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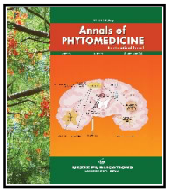

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Pharmacognostic investigation and biological evaluation of *Allium sativum* L.

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Abstract

A phrase used in medicine to encourage lowering blood lipid levels is "hypolipidemic." The goal of the current study was to examine the biological effects of *Allium sativum* L. (AS) on lipid concentration by pharmacognostic analysis. For the experimental investigation, 42 male rats were chosen, and they were split into seven groups. By calculating lipid markers such serum cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, and VLDL cholesterol levels, the evolution of hypolipidic activity was assessed. In hyperlipidemic rats, our findings demonstrated that the ethanolic and aqueous extracts of AS root decreased cholesterol levels. In hyperlipidemic rats given AS ethanolic and aqueous extract as compared to triton-induced groups, high levels of plasma HDL-cholesterol have recently been detected; demonstrating the effectiveness of these drugs in raising HDL-cholesterol levels. Our results indicate that, in terms of hypolipidemic activity in the experiments conducted with triton-induced hyperlipidemic model; the ethanolic extract of AS in various doses is superior to the aqueous extract of *A. sativum* in lowering the lipid parameters such as total cholesterol level, triglycerides level, LDL and VLDL level with increase in HDL-cholesterol.

1. Introduction

Saponin is a group of chemical compounds and secondary metabolites found in various natural sources. Phenomenologically, when agitated in aqueous solution, saponin created foam that resembled soap, and structurally, it is made up of one or more hydrophilic glycoside groups and derivatives of lipophilic triterpenes (Hostettmann *et al.,* 1991). Historically, saponins have been understood to originate from plants, but have also been isolated from marine organisms (Hostettmann *et al.,* 1991; Riguera 1997).

Hyperlipidemia has become a widespread condition worldwide, particularly in industrialized nations, as a result of irregular and unusual eating practices, lifestyle choices, and sources of stress. (Alvarez Ramirez *et al.,* 2020). Numerous people's lives are at danger, and this issue has an impact on their health and ability to work. There has been a lot of studies done globally to address the issue of testing robust hypolipidemic medications; the majority of the plants are of Indian origin (Nelson 2013; Srivastava *et al.,* 2019).

In the world, hyperlipidemia causes atherosclerosis, which is the leading cause of death (Sampath Kumar *et al.,* 2011). As a result of changes in lifestyle and an increase in stress, atherosclerosis is also becoming a significant illness in India. There is a significant link between hyperlipidemia and coronary heart disease, as demonstrated by population research and clinical trials (Shabana and Shahid 2020). "Increased risk of CHD is associated with elevated serum concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides" (Gordon *et al.,* 1977). In the other aspect, coronary heart disease is also brought on by low levels of high-density lipoprotein (HDL) cholesterol (Nelson, 2013).

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2. Methods and Materials

2.1 Plant materials

The AS was purchased from local distributor of herbal crude drugs in Kanpur, India in January. The bulbs are dried, washed, crushed to moderately coarse powder and stored in air tight containers. The AS plant bulb is authenticated by Dr. Siddh Malya, Department of Ayush Bangalore. The AS plant bulbs were identified and validated with pharmacognostic properties.

2.2 Extraction

For the purpose of extraction, the AS bulbs are gathered, cleaned, and pulverised. A Soxhlet system was used to extract 250 g of powdered material using distilled water and pure ethanol. For roughly 20 h, extraction is done using hot continuous extraction. After extraction, the extract was filtered to eliminate any contaminants using Whatman filter paper (# 42). Vacuum distillation was used to concentrate the extract and cut the volume in half. The leftover solvent was evaporated on a water bath, collected, and put in desiccators to remove the additional moisture before the concentrated extract was transferred to a 100 ml beaker. The dried extract was used to screen for phytochemicals and was packaged in an airtight container (Pendbhaje *et al.,* 2011; Ranjani and Raju 2012).

