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IMPACT OF AGAVE EXTRACT ON ATHEROSCLEROSIS IN RAT MODEL: ASSESSING ITS THERAPEUTIC POTENTIAL, ELUCIDATING ITS MECHANISMS, IMPLICATIONS FOR CARDIOVASCULAR HEALTH, AND ENHANCING TREATMENT OPTIONS

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Abstract
Atherosclerosis is the deposition of fat within the wall of arteries. Risk factors include increased consumption of saturated fat. In this study, a rat model of atherosclerosis was established using high fat diet, the effect of *Agave sisalana L.* was analyzed on the experimental model and results were compared with standard drug by biochemical analysis which included lipid profile test and liver function tests. Rats were divided into five groups, all groups except control were given high fat for 8 weeks followed by a treatment for 6 weeks; control (normal rat feed),positive control (high fat diet 1ml/day), drug treated (high fat 1ml/day for 8 weeks + Lipiget 1ml/day for 6 weeks), extract treated (high fat 1ml/day for 8 weeks+ Agave sisalana L. extract 0.5ml/day for 6 weeks) and combined group (high fat 1ml/day for 8 weeks+ Lipiget 0.5ml/day and agave extract 0.25ml/ day for 6 weeks) respectively. Weights were observed on daily basis and a significant increase in weight was observed in all groups fed with high fat diet. After 8 weeks, treatment was given and a decrease in weights were observed in drug treated, extract treated and combined group. Upon biochemical analysis, triglycerides were seen to be high in positive control due to consumption of high fat diet and were decreased in drug, extract and combined groups which shows that both drug and extract have positive effects in reducing atherosclerosis. ALT concentration was low in positive control group and shown to be increased in all treatment groups. Bilirubin was in high concentration in positive control group as compared to other groups. No significant difference was observed in other parameters. Hence, we conclude that consumption of *Agave sisalana L.* by high fat diet fed rats attenuates the progression of atherosclerotic lesions.

1. Introduction
Cardiovascular disease (CVD) is a disorder that has an impact on the heart and circulatory system of people. It is connected to the buildup of fat molecules in the arteries, which leads to the development of blood clots and arterial damage in a number of organs, including the kidney, brain, heart, and eyes.

Prior to the 20th century, less than 10% of fatalities worldwide were attributable to CVD; nevertheless, in 2001, the death rate rose to 30%. Murray and Lopez predicted that the leading cause of disability and mortality in developing nations by the year 2020 will be cardiovascular disease in 1996 (Gaziano *et al*., 2006). Cardiovascular disease risk factors include high blood sugar, high blood pressure, smoking, obesity, abnormal lipids, and inactivity. Age, race, and family history are a few risk factors that cannot be altered. While certain conditions, including smoking, diabetes, excessive blood cholesterol levels, etc., can be changed (Gaziano *et al.*, 2006).

The most common cardiovascular condition is atherosclerosis which causes plaque, or atheroma development in the endothelium lining of the artery wall ultimately leading towards the blockage of arteries. Inflammatory cells and lipids fat components can be found in plaque. Atherosclerosis is treated with antiplatelet and anti-atherogenic medications (Aziz and Yadav, 2016). Lipid retention is the first step in the pathogenesis of atherosclerosis, followed by inflammation. These fatty streaks keep growing and transforming into fibrous plaque. Increased blood flow and reduced NO production result from endothelium lining damage, which in turn promotes inflammatory cells. T lymphocytes and monocytes migrate to sub-endothelial layers after adhering to endothelial cells. LDL and VLDL bind to and oxidize on walls. The oxidized LDL is engulfed by macrophages, which create foam cells that turn into fatty streaks. As additional proinflammatory cytokines are drawn to macrophages, the lesion develops into a fibrous plaque made of lipid. Large nodules in the wall are caused by calcium deposits in the plaque. The plaque is ruptured by the enzymes secreted by activated macrophages, and the contents are then exposed, leading to thrombosis (Aziz and Yadav, 2016).

