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**Research Article****Assessing the therapeutic potential of *Zinnia elegans* in mitigating atherosclerosis in mice model****Hafsah Shafiq, Sania Riaz, Areej Talat, Farah Ashfaq, Aqsa Zafar, Mahnoor Tariq***Department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology, Islamabad.***ABSTRACT**

Atherosclerosis, a major contributor to cardiovascular disorders, marked by the accumulation of plaques within arterial walls, potentially resulting in heart attacks or strokes. Considering its widespread impact, the present study explores the potential of *Zinnia elegans* extract in mitigating atherosclerosis in a murine model. Five groups of BALB/c mice were established: a control group, a positive control group receiving a high-fat diet (1ml/day), a drug-treated group administered Lipiget (0.2ml/day), an extract-treated group receiving *Zinnia elegans* (0.2ml/day), and a combined-treatment group receiving both Lipiget (0.1ml/day) and *Zinnia elegans* (0.1ml/day). Following a six-week induction of atherosclerosis via high-fat diet, treatments were administered for four weeks. Notably, biochemical analysis revealed significant reductions in triglyceride levels (134.5 ± 0.5 mg/dL, $p < 0.05$) and weight gain in the extract-treated and combined-treatment groups. Histopathological examination revealed normal tissue morphology in the plant extract treated group. These findings suggest that *Zinnia elegans* extract exhibits anti-atherosclerotic properties, warranting further investigation into its therapeutic potential for managing hyperlipidemia and preventing atherosclerosis.

Keywords: *Zinnia elegans*; atherosclerosis; lipiget; high-fat diet.**INTRODUCTION**

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. These are indeed a major global health concern, responsible for a significant number of deaths each year. CVDs encompass a range of heart and blood vessel disorders, including coronary heart disease, cerebrovascular disease, rheumatic heart disease, and other related conditions. Among the leading causes of CVD-related deaths, heart attacks and strokes are prominent, and unfortunately, a substantial proportion of these fatalities occur prematurely in individuals younger than 70 years of age (WHO, 2021; Dichgans et al., 2019).

A protein known as apolipoprotein E exists in different forms, and is coded by a gene found on chromosome 19. This protein facilitates the removal of excess cholesterol from the bloodstream by transporting it to LDL receptors on hepatocytes for clearance and metabolic processing. When apolipoprotein E is dysfunctional, it can't attach to these receptors and in turns, initiates the disease (National Center for Biotechnology Information, 2011). The onset of atherosclerosis begins with damage to the endothelium, the artery's inner lining (Poznyak et al., 2020).

Oxidized LDL triggers an inflammatory response, attracting monocytes that differentiate into macrophages within the arterial wall. These macrophages engulf oxLDL particles, becoming foam cells and forming fatty streaks, early lesions in atherosclerosis. This process releases various cytokines and growth factors, perpetuating inflammation and attracting more immune cells (Gisterå & Hansson, 2017). Synthetic medications are categorized into lipid-lowering, antiplatelet,

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antihypertensive, and antidiabetic agents. Additionally, anti-inflammatory drugs are prescribed to mitigate atheroma formation. Among these, low-dose colchicine has been found to be effective in reducing the adverse effects of cardiovascular disease in individuals with stable atherosclerosis (Ridker, 2023). The Lipid lowering drug is actually the statin-based drug with addition to fibrates. Whereas, the anti-hypertensive drugs are composed of agents which are NO donors, β -blockers, Ca^{+2} channel blockers, and ACE inhibitors. The agent present in anti-platelet clotting drug is acetylsalicylic acid which acts on COX-1 receptor.

The *Asteraceae* family, also known as the sunflower family, reigns supreme as the largest flowering plant family on Earth. *Asteraceae* members have spread far and wide across the globe, with a particular fondness for South Asia, South Africa, and South America. The family, additionally, claims a dazzling array of ornamental plants, including *Cosmos sulphureus* and the ever-popular *Zinnia elegans* (Gomaa et al., 2019). *Zinnia* itself is a genus within *Asteraceae*, comprising about 20 species of annual and perennial plants native to South America and Mexico. *Zinnia elegans*, commonly known as common zinnia or simply zinnia or Gul-e-zar is likely found throughout Pakistan wherever ornamental flowers are grown (Gomaa et al., 2019).

Studies have shown that *Zinnia* species contain a rich variety of secondary metabolites, falling into several chemical classes. These include flavonoids, sesquiterpenes, sterols, and anthocyanins. Among these, sesquiterpene lactones stand out as the most abundant compounds isolated from *Zinnia* (Gomaa et al., 2019).

Zinnia elegans demonstrated a notable protective effect against ethanol-induced gastric mucosal damage in mice. Ethanol-induced stomach mucosal injury is associated with increased levels of TNF and IL-1. However, pre-treatment with *Zinnia elegans* resulted in a significant reduction of IL1 β and TNF α levels, indicating its anti-inflammatory effects against gastric mucosal damage (Hameed et al., 2022).

In a related study, the effects of *Zinnia elegans* leaves were examined both in vitro and in vivo as a treatment for CCl₄-induced toxicity in albino rats. The findings demonstrated that the leaves contain a high level of total phenols. Additionally, the *Zinnia elegans* leaf extract improved NO, Hydrogen peroxide, alanine transaminase (ALT), malondialdehyde (MDA), urea, creatinine, glutathione S-transferase (GST), and superoxide dismutase (SOD) levels (Mohamed et al., 2015).

