation on the basis of their traditional claim. The Triton-induced obesity model was used to evaluate the effect of PHF.

MATERIAL AND METHODS

Plant collection and identification:

All herbal plant parts of *Zingiber officinale, Allium sativum*, *Glycorizza glabra*, and *Gymnema sylvestre* were collected and authenticated from Mankarnika Aushdhalaya, Pune, Maharashtra, India, in the form of coarse powder.

Extraction

The extraction of *Allium sativum, Gymnema sylvestre, Glycyrrhiza glabra, and Zingiber officinale* was performed using a maceration method with a solvent mixture of 80:20 ethanol and distilled water. The solvent mixture was prepared by combining ethanol and distilled water in the 80:20 ratio. Coarse powders of the respective plants were accurately weighed and placed into a different clean, dry extraction vessel. The prepared solvent mixture was then added to each vessel, ensuring the plant materials were fully submerged, typically using a solvent volume ten times the weight of the plant material. The extraction vessel was sealed, and the mixture was left to macerate for seven days, with occasional stirring to ensure thorough mixing and optimal solvent penetration. After the maceration period, the mixture was filtered to separate the liquid extract from the plant residues. The resulting filtrate was concentrated using an evaporator to remove the solvent. The concentrated extract was then further dried in a drying oven at a temperature not exceeding 50°C to eliminate any residual water, yielding a dry powder. The final dried extract was stored in airtight containers, protected from light and moisture, for future use [15].

Phytochemical Screening of Extract

The ethanolic extracts of *Allium sativum (AS), Gymnema sylvestre (GS), Glycyrrhiza glabra (GG), and Zingiber officinale (ZO)* were subjected to preliminary phytochemical testing to detect major preliminary phytoconstituents such as alkaloids, glycosides, phenols, tannins, flavonoids, and steroids.

Preparation of Polyherbal formulation (PHF)

The process of preparing the polyherbal formulation involved several meticulous steps to ensure its uniformity and effectiveness. Initially, precise amounts of herbal extracts from *Zingiber officinale, Allium sativum, Glycyrrhiza glabra, and Gymnema sylvestre* were dissolved in 15 ml of alcohol. At the same time, sodium saccharin was dissolved in 20 ml of water with continuous stirring until completely dissolved. The alcohol-soluble ingredients were then combined with the water-soluble ingredients in a clean container, ensuring uniform dispersion of all active components. To improve the stability and flavour of the elixir, methyl paraben and propyl paraben were added as preservatives, and orange syrup was included for a pleasant taste and aroma. The mixture was then diluted to the desired final volume with water, and a magnetic stirrer was used to thoroughly mix all ingredients. The final polyherbal formulation was labelled properly and then transferred to airtight containers. To maintain its stability and efficacy, it is kept in a cool and dry place away from direct sunlight [16].

Acute oral Toxicity

The OECD Guideline 425 was utilized to assess the acute oral toxicity of the formulation. Overnight-fasted female Wistar rats were administered the polyherbal formulation orally in accordance with the guidelines. The rats were monitored individually for approximately 48 hours for any behavioral or neurological abnormalities, including diarrhea, feeding behavior changes, convulsions, tremors, lacrimation, sleep disturbances, and salivation as indicators of toxicity. To verify any potential mortality, the rats were observed for an additional two weeks [17].

Triton X-100 induced hyperlipidemia

Experimental animal:

Thirty Wistar albino male rats, each weighing between 150 and 180 grams, were acclimatized to the laboratory environment over a period of 7 days. The temperature was kept at 25 ± 1°C, and the light-dark cycle was set for 12 hours. The rodents were fed conventional rat chow and water ad libitum.

Induction of Hyperlipidemia

Hyperlipidemia was induced in twenty-four experimental rats by administering freshly prepared Triton X-100 solution through a single intraperitoneal injection at a dosage of 100 mg/kg body weight. The Triton X-100 was dissolved in physiological saline (0.9% NaCl solution). This procedure was carried out after the rats had fasted overnight for 18 hours [17].