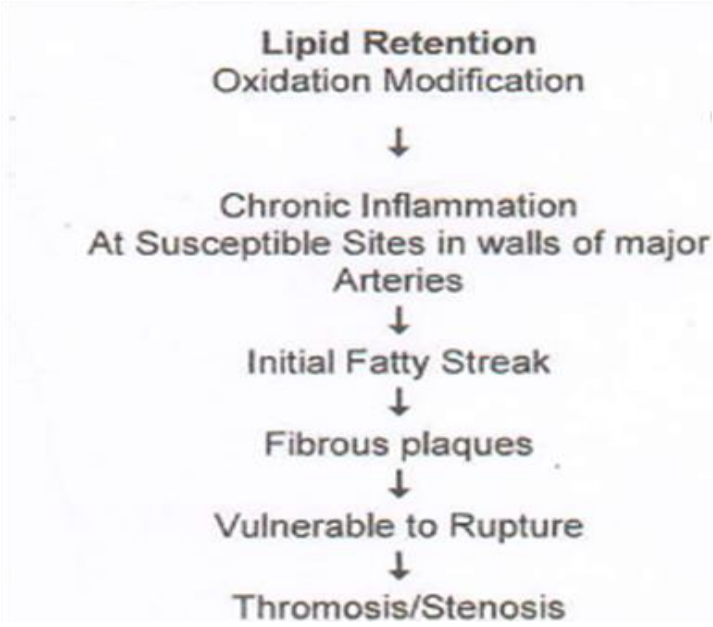

Figure 1: Stages of atherosclerosis (Aziz and Yadav, 2016)

Using the lipid-lowering medication class known as Statins, atherosclerosis can be treated. On the other hand, there are certain naturally occurring, disease-fighting bioactive substances known as phytochemicals that are present in fruits, vegetables, and medicinal plants that serve to protect the heart. The flavonoids, saponins, polyphenols, vitamins, and organo-sulfur compounds that are naturally found in plants are phytochemicals (Vasanthi et al., 2012). Heart disease risk is decreased by antioxidant, anti-hypercholesterolemic, anti-angiogenic, platelet aggregation inhibition, and anti-inflammatory characteristics. They perform a variety of tasks, including removing reactive oxygen species (ROS) and avoiding cellular damage (Pagliaro et al., 2015).

The *Asparagaceae* family includes the genus Agave. It is indigenous to several regions of the Americas, including the Caribbean and Mexico. Succulent plants that flourish in dry or semi-dry settings include the agave family. *Agave americana*, often known as maguey plant, and blue agave plants are the sources of agave nectar. Essentially, agave nectar is a sweetener that is used in place of

sugar (Pérez et al., 2020). *Agave sisalana* and *Agave americana* are members of the *Asparagaceae* family. These species are commonly cultivated all over the world. The herbs under consideration are analgesic, anti-inflammatory, and insecticidal {Shahzad et al., 2017).

They have been examined for the low-toxicity analgesic and anti-inflammatory effects of the hexanic fraction (Tewari et al., 2014). Three agave species *Agave sisalana, Agave americana,* and *Agave cupreata* have all been researched for their possible therapeutic use in treating a range of conditions. Previous research has also shown that these plants may contain compounds that are anti-inflammatory (Pineda et al., 2017). The phytochemicals included in *Agave sisalana* are extensively used in the medicinal sector. In addition, it stands out due to the presence of flavonoids and saponins in its structure (Sarwar et al., 2020).

Agave americana plants can also be used medically to treat a number of diseases. Phytochemical components from numerous medicinal plants are employed to achieve a variety of pharmacological objectives. Alkaloids, flavonoids, and saponins are some of the bioactive compounds found in the plant's leaves, and together they have the ability to protect against a wide range of ailments (Singh et al., 2018).

The manufacturing of alcoholic beverages including raicilla, tequila, pulque, bacanora, and mezcal is said to produce agave leaves as a by-product. These by-products are where agave extracts, which contain terpenes, saponins, and phenolic compounds, get their bioactive components from. Numerous pharmacological effects of these bioactive compounds include antihypertensive, anti-inflammatory, immunomodulatory, antiparasitic, antibacterial, antifungal, antioxidant, and anticancer activities. Agave plants are employed in the treatment of many ailments and as food additives (López et al., 2018).

Worldwide, animal models are employed to investigate human illnesses. Mice, *Mus musculus*, are frequently used as models to study human biology and illness because of their evolutionary similarity to people, their physiological similarity to humans, and because they are easy to care and reproduce in the lab. Genomic studies have shown the great genetic similarity between the two species, which is the major justification for the widespread use of mice in human research (Perlman, 2016)

2. Methodology

2.1. Experimental Animals

The study employed 15 adult male albino rats, with an average weight of 140-160g and an age of 4-5 weeks. The animals were seized from the Animal House, Pharmacy Department, Capital University of Science and Technology, Islamabad and were housed in group of 3 each for 1week acclimatization. They were provided unrestricted access to food and drink while being kept in a temperature and humidity-controlled space with natural light and dark periods. The animals were employed in the study after a week-long acclimation phase.