The mice/rats and rabbits are commonly used as animal models in this case because of quick lesion formation; ease in maintenance and breeding of mice and rabbits; transgenic and genetic flexibility. Thompson used an inbred strain of mice, known as (C57BL/6) and fed them with high-fat diet and it was concluded that this mice strain developed atheroma in aortic valve region. So, in this way, this strain of mice has been commonly used for genetic, biochemical, and lipoprotein profile analysis after induction with atherosclerosis (Zhang et al., 2021).

The purpose of this study is to develop atherosclerosis-induced mice model via high fat diet and then evaluate the potential of *Zinnia elegans* extract against atherosclerosis and to also compare those effects with that of a standard treatment. The parameters for evaluation were serum triglyceride levels, body weight, and histopathological changes in mice model. This study would pave a way for the development of herbal drugs against the atherosclerosis disease.

MATERIALS AND METHODS

Animal Model Selection

Ten adult male BALB/c mice were utilized each varying between 25 to 28 g and aged 3 to 4 weeks were used in this study. These mice were obtained from the Animal House of the Department of pharmacy, Capital University of Science and Technology. After retrieving, the mice were acclimatized to the surrounding environment for a period of one week. They were divided into two groups: a control group and an experimental group given unrestricted access to food and water, and housed in a controlled environment with proper temperature, humidity, and natural light-dark cycles. After this acclimatization phase, the animals were treated according to the protocols assigned to each group. This ethical research work on mice model received ethical approval from Ethical Review Committee of the Department of Bioinformatics and Biosciences, Capital University of Science and Technology, Pakistan.

Normal Dietary Components

The typical diet consisted of various components, including vitamins, powdered milk and chokar (wheat bran), flour, and appropriate amount of dried fish powder. These ingredients were mixed to create a required amount of feed, which was later portioned into smaller portions. Each piece weighed around fifty grams and subsequently, a single mouse consumed around a gram of portion on daily basis.

Fat-Rich Diet Composition

Over the course of six weeks, a high-fat diet was orally administered to the mice, comprising the mixtures of coconut and vegetable oil in a 2:1 ratio. A total of 50ml of this mixture was made and then stored in a bottle. Initially, each mouse received a daily dose of 0.2ml of this composition for the period of a week on the basis of weight. This dose was then increased to 0.3ml and then to 0.4ml in the next two weeks. Thus, 0.3 to 0.4ml of diet was then orally administered to experimental mice group daily for the remaining three weeks, approximating six weeks of the fat-rich diet administration (Amjad et al., 2023).

***Zinnia elegans* Extract Preparation**

The fresh leaves of *Zinnia elegans* locally named as “dreamland” in mix and scarlet color was obtained from a nursery at Tulsa Road, Rawalpindi, and was sterilized, washed, and leaves were obtained. These leaves were air dried for two days, away from sunlight and then these dried leaves were ground to powder. The green powder was then weighed on a digital scale. The powder weighed 8.75 grams. This powder was then macerated with 80% ethanol (comprising 80 mL ethanol and 20 mL distilled water) and kept on rotary shaker for 48 hours. Extract was filtered, and the filtrate was then dried in petri plate for the purpose of obtaining the dried powder. The dilutions of dried plate powder were prepared by adding distilled water for oral administration. Each mouse received 3.8 mg of plant powder in these dilutions (Misra et al., 2018).

FTIR Analysis

The FTIR (Fourier Transform Infrared Spectroscopy) was performed on the powdered plant leaves and the subsequent results were analyzed. The main purpose of this analysis was to identify and analyze the functional groups present in the *Zinnia elegans* leaf powder. This analysis confirmed the chemical composition of the extract, revealed bioactive compounds that contributed to the plant’s therapeutic properties. Specifically, FTIR identified the different bonds and molecular structures in the plant extract, which are linked to its potential antioxidant, anti-inflammatory, and lipid-lowering effects (Pharmawati and Wrasati, 2020).

Drug Preparation

Atorvastatin, commonly recognized as Lipiget, was procured from a local pharmacy and employed as the standard medication in this study. Ten tablets, each containing 10mg of Atorvastatin, were crushed, and subsequent dilutions were prepared using distilled water. The dilutions were prepared by crushing half of a tablet (i.e., 5mg) and dissolving the powder in 50ml distilled water.

Experimental Framework

After being weighed, documented, and tagged (by marking group number on their tails), all mice were divided into five groups i.e., each group having two mice. All mice received ethical treatment. There may also be possibility of checking different doses of plant extract on mice models.