Animal Grouping and Treatment

The rats were randomly divided into five groups. Four of these groups consisted of hyperlipidemia-induced rats by Triton X-100, while the remaining group served as a normal control, receiving only water orally for 7 days. The other four groups received respective treatments for the next 7 days as follows:

- Group I (Normal Control): Received water orally.
- Group II (Induction Control): Induced with hyperlipidemia through a single intraperitoneal injection of Triton X-100.
- Group III (Reference Standard): Hyperlipidemic rats administered atorvastatin at a dose of 20 mg/kg orally daily for 7 days.
- Group IV (Treatment Lower Dose): Hyperlipidemic rats treated with PHF 150 mg/kg orally every day for 7 days.
- Group V (Treatment Higher Dose): Hyperlipidemic rats treated with PHF 300 mg/kg orally every day for 7 days.
- Throughout the study, rats were observed daily for signs of toxicity or adverse effects, with body weight (BW), food intake, and water consumption recorded daily [18].

Blood Sample Collection and Serum Preparation

Blood samples were drawn from rats' retro-orbital plexuses by the end of the study. The serum samples were collected and proceed for biochemical analysis.

Serum Lipid Profile Analysis

The serum lipid profile, including total cholesterol, total lipids, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), was measured using a semi-automated bioanalyzer.

Liver Function Tests

The activities of serum alanine transaminase (ALT) and aspartate transaminase (AST) were tested using a semi-automated analyzer.

Histopathological Examination

Liver and adipose tissue specimens were dissected from the rats and proceed for histopathological studies [18].

RESULTS

Phytochemical test

Phytochemical analysis of hydroalcoholic herbal extract of *Allium sativum* (AS)*, Gymnema sylvestre* (GS), *Glycyrrhiza glabra* (GG)*, and Zingiber officinale* (ZO) showed the occurrence of carbohydrate and flavonoid. GS indicates the presence of terpenoids, tannins, saponins, and alkaloids. AS and ZO showed the presence of saponins and alkaloids.

Effect of PHF on Body weight of rats

The study assessed the effects of a polyherbal formulation (PHF) on body weight (BW) in rats with Triton X-100-induced hyperlipidemia, with weights measured on day 0 and day 7. The normal control group, which received only water, maintained stable BW throughout the study. Conversely, the induction control group, treated with Triton X-100, showed significant weight gain by day 7. Rats administered atorvastatin (20 mg/kg) experienced a reduction in BW by the end of the study, approaching towards normalisation. The group treated with a low dose of PHF (150 mg/kg) also exhibited a decrease in weight gain, though to

a lesser extent than the atorvastatin group. Importantly, the high-dose PHF group (300 mg/kg) showed a significant reduction in body weight by day 7, comparable to the results seen with atorvastatin