2.2. Normal diet composition

The normal diet is composed of:
Vitamins - 500g
Milk - 1.5kg
Chokar - 1kg
Flour - 6kg
Fish - 3.5kg
All these ingredients are combined to make 12000 g feed which is then divided into small pieces. Each piece weighs approximately 110g. One rat consumes 9.2g of this piece in 1 day.

2.3. High fat diet composition

For a period of six weeks, an oral high-fat diet was made using a 2:1 blend of coconut oil and vegetable ghee. It was first administered to each rat at a dose of 2 ml/day for 2 weeks before being lowered to 1 ml/day (Shokouh et al., 2017).

2.4. Preparation of *Agave sisalana L* extract

Agave sisalana L. fresh leaves were collected from the local vicinity of Islamabad, Pakistan and after that get them counter verified from the Botany Department, Quaid-e-Azam University, Islamabad. The leaves were washed with tape water, cut in to small slices, then they were crushed and macerated for 48 hours in a rotatory shaker with 70% ethanol solvent. The extract was then filtered using the percolation process, and the resulting mixture was scraped into powder before drying in petri plates. The *Agave sisalana L.* extract was dissolved in distilled water to create a fresh solution, which was then administered orally to the test animals. (Misra *et al.,* 2018).

2.5. Preparation of drug

Atorvastatin, also known as Lipiget, was purchased from a nearby pharmacy and served as the reference medication in this experiment. 10 pills weighing 10 mg each were crushed, and distilled water dilutions were created.

2.6. Experimental design

Animals were placed into five groups of three each after being weighed, recorded, and tagged. All of the animals received treatment in accordance with ethical standards.

Group 1 Animals were given a regular feed and clean water for 24 hours, and they were regarded as the control group.

Group 2 Animals were used as a positive control group; they were fed a normal diet plus a high-fat diet for 24 hours every day for a period of 6 weeks.

Group 3 Animals were fed a typical diet plus a high-fat meal every day for six weeks. After six weeks, Lipiget dilutions were given at a dose of 1 ml/day for a period of four weeks; this group was regarded as the drug-treated group.

Group 4 Over the course of six weeks, the animals were fed a typical food plus a high-fat meal each day. Agave sisalana L. extract was given at a dose of 0.5 ml/day for a period of 4 weeks, and the group that received the extract was determined to be the treated group after 6 weeks.

Group 5 Over the course of six weeks, the animals were fed a typical food plus a high-fat meal each day. After 6 weeks, a combined group of patients received Agave extract and medication dilutions, each at a dose of 0.25 and 0.5 milliliters per day, respectively, for a period of 4 weeks.

2.7. Weight measurement

Every day, the body weights of all the rats were recorded. Following the provision of a high-fat diet for 8 weeks, a considerable rise in body weight has been reported.

2.8. Biochemical Testing

At the end of experiment, all the animals were taken group wise and blood was collected by cardiac puncture. All rats were given a chloroform anesthetic before blood was drawn, centrifuged, and used to create serum. For each group, tests on liver functioning and lipid profiles were conducted, and the findings were compared.

2.9. Statistical Analysis

All data will be analyzed and presented as a mean ± SM in one-way ANOVA.

3. Results

3.1. Body weight measurement

3.1.1. Weight before treatment

In comparison to the control group, which got a normal diet, a substantial rise in body weight was shown after the administration of a high fat diet for 8 weeks in the positive control group, drug group, extract group, and combination group. The weight gain was almost two times as much as in the control group, which is a blatant sign that rats had developed atherosclerosis.