1. Control Group: Animals in group 1 were provided regular feed and clean water for 24 hours, serving as the control group.
2. Positive Control Group: Animals in group 2 were given a regular diet along with a high fat diet daily for six weeks.
3. Drug Treated Group: Animals in group 3 received a standard diet with a meal based on high-fat daily for nearly six weeks. After this period, Lipiget dilutions were administered at a dosage of 0.2 ml per day for four weeks.
4. Extract-Treated Group: Over four weeks, animals in group 4 were fed a standard diet with a high-fat meal. *Zinnia elegans* extract was given at a dosage of 0.2ml per day for nearly four weeks.
5. Combined-Treatment Group: Animals in group 5 were fed a standard diet with a daily high fat meal for six weeks’ period. Afterward, a combined treatment was administered, consisting of *Zinnia elegans* extract and medication dilutions at doses of 0.1 to 0.15 ml per day for four weeks (Kumar & Sudha, 2023).

Morphological Analysis and Weight Measurement

The body weights of the mice were recorded daily via the digital scale. Each mouse was kept in the large glass beaker and placed on digital scale to restrict its movement. Following a six-week period of feeding with a high-fat diet, a significant increase in body weight was observed. Other morphological and physical aspects like the physical health, movement, posture, fur etc. was observed before and after the experiment and comparison was made.

Biochemical Based Analysis

At the conclusion of the experiment, the mice were grouped, and blood was obtained via cardiac puncture. Prior to blood collection, each mouse was anesthetized with chloroform, and the collected blood was later centrifuged to provide serum. Serum triglycerides test was subsequently performed for each group and the results was subsequently compared (Amjad et al., 2023).

Histopathological Analysis

For histopathology, after obtaining of blood via cardiac puncture, dissection was done using scissors and the organs were cut and isolated from the body using forceps. Stomach of plant treated mouse, intestines of control group mouse, and positive control group mouse were obtained and placed in the tube containing formalin. These samples were sent to lab for histopathology analysis (Rathnamali, 2022).

Statistical Evaluation

All the data was analyzed and presented as a mean \pm standard deviation in one-way ANOVA.

RESULTS AND DISCUSSION

FTIR Analysis

FTIR spectra of dried leaves powder of *Zinnia elegans* is shown in Figure 1. In FTIR spectra, several peaks are observed that are at 2359.84 $[\text{cm}]^{-1}$, 2203 $[\text{cm}]^{-1}$, 2033 $[\text{cm}]^{-1}$, 1977 $[\text{cm}]^{-1}$, 1020.47 $[\text{cm}]^{-1}$, 673.2 $[\text{cm}]^{-1}$, 531.4 $[\text{cm}]^{-1}$, 460.6 $[\text{cm}]^{-1}$ wavelength. Peak observed at 2359.84 $[\text{cm}]^{-1}$ indicates an N-H bond that depicts the presence of amino acids and related compounds (Pharmawati and Wrasati, 2020). The peak that is formed at 2203.93 $[\text{cm}]^{-1}$ C triple (\equiv) C bond which depicts the presence of terminal alkynes (Kainat et al., 2022). Peak formed at 2033.85 $[\text{cm}]^{-1}$ that represents NH₃ bond which indicates the presence of free amino acids and their hydro halides (Pharmawati and Wrasati, 2020). Peak at 1977.16 $[\text{cm}]^{-1}$ indicates C=O bond stretch that depicts the presence of conjugate acid and aldehyde (Kainat et al., 2022). Peak observed at 1020 $[\text{cm}]^{-1}$ represents the presence of C-O that are alcohols and phenols (Rani et al., 2021). Peak formed at 673.2 $[\text{cm}]^{-1}$ represents the presence of C triple bond with hydrogen (C \equiv H) that shows the presence of vinyl hydrocarbon compounds (Rani et al., 2021). Peak observed at 531.4 $[\text{cm}]^{-1}$ represents C-I stretch that indicates the presence of halo compounds (Rani et al., 2021). Peak observed at 460.6 $[\text{cm}]^{-1}$ indicates C-OH bond that shows the presence of phenols (Kainat et al., 2022).