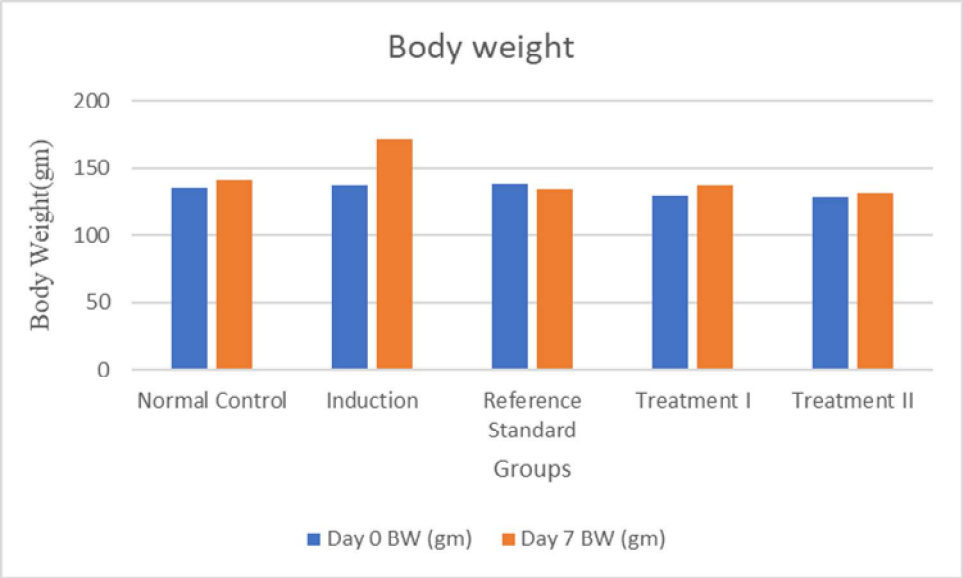

Fig. 1: Effect of PHF on body weight of triton X 100 induced rats

Serum Lipid Profile

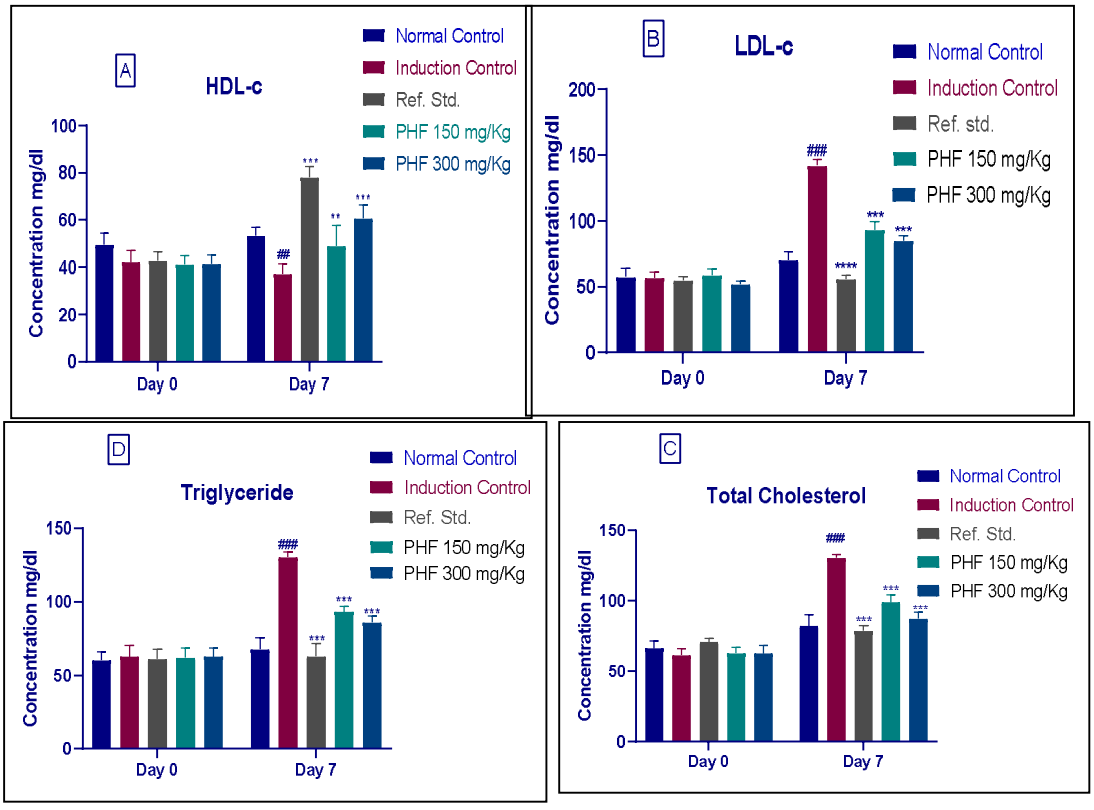

Fig. 2: Effect of PHF on Lipid Profile A) HDL-c level, B) LDL-c level, C) TC level, and D) TG level Values are expressed as mean ± SEM; n=6; data analyzed by one-way ANOVA test followed by Dunnett Multiple Comparisons Test. Level of significance ### P< 0.001 when compared to the normal control group and * P<0.05, ** P<0.01 and *** P<0.001 when compared to the induction control group.