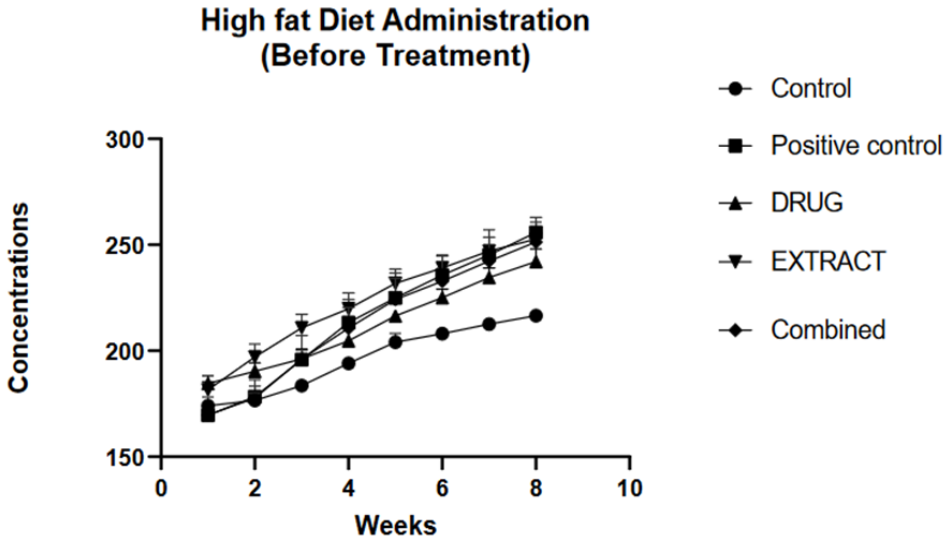

Figure 2: Weight measurement before treatment

3.1.2. Weight after treatment

Rats received therapy for 6 weeks after receiving high fat diets consistently for 8 weeks. It was noted that the weight of the drug-treated, extract-treated, and combination group gradually decreased after treatment. A combined group's decline was the greatest.

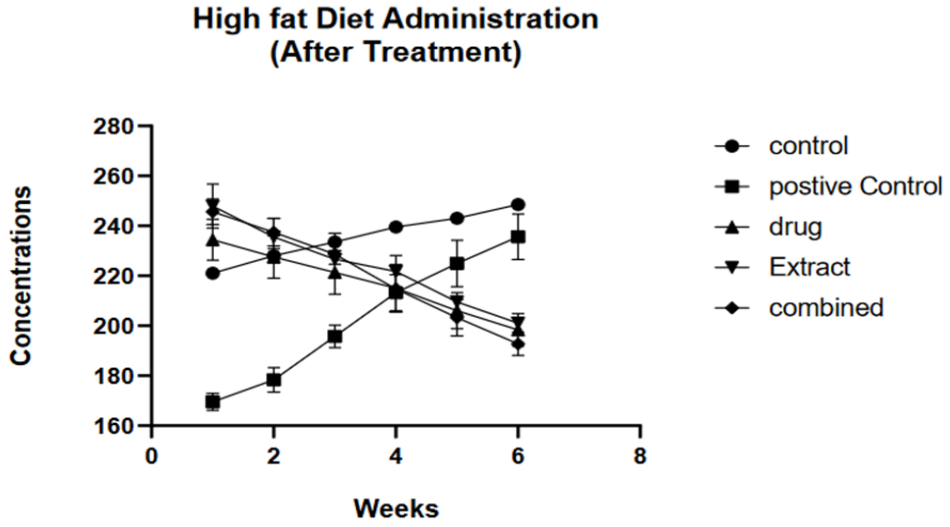

Figure 3: Weight measurement after treatment

3.2. Biochemical Analysis

3.2.1. Triglycerides

Increased triglyceride levels may lead to artery stiffening, which raises the risk of heart disease. After administering a high-fat meal, a positive control group's triglyceride levels significantly increased as compared to the control group, according to the results of a biochemical examination. The concentrations were lower in the drug-treated, extract-treated, and combination groups after treatment with the drug and extract, respectively.

Table 1: ANOVA: Mean of triglycerides in all groups of rats

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	8.543	4	2.136	7.963	.005
Within Groups	2.414	9	.268		
Total	10.957	13			