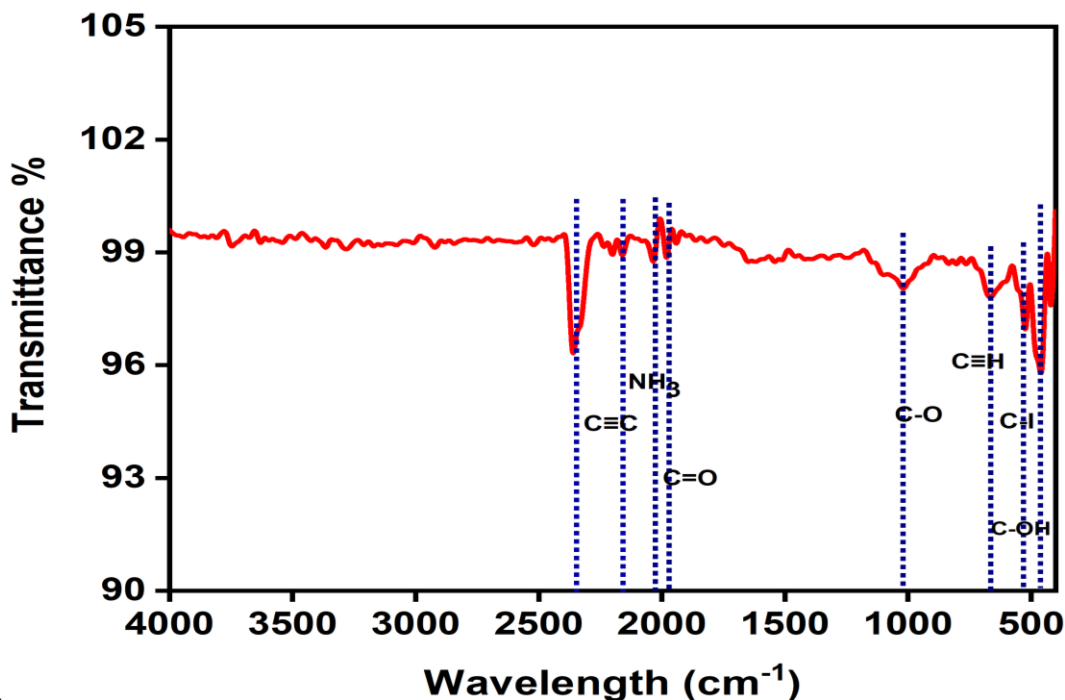


Figure 1. FTIR spectra of dried leaves powder of *Zinnia elegans*.

Morphological Analysis

Mice subjected to a high-fat diet (HFD) exhibited a significant increase in body weight and fat mass compared to those on a standard diet. This weight gain is primarily attributed to the accumulation of body fat, particularly noticeable in the abdominal region. These changes render the mice visibly larger and bulkier, with pronounced fat deposits (adiposity) that contribute to a rounded appearance. Overweight mice often struggled to reach all areas of their bodies for grooming, leading to unkempt fur. This can result in the fur appearing dull or greasy due to reduced grooming activity

and potential metabolic changes associated with obesity. The reduced grooming efficiency is a direct consequence of their increased body mass and reduced flexibility. Due to the substantial weight gain, HFD-fed mice exhibited decreased mobility and a more sedentary lifestyle. This reduced activity level was evident when these mice were housed together, as they showed less movement and exploratory behavior compared to control group (Gao et al., 2015). This was evidenced by a reduction in resistance to oral gavage (administration) and a general decline in mobility. Furthermore, a decrease in the consumption of regular chow was observed. These changes are indicative of a disorder such as obesity, or cardiovascular disease. Additionally, the high-fat diet induced a decrease in overall activity levels and changes in sleep pattern (increased sleep duration) (Gao et al., 2015).

Behavioral Analysis

Mice on a high-fat diet were observed to be exploring their environment less, spending more time in familiar areas and showing diminished interest in new objects. This reduced exploratory behavior is indicative of cognitive impairment, which is a common consequence of diet-induced obesity. There was a noticeable decrease in social interactions among HFD-fed mice. These mice exhibited less playfulness and grooming of cage mates, indicating a reduction in social behavior and increased immobility. These depressive behaviors are apparent during regular handling and observation, where the mice appear less responsive and more lethargic. Such mice exhibited less interaction with other mice, highlighting a significant change in their social behavior patterns (Leuven et al., 2020).



Figure 2. Observed weight gain and disheveled fur in experimental mice.

Body Weight Analysis

Body weight was monitored daily throughout the six-week high-fat diet intervention period. Mice in the high-fat diet group exhibited a significant increase in weight compared to the control group. At the beginning of the study the average body weight of the mice was approximately between 25 and 28 grams, indicating a healthy and normal weight range. Following a one-week acclimatization period, daily oral administration of the high-fat oil resulted in an average weight gain of 8 grams per mouse. Mice in control group were not given high fat diet.

Weight of all groups were measured using a digital weight balance. Gradual increase in the weight was observed in all four groups. By the end of the intervention, mice in the high-fat diet group displayed an average weight of approximately 38-42 grams. The weight change for all groups is depicted graphically below in figure 4. The body weights were analyzed via one-way ANOVA. Table 1 shows the body weights during high fat diet administration i.e., before treatment. The body weights were not significantly different among the groups initially, except by Day 42 where significant differences emerged. All mice were subjected to a high-fat diet regimen for six weeks, following which body weight measurements were recorded for each experimental group. Subsequently, a four-week treatment phase commenced. The control group received no intervention, while the positive control group continued on the high-fat diet. The plant extract group was administered an appropriately diluted *Zinnia elegans* extract, the standard group received Lipiget dilution, and the combined group was given a mixture of *Zinnia elegans* extract and Lipiget dilution. Body weight was monitored daily throughout the treatment period and analyzed using one-way ANOVA as shown in Table (2).

Significant differences in body weights among the groups were observed on Days 1, 6, 10, and 16, indicating that the treatments had a significant impact on body weight early in the treatment phase as indicated in Figure 2. This suggests that the interventions (Lipiget, *Zinnia elegans* extract, or their combination) influenced body weight compared to the control and positive control groups.

Serum Triglycerides

Serum triglycerides are a type of fat found in the blood, and they play a significant role in the diagnosis and management of atherosclerosis. In clinical practice, measuring serum triglycerides helps assess an individual's risk for developing atherosclerosis and related cardiovascular events. High triglyceride levels often indicate the presence of other lipid abnormalities, such as low high-density lipoprotein (HDL) cholesterol and small, dense low-density lipoprotein (LDL) particles, which are more atherogenic. They are carried in the blood by lipoproteins, particularly very-low-density lipoproteins (VLDL) and remnant lipoproteins, which can penetrate the arterial wall and promote inflammation and plaque formation. This process is facilitated by the presence of apolipoprotein B containing lipoproteins, which are key players in atherogenesis (Ginsberg and Hegele, 2020).