The induction of hyperlipidemia with Triton X-100 led to a significant increase in LDL, TG, and TC levels and a decrease in the HDL level in the induction control group compared to the normal group.

Administering atorvastatin at a dose of 10mg/kg significantly lowered these LDL, TG, and TC while increasing HDL levels, bringing them closer to those of the normal control. Treatment with the polyherbal formulation (PHF) at a lower dose of 150 mg/kg also decreased LDL, TG, and TC levels and raised HDL levels, although not as effectively as atorvastatin, while the higher dose of PHF at 300 mg/kg resulted in a marked reduction in LDL, TG, and TC and a significant increase in HDL levels, nearing the effectiveness of atorvastatin. All these lipid profiles expressed in Fig.2

Liver Profile Test

The induction of hyperlipidaemia with Triton X-100 resulted in a significant increase in the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the induction control group compared to the normal control group ($P < 0.001$). Administering atorvastatin at a dose of 10 mg/kg significantly lowered ($P < 0.001$) the levels of ALT and AST, bringing them closer to those observed in the normal control group. Treatment with the polyherbal formulation (PHF) at a lower dose of 150 mg/kg also decreased ALT and AST levels, although not as effectively as atorvastatin. However, the higher dose of PHF at 300 mg/kg resulted in a marked reduction ($P < 0.001$) in ALT and AST levels, nearing the effectiveness of atorvastatin. All these liver profile results are summarized in Fig.

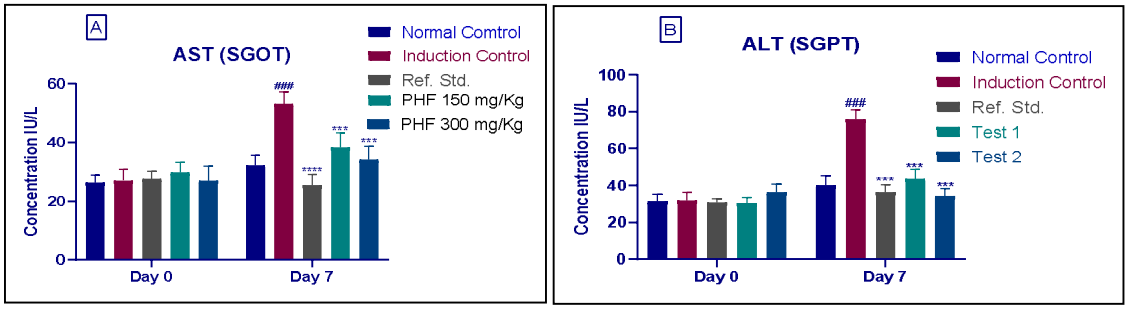

Fig. 2: Effect of PHF on Liver function A) AST level B) ALT level

The data was analyzed using a one-way ANOVA test and then the Dunnett multiple comparisons test. The values are shown as mean ± SEM, with n = 6. Level of significance * P<0.05, ** P<0.01, and *** P<0.001 in comparison to the induction control group and ### P<0.001 in comparison to the normal control group.