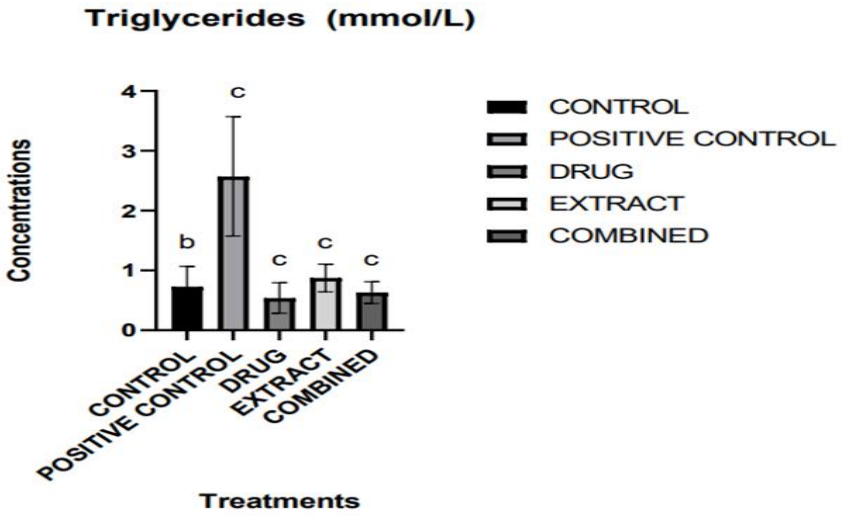

Figure 4: Concentrations of Triglycerides (mmol/L) in all groups

3.2.2. Total Cholesterol

Our bodies need cholesterol to create healthy cells, but having too much of it might put you at risk for heart disease. In blood arteries with excessive cholesterol, fatty deposits may form. The results of the biochemical analysis of cholesterol showed that the concentration of cholesterol was higher in the drug-treated and combined groups than in the positive control group, while the concentration of cholesterol was slightly lower in the extract-treated group than in the positive control group.

Table 2: ANOVA: Mean of total cholesterol in all groups of rats

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	.233	4	.058	.964	.472
Within Groups	.544	9	.060		
Total	.777	13			

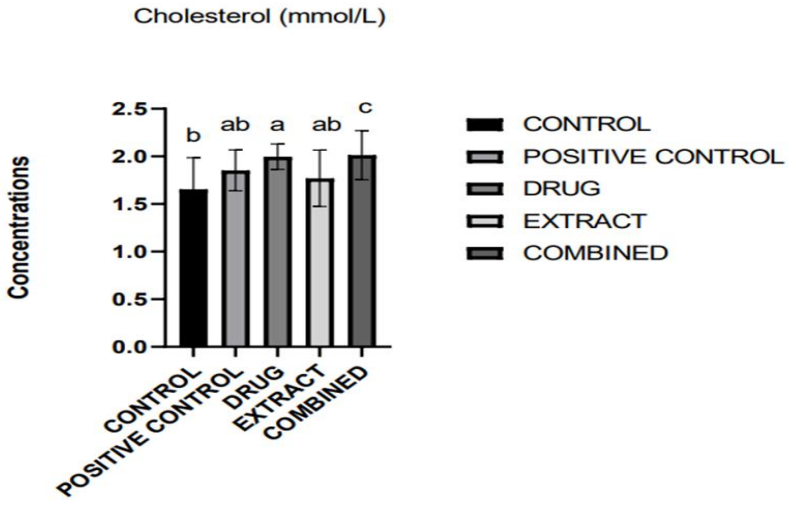

Figure 5: Concentrations of total Cholesterol (mmol/L) in all groups

3.2.3. HDL

The good cholesterol known as high-density lipoproteins (HDLs) fights atherosclerosis by eliminating cholesterol from foam cells, preventing the oxidation of LDLs, and reducing the inflammatory processes that cause atherosclerosis. When compared to a positive control group, the findings of the biochemical examination of HDL indicated a rise in the drug-treated and combination groups. Whereas the HDL levels of extract-treated group content remained the same.

Table 3: ANOVA: Mean of HDL in all groups of rats

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	0.266	4	0.067	1.703	.233
Within Groups	.351	9	.039		
Total	.618	13			

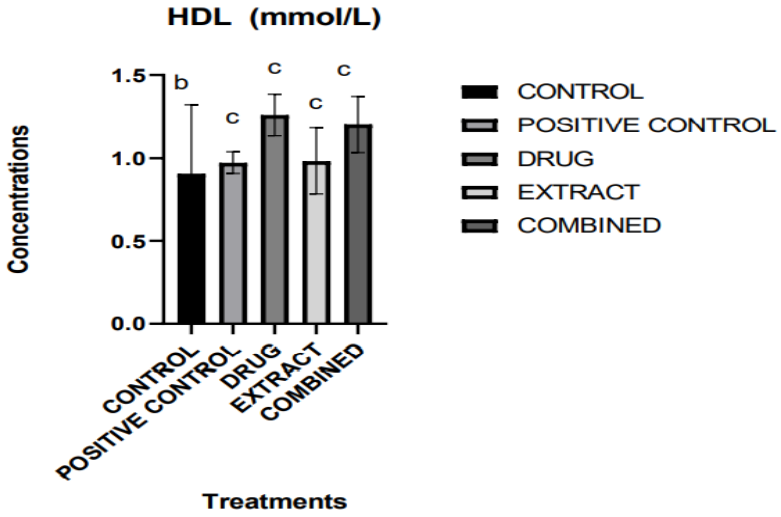

Figure 6: Concentrations of HDL (mmol/L) in all groups

3.2.4. LDL

A variety of bioactivities may be triggered by the uptake and storage of LDL (low-density lipoprotein) by macrophages and lead to the formation of atherosclerotic plaques. When compared to the positive control group, the LDL concentration was found to be somewhat lower in the extract treated group while slightly higher in the medication treated and combination groups.