Table 1. Body weights (g; Mean± SD) during high fat diet administration P<0.05 is significant; one-way Anova.

Groups	Days						
	Day-1	Day-5	Day-11	Day-16	Day-21	Day-32	Day-42
Control	31.35± 2.33a	32.6± 3.67a	33.06± 3.87a	34.8± 5.8a	25.9± 7.1a	28.5± 5.8a	31.35± 1.34c
Standard	34.3± 0.282a	34.9± 0.98a	34.5± 1.69a	34.5± 0.14a	36.1± 0.56a	36.3± 0.14a	36.6± 0.84b
Combined	33.35± 0.77a	34.2± 0.77a	34.2± 0.70a	35.3± 0.14a	36.9± 0.49a	37.4± 0.56a	37.35± 0.49b
Plant Extract Treated	33.9± 2.26a	35.6± 1.97a	35.8± 2.4a	36.6± 3.11a	38.4± 3.11a	38.9± 2.12a	39.5± 2.12a
Positive Control	34.1± 0.282a	34.6± 0.28a	34.8± 0.14a	35.4± 0.56a	37.05± 0.35a	36.85± 0.63a	36.25± 1.06b
P value	0.39	0.65	0.79	0.95	0.07	0.075	0.011

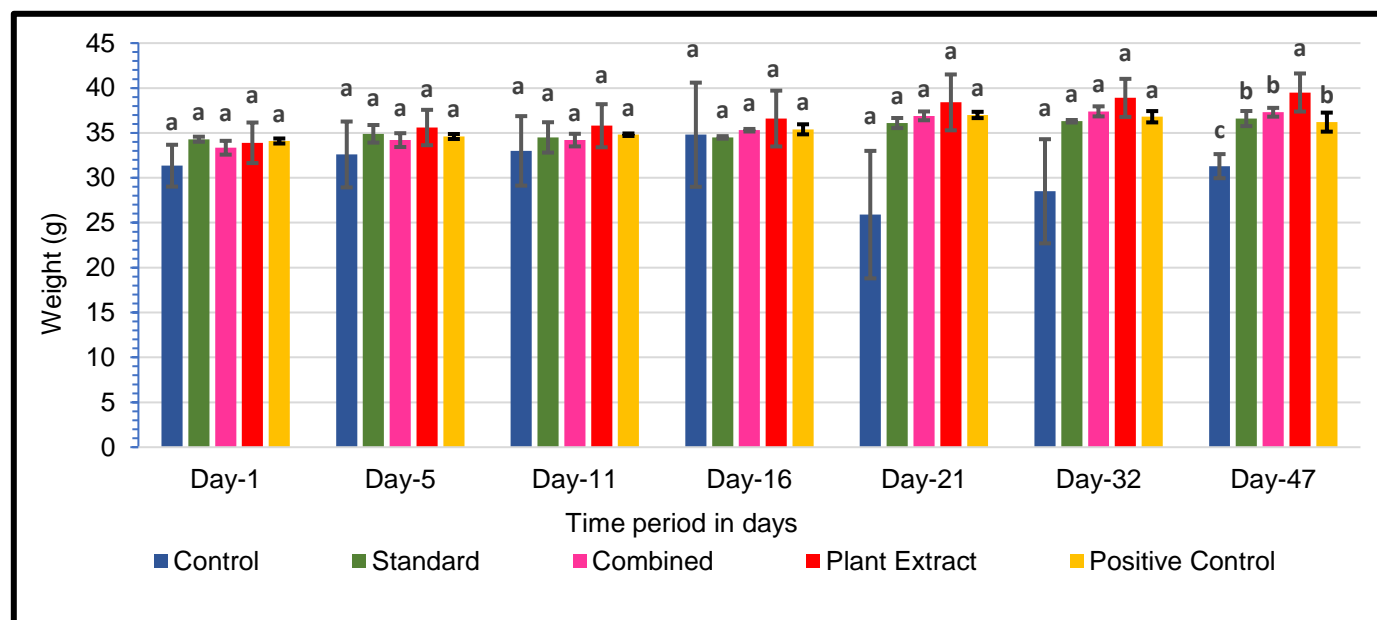


Figure 3. Weights before treatment

Table 2. Body weights during treatment.

Groups	Days (Treatment)					
	Day-1	Day-6	Day-10	Day-16	Day-22	Day-27
Control	30.55±1.62c	32±1.13c	33.35±0.91c	31.5±1.83c	29.6±2.26a	30.2±1.34a
Standard	37.35±0.49ab	36.7±0.28b	36.6±0.424b	36.65±0.91b	34.4±0.56a	34.3±0.00a
Combined	35.65±0.07b	37.4±0.00b	37.35±0.49b	36.4±1.13b	34.8±0.70a	34.6±1.69a
Plant extract Treated	39.7±2.96a	39.1±2.54a	38.5±1.83a	38±1.41a	35.6±1.91a	35±2.12a