Triton-X 100 Liver Histopathological study

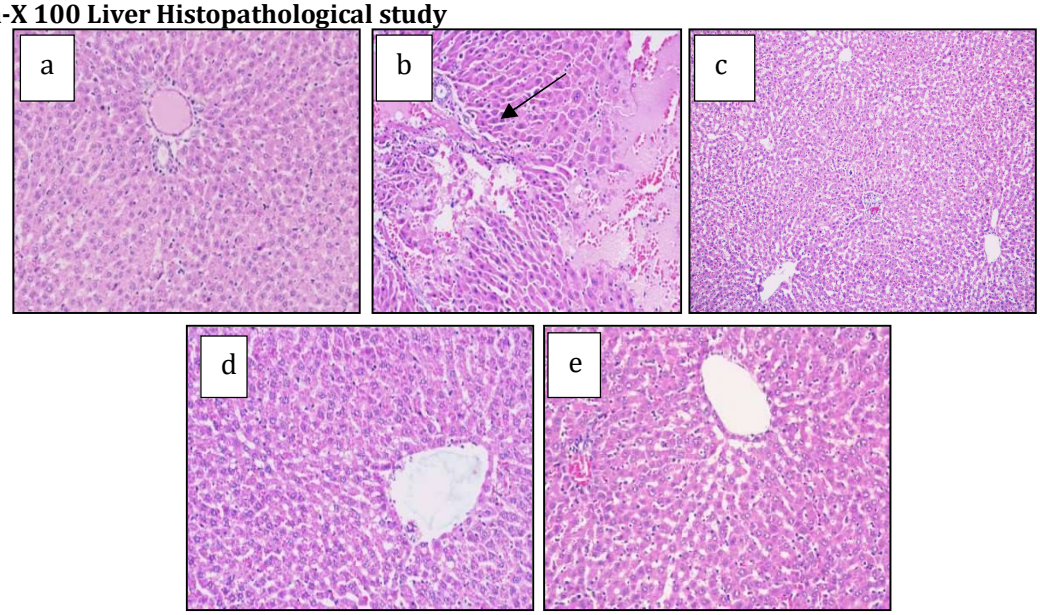

Fig: Effect of PHF on Histopathology of Hepatocytes of Triton X-100 Induced Hyperlipidaemic Rats

a) The result shows normal architecture of hepatocytes in normal control. b) Microscopic examination of liver tissue of induction shows necrosis (arrow) and infiltration of inflammatory cells in hepatocytes. c) The result shows normal architecture of hepatocytes in the reference standard. d) Microscopic examination of liver tissue shows normal architecture of hepatocytes, Kupffer cells, central veins, sinusoid space, bile duct cells, and portal triad. The hepatocyte nucleus rounded and pale cytoplasm located radiated. e) Microscopic examination of liver tissue shows normal architecture of hepatocytes, Kupffer cells, central veins, sinusoid space, bile duct cells, and portal triad. The hepatocyte nucleus rounded, and the pale cytoplasm located radiated.