Table 4: ANOVA: Mean of LDL in all groups of rats

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	.025	4	.006	4.186	.035
Within Groups	.013	9	.001		
Total	.039	13			

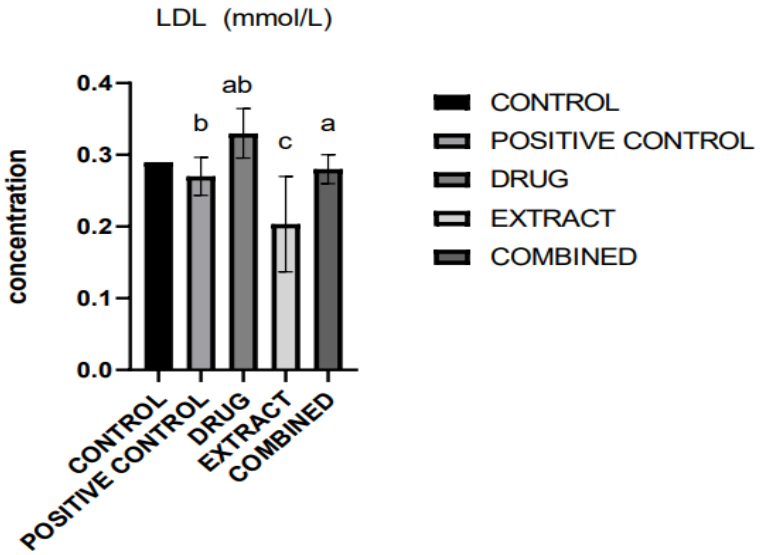

Figure 7: Concentrations of LDL (mmol/L) in all groups

3.2.5. ALP

The quantity of alkaline phosphatase (ALP) in the blood is determined by an ALP test. ALP tests are used to screen for, identify, and keep track of liver illness and dysfunction. The graph's ALP concentration reveals that both the control and the positive control groups had similar ALP levels. ALP levels may be noted to be somewhat higher in the combination treatment group than in the extract treated group when compared to the position control group.

Table 5: ANOVA: Mean of ALP in all groups of rats

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	314.762	4	78.690	.522	.722
Within Groups	1356.667	9	150.741		
Total	1671.429	13			

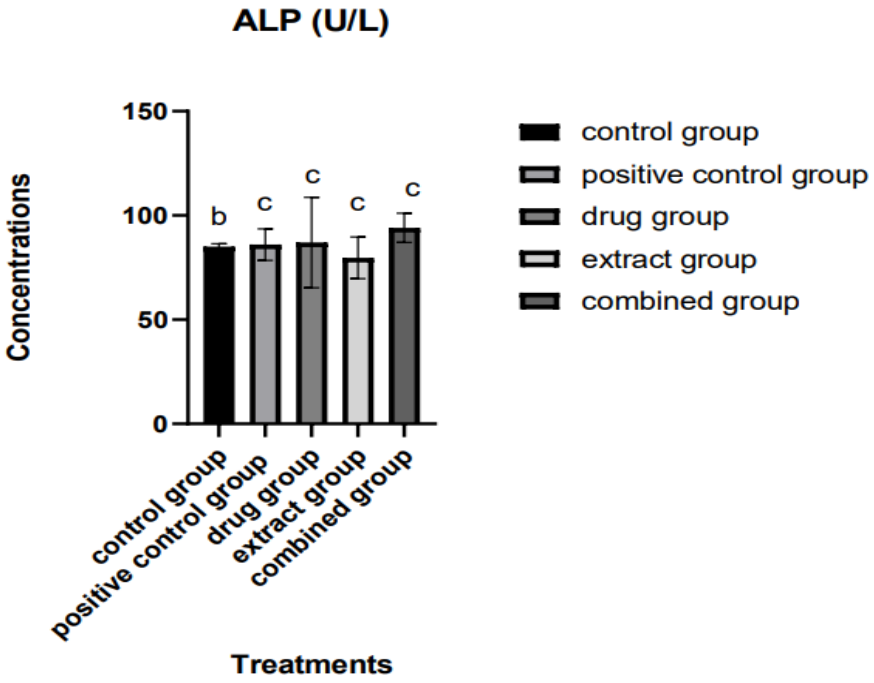

Figure 8: Concentrations of ALP (U/L) in all groups

3.2.6. ALT

The blood test alanine transaminase (ALT) examines the amount of the ALT enzyme in the blood. Generally speaking, elevated ALT levels may be a symptom of liver disease. A lack of blood supply to the liver, certain medications, or chemicals may also be to blame for the damage. In our study, ALT levels are lower in the positive control group than in the control group. As compared to the positive control group, all treatment groups—drug, extract, and combination groups—show elevated levels