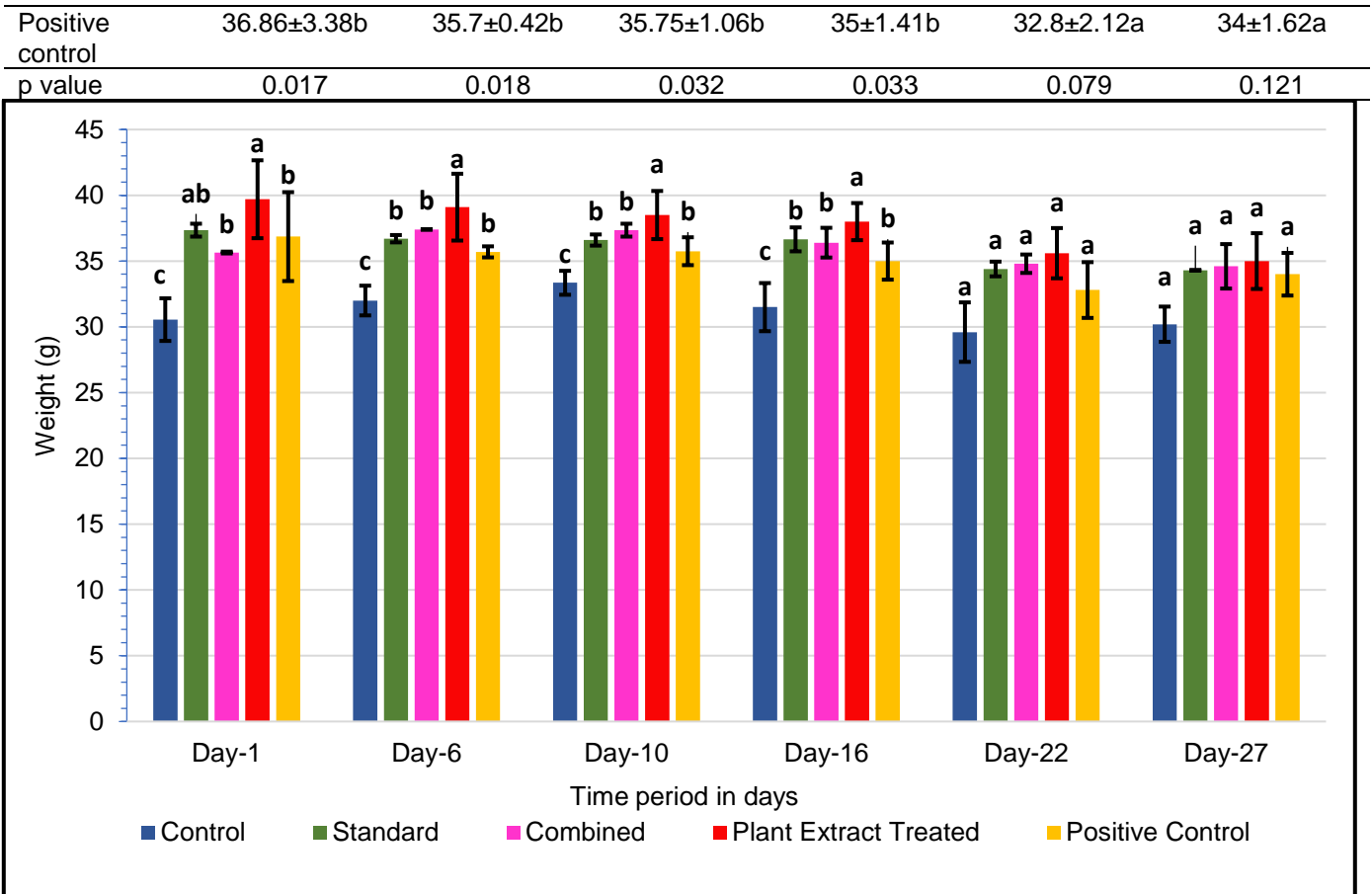


Figure 4. Weights during treatment.

Table 3. Mean value for triglyceride level (mg/dL) in all groups.

Parameter	Groups					P value
Triglycerides	Control	Standard	Combined	Plane extract	Positive control	
	173±1a	155.5±0.5b	212±1e	134.5±0.5d	182.5±0.5c	<0.001