Triton-X 100 Adipose Tissue Histopathological study

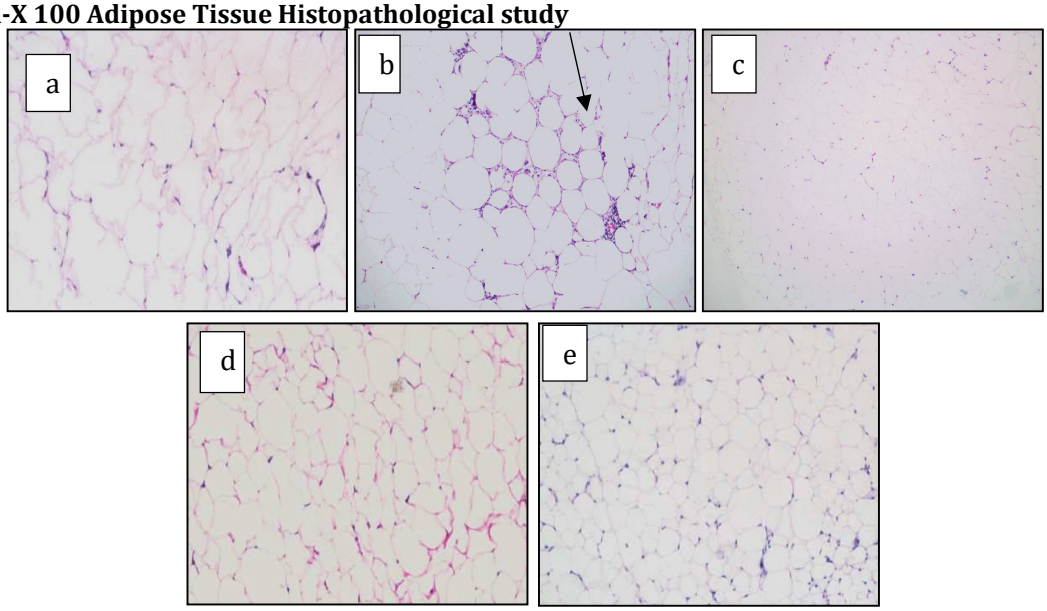

Fig: Effect of PHF on Histopathology of Adipose tissue of Triton X 100 induced hyperlipidaemic rats
a) Microscopic examination of normal control fat tissue shows normal architecture of cells and nucleus at the peripheral site. b) Microscopic examination of induction control fat tissue shows inflammatory cell (arrow) infiltration in adipose cells. c) Microscopic examination of ref. standard fat tissue shows normal architecture of cells and nucleus at peripheral site. d) Microscopic examination of PHF 150 mg/kg fat tissue shows normal architecture of cells and nucleus at peripheral site. e) Microscopic examination of PHF 300 mg/kg fat tissue shows normal architecture of cells and nucleus at peripheral site

DISCUSSION

This study evaluated the potential of a polyherbal formulation (PHF) containing *Zingiber officinale (ginger), Allium sativum (garlic), Glycyrrhiza glabra (licorice),* and *Gymnema sylvestre (Gurmar)* in managing Triton X-100 induced hyperlipidemia in rats. Hyperlipidemia, marked by increased serum lipids, is a major risk factor for cardiovascular diseases and obesity. Triton X-100 effectively induces hyperlipidemia by disrupting lipid metabolism, leading to elevated triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), and reduced high-density lipoprotein cholesterol (HDL-C) [19].

Efficacy of PHF in Hyperlipidemia

The PHF treatment significantly lowered TG, TC, and LDL-C levels while increasing HDL-C levels. Ginger, garlic, licorice, and gurmar, each known for their lipid-lowering properties, contributed synergistically to these effects. Ginger enhances lipid metabolism and bile acid secretion [20], garlic inhibits cholesterol synthesis [21], licorice reduces oxidative stress and enhances lipoprotein lipase activity [22], and Gurmar inhibits lipid absorption and modulates lipid metabolism [23].

Liver Function Tests (LFTs)

Liver function tests showed elevated aspartate transaminase (AST) and alanine transaminase (ALT) levels due to Triton X-100, indicating liver damage. PHF treatment significantly reduced ALT and AST levels, suggesting hepatoprotective effects and mitigation of Triton X-100-induced liver damage.

Body Weight Parameter

Triton X-100 induced hyperlipidemia also led to increased body weight, reflecting lipid accumulation and obesity. PHF treatment significantly reduced body weight, indicating anti-obesity effects through modulation of lipid metabolism and reduced lipid accumulation.

The lipid-lowering and hepatoprotective effects of the PHF can be attributed to inhibited cholesterol synthesis, enhanced lipid catabolism, and increased lipid excretion. The reduction in LDL-C and increase in HDL-C levels suggest decreased cardiovascular risk. Due to these activities highlighting its potential as a natural therapeutic option for managing hyperlipidemia and obesity. Further research, including clinical trials, is required to confirm these findings and understand the underlying mechanisms.

CONCLUSION

The polyherbal formulation (PHF) comprising *Zingiber officinale, Allium sativum, Glycyrrhiza glabra, and Gymnema sylvestre* demonstrated significant therapeutic potential in managing Triton X-100 induced

hyperlipidemia in rats. The PHF effectively lowered serum triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels while increasing high-density lipoprotein cholesterol (HDL-C) levels, thereby improving the lipid profile. Additionally, PHF treatment reduced elevated aspartate transaminase (AST) and alanine transaminase (ALT) levels, signifying hepatoprotective effects. Overall, this indicates its anti-obesity properties.

The combined lipid-lowering, hepatoprotective, and anti-obesity effects of the PHF can be attributed to the synergistic actions of its herbal components, each known for their beneficial impact on lipid metabolisms. These findings suggest that PHF could be a valuable natural therapeutic option for managing hyperlipidemia, and obesity. Further research, including clinical trials, is required to validate these results and explore the underlying mechanisms of action.

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