Table 6: ANOVA: Mean of ALT in all groups of rats

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	1932.929	4	483.232	8.912	.003
Within Groups	488.000	9	54.222		
Total	2420.929	13			

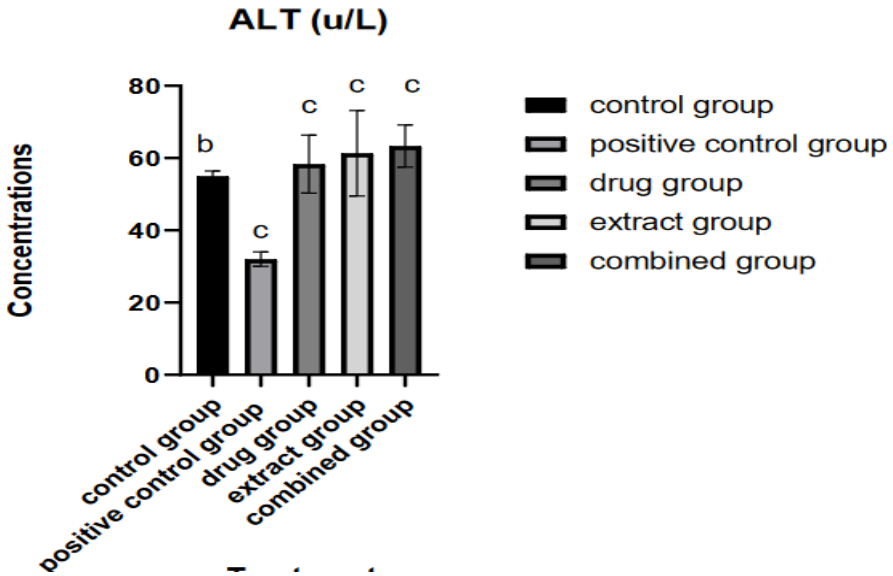

Figure 9: Concentrations of ALT (u/L) in all groups

3.2.7. Bilirubin

A yellowish pigment called bilirubin helps the body break down old red blood cells. Bile aids in food digestion and contains bilirubin. The majority of the bilirubin will be eliminated from the body by a functioning liver. Our experiment's findings demonstrate that the positive control group had higher levels than the control group. Bilirubin levels in the extract-treated group were lower than in the positive control group, but they were higher in the extract group when compared to the drug-treated and combination groups.

Table 7: ANOVA: Mean of Bilirubin in all groups of rats

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	30.857	4	7.714	6.943	.008
Within Groups	10.000	9	1.111		
Total	40.857	13			

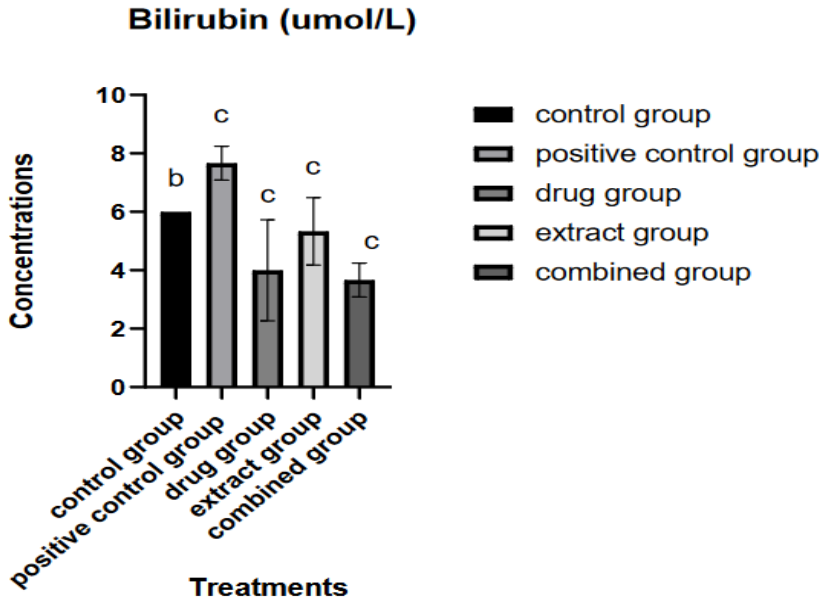

Figure 10: Concentrations of Bilirubin (umol/L) in all groups

4. Discussion

In this experiment, atherosclerosis was induced in rats using a high-fat diet. According to earlier studies, a high-fat diet can successfully cause atherosclerosis. After consuming a high-fat diet, weight gain is a sign of atherosclerosis (Ilyas et al., 2022).