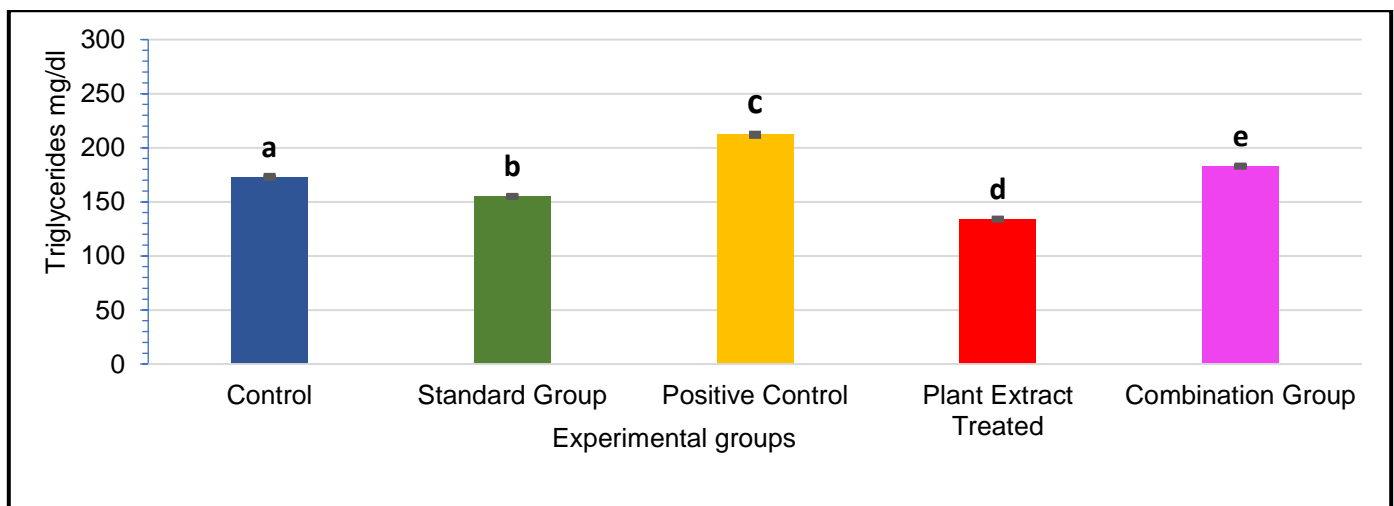


Figure 5. Concentration of triglycerides (mg/dL) in all groups.

The positive control group has significantly higher triglyceride levels (mg/dL) compared to the control group. The plant treated group has the lowest triglyceride levels among all groups (Figure 5). The standard group shows a reduction in

triglyceride levels compared to the control group. The combination group shows elevated triglyceride levels (mg/dL) compared to the standard group but lower than the positive control group. Mean values depicting triglyceride levels is also shown in Table 3.

Histopathology Analysis

A histopathology test involves the examination of tissues and cells under a microscope to diagnose and study diseases. This process includes obtaining a tissue sample, often through a biopsy, and then carefully examining it for cellular changes that could indicate disease, such as cancer or inflammatory conditions (for example, atherosclerosis). Histopathologists, who are specialized doctors, prepare and stain the tissue samples using various techniques to highlight different cellular components. The intestine of mouse from control and positive control group in addition to the stomach from the mouse belonging to plant extract treated group was given for histopathological analysis and the resulting images were subsequently compared.

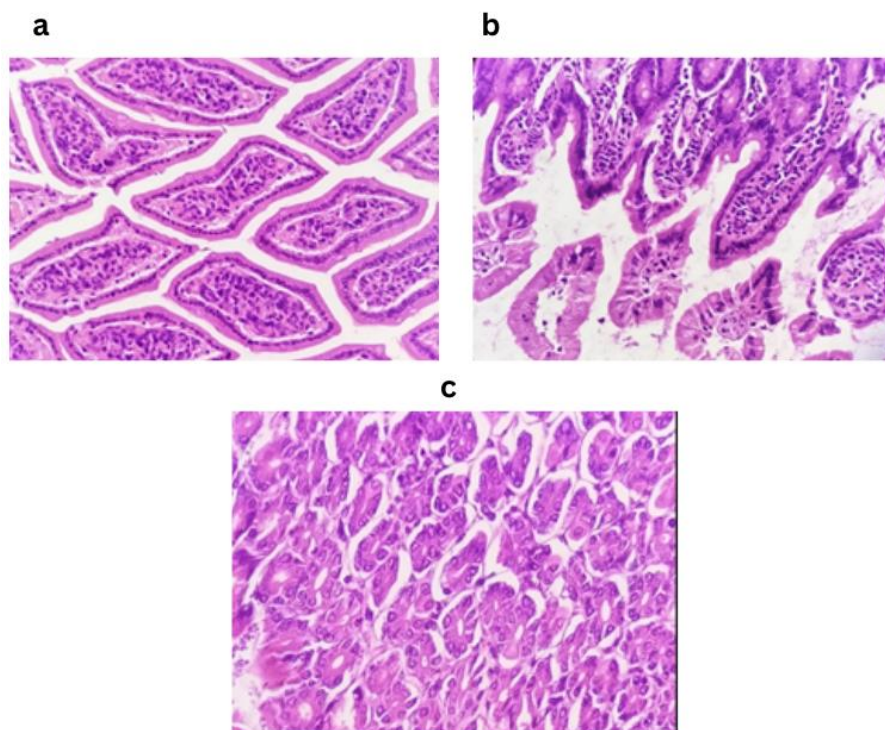


Figure 6. The microscopic images of the intestine of mouse from (A) control group, (B) positive control group, (C) plant treated group.

The histopathological section of intestine from control group mouse reveals some goblet cells, absorptive cells and enterocytes. Necrosis and degenerative changes are not seen. The histopathological section of intestine from positive control group mouse reveals mild degeneration along with some sloughing of villi. The histopathological section of stomach from plant extract treated mouse reveals normal structure of submucosa and mucosa along with muscularis mucosae. Goblet cells are also normal in structure along with absorptive cells. There will be no necrosis and degeneration seen as indicated in Figure (6).