Daily weight measurements were used in our study, and after the high-fat diet was administered to all of the groups for 8 weeks, a substantial rise in weight was seen. This demonstrates the introduction of the illness, which was further supported by an analysis of the levels of several biomarkers in serum. The rats' weights gradually decreased after being given both the medication and the Agave sisalana L. extract, confirming the efficacy of both therapies for treating atherosclerosis. The statin medication class, which includes Lovastatin, Atorvastatin, and Fluvastatin, is used to treat atherosclerosis. One of the most widely accessible medicines, Lipiget (Atorvastatin), was employed in this investigation.

We measured the levels of several lipid profile and liver function test indicators in the serum. A lipid profile test measures the levels of HDL, LDL, triglycerides, and total cholesterol in the serum. Bilirubin total, ALT, and alkaline phosphatase quantities are measured during a liver function test. Talayero, B. G., and Sacks, F. M., reported eating a high-fat diet significantly affects TG levels, as does being overweight and having uneven body fat distribution. For the therapy of hypertriglyceridemia, they concentrated on weight loss. However, their findings indicated that both high-carbohydrate and low-carbohydrate diets resulted in reduced plasma TG in the setting of sustained weight reduction (Ilyas et al., 2022).

In our investigation of atherosclerosis induction, the positive control group's TG levels considerably rose. The TG levels dramatically decreased after the daily treatment dose was administered, demonstrating the efficacy of statins and agave extract as therapeutic lipid-lowering agents. As indicated by Getz, G., and Reardon, C., eating a high-fat diet can noticeably speed up atherogenesis and cause a large rise in plasma lipid levels. Another study by Kapourchali *et al*. revealed that, in contrast to humans, mice have high density lipoprotein (HDL) particles that contain the majority of the circulating cholesterol, whereas humans have low density lipoprotein (LDL) particles. They came to the conclusion that this is probably the key factor preventing atherosclerosis from developing fast in wild-type mice. The mouse does not significantly absorb dietary cholesterol because it lacks the cholesterol-ester transfer protein (CETP). Our research, which is relevant to their experiment, revealed a minor rise in cholesterol concentration following a high-fat meal, which may be caused by rats' absence of cholesterol-ester transfer protein or by their reaction to a high-fat diet (Getz and Reardon, 2012). The concentrations were reduced by treating with *A. sisalana L* extract.

There is supporting evidence from a number of epidemiological studies showing those with greater blood bilirubin levels had a decreased risk of cardiovascular events. Bilirubin seems to inhibit the formation of atherosclerotic plaque in Ldlr/- mice by scavenging ROS signaling intermediates and interfering with endothelial VCAM-1 and ICAM-1-mediated leukocyte migration (Vogel *et al.*, 2017). However, we discovered a rise in bilirubin levels in the positive control group compared to falls in levels in the drug-treated, extract-treated, and combination groups. We have come to the conclusion that our results are in conflict with earlier research.

The considerably elevated levels of ALT and ALP in the extract- and drug-treated groups show that *A. sisalana L* may have harmed rats' livers in some way. Concentrations that are higher indicate liver damage. When selecting a suitable mouse model for the research of atherosclerosis, various aspects must be taken into account. These considerations include stress, gender, nutrition, age, circadian rhythm, simplicity of maintenance, and similarity with the pertinent human situation (Ilyas *et al.*, 2022). Therefore, the variations in these characteristics, such as rat age and weight, may account for the variations in our results.

5 Conclusion

We come to the conclusion that lipiget and *Agave sisalana L.* both work well to treat atherosclerosis. Since rats given extract showed some toxicity, lipiget has produced superior outcomes than *A. sisalana L* extract. Our research has revealed concerning liver toxicity linked to the crude *Agave*

sisalana L extract. To harness its therapeutic potential while ensuring safety, we must focus on isolating and characterizing its phytochemicals. By doing so, we can pinpoint the compounds responsible for toxicity, establish optimal and safe dosages, maintain consistent quality, evaluate safety profiles, enhance drug efficacy, and meet stringent regulatory requirements. This approach is essential for responsible drug development, enabling us to unlock the medicinal benefits of *Agave sisalana L.* while minimizing associated risks.

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