The use of high fat diet has been successful in inducing atherosclerosis as proven by previous researches. An increase in weight after consumption of high fat diet shows indication of atherosclerosis (Bhandari et al., 2011). After the introduction of high fat diet for a duration of nearly six weeks, weights of mice were documented daily and a significant increase was observed. This weight gain was primarily attributed to the accumulation of body fat, particularly noticeable in the abdominal region. Furthermore, a decrease in the consumption of regular chow was observed (Rosen et al., 2014). These changes are indicative of a disorder such as obesity, or cardiovascular diseases. Pharmaceutical drug used for the treatment of atherosclerosis included Lipiget 10mg, it was used as a standard treatment. After treating the mice with both drug and *Zinnia elegans* extract, there was a gradual decrease in the weights of the mice which confirmed the role of both the treatments in treating atherosclerosis. Similar findings have been reported in studies examining the lipid-lowering effects of plant extracts (Wang et al., 2016).

Biochemical analysis of triglyceride levels revealed significant differences among the groups. The positive control group, which was subjected to a high-fat diet, exhibited significantly higher triglyceride levels compared to the control group, indicating the adverse effects of the high-fat diet. In contrast, the plant-treated group showed the lowest triglyceride levels among all groups, suggesting a potential protective effect of the *Zinnia elegans* extract against diet-induced hyperlipidemia. In the control group triglyceride's level was around 173 ± 1 mg/dL. This group represents the baseline triglycerides levels, mice in this group which received no treatment. In the standard group triglycerides level was 155.5 ± 0.5 mg/dL. Mice in this group received a standard treatment (Lipiget), which resulted in a reduction of triglycerides levels compared to the control group. In positive control triglycerides Level was around 212 ± 1 mg/dL. This group represents a positive control, triglycerides level increased due to a high-fat diet. In plant treated triglycerides level was 134.5 ± 0.5 mg/dL, this group received a plant-based treatment, which resulted in the lowest triglycerides levels among all groups, indicating a significant reduction. For combined group triglycerides Level was 182.5 ± 0.5 mg/dL. This group received a combination of treatments (Plant extract and standard drug), leading to triglycerides levels that are higher than the standard and plant-treated groups but lower than the positive control. A similar study carried out by (Talayero and Sacks, 2011) showed that TG levels are markedly affected by overweight and body fat distribution due to high fat diet consumption. In this study of atherosclerosis induction, levels of triglycerides were significantly increased in the positive control group. After the administration of daily dose of drug and extract, triglycerides levels significantly reduced, which showed that plant extract has an effective lipid lowering therapeutic potential. The p-value indicates that the differences in triglycerides levels among the groups are statistically significant. Overall, the plant-based treatment appears to be the most effective in reducing triglycerides levels, followed by the standard treatment (Wang et al., 2016). The presence of certain phytochemicals such as the tannins, terpenoids, saponins, flavonoids, alkaloids, and/or phenolic compounds in the plant extract may be responsible for such antioxidant, anti-inflammatory, endothelial protection, and lipid metabolism regulation in the diseased mice model. Flavonoids exhibit significant anti-inflammatory properties by inhibiting enzymes such as lipoxygenase and cyclooxygenase involved in the inflammation. This reduces the production of pro-inflammatory mediators, thereby mitigating inflammation associated with atherosclerosis (Rathee et al., 2009). By neutralizing free radicals, tannins help prevent the oxidative modification of low-density lipoprotein (LDL), a critical step in the initiation of atherosclerosis. Certain alkaloids act as vasodilators by activating the endothelial nitric oxide synthase (eNOS) pathway, leading to the production of nitric oxide (NO) which are involved in vascular smooth muscle cells relaxation (Munteanu et al., 2024). Along with this, the intestines of mice from the control and positive control groups, as well as the stomach from the plant extract-treated group, were subjected to histopathological analysis. The resulting images were subsequently compared to assess the impact of different treatments on tissue morphology. These findings highlight the efficacy of *Zinnia elegans* extract in mitigating the adverse effects of a high-fat diet, as evidenced by both biochemical and histopathological analyses. The plant extract's ability to lower triglyceride levels suggests it's potential as a therapeutic agent for managing hyperlipidemia (Rosen et al., 2014).

CONCLUSION

Atherosclerosis-induced mice model via high fat diet was the first step in assessing the therapeutic efficacy of *Zinnia elegans* having anti lipidemic potential. This was evaluated with the potential biomarkers' serum triglyceride levels, morphological assessment through body weight, and histopathological changes in mice model of control and treated groups. The phytotherapy of *Zinnia elegans* is found to have promising impact on the disease cure and prevention. Future scientific studies on underlying molecular mechanism involved in Atheroma progression in blood vessels and their cure with zinnia elegans can help in revolutionizing the pharmaceutical area.

DECLARATIONS

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

H.S., A.T., F.A. carried out the experimental work, analyzed the data, and wrote the manuscript. S.R., supervised the project, wrote and reviewed the manuscript. A.Z. and M.T wrote and reviewed the manuscript.

FUNDING

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethical Review Committee of the Department of Bioinformatics and Biosciences, Capital University of Science and Technology, Islamabad, Pakistan. All the experiments were performed in the Animal house of Department of Pharmacy according to the ethical guidelines provided by the institution.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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