2.3 Drugs

To get the final concentration of the suspension 20 ml, distilled water was added after the weighed quantity of fractionated ethanolic

extract and aqueous extract was obtained and triturated with polyvinyl pyrrolidine (2% w/v). The refrigerator was used to keep the suspension in an airtight bottle. Isooctyl-polyoxy-ethylene phenol, also known as Triton WR 1339, was purchased from Sigma Chemicals Co. (St. Louis, Missouri, U.S.A.). Triton WR 1339 was made in 7% solution in ordinary saline and given at a dosage of 2 mg/kg body weight intraperitoneally. Triton was administered to the group at a dosage of 2 mg/kg body weight intraperitoneally as the triton control group.

2.4 Pharmacological evaluation of hypolipidemic activity

2.4.1 Acute toxicity study

In this investigation, several serum lipid parameters were measured and compared with the standard group and other groups in order to identify the dose-dependent hypolipidemic activity of various dosages of the same extract under the same circumstances. To regulate and simulate triton-induced hyperlipidemia, albino rats were given ethanol and water extract of AS dosages of 1 mg/kg and 2 mg/kg body weight (i.p.).

2.4.2 Experimental design

Animal experiments were carried out with approval from the Institute's Institutional Animal Ethics Committee (Registration No. 778/C/CPCSEA). Swiss albino rats (weighing between 100 and 200 g) were housed in a climate-controlled environment with regular food and water availability. All additional analytical-grade compounds, including Triton WR 1339, were also utilised.

The following groups of six animals (n = 6) each were created from adult albino rats of either sex (weighing between 100 and 200 g) for the study:

Group I: Control (without any drug treatment, given food and water intermittently)

Group II: Triton control subjects received (200 mg/kg body weight i.p.)

Group III: Standard drug - fenofibrate treated (50 mg/kg body weight i.p.)

Group IV: Triton with an ethanolic extract of AS (200 mg/kg + 1 mg/kg body wt. i.p.)

Group V: Triton with an ethanolic extract of AS (200 mg/kg + 2 mg/kg body wt. i.p.)

Group VI: Triton with aqueous extract of AS (200 mg/kg + 1 mg/kg body wt. i.p.)

Group VII: Triton with aqueous extract of AS (200 mg/kg + 2 mg/kg body wt. i.p.)

2.4.3 Effect of triton WR 1339 on serum lipid parameters

Plasma cholesterol and triglycerides rose biphasic when albino rats were given the surfactant Triton WR 1339. In this hyperlipidemic paradigm, adult albino rats weighing between 100 and 200 g were administered Triton WR 1339 (200 mg/kg) intravenously after fasting for 18 h. In the first phase, blood cholesterol levels rise immediately after delivery by 2-3 times after 24 h. In the second stage, hypercholesterolemia drops to control levels during the course of the following 24 h. The test substance or plant extract used as the control is administered concurrently with the triton injection. The next step was to analyze the serum lipids at 6, 24, and 48 h following the triton injection.

By blocking the tissues' ability to absorb plasma lipids, triton-triton causes hypercholesterolemia in a phase-dependent manner. Drugs that block cholesterol production are active during Phase I, whereas those that block cholesterol metabolism and excretion are active during Phase II.

2.4.4 Studies on hypolipidemic activity

When the test drugs were administered to experimental animals, we assessed hypolipidemic activity by changes in serum lipid levels (such as total cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C). The parameters are determined before and after regular drug use. Screening of activity with fractionated ethanolic and aqueous extracts of AS in triton-induced hyperlipidemic animal models was employed.

Initial lipid parameters were established, including total cholesterol, triglyceride, and high-density lipoprotein values. As a control, only sporadic access to food and water was given to Group I. In the second instance, Group II is treated with triton WR 1339 (7% solution in normal saline) at a dosage of 200 mg/kg body weight intraperitoneal (i.p.). Fenofibrate is administered intraperitoneal at a dosage of 50 mg/kg body weight in Group III. triton + extract is administered to Groups IV and V at doses of 200 mg/kg + 1 mg and 200 mg/kg + 2 mg/kg body weight, respectively. Serum lipid levels in each group are examined following the delivery of Triton and extractive fraction dosages. Here, the Triton control-treated groups and the groups that received extract are contrasted with the control group. The blood lipid levels are analyzed at 6, 24, and 48 h following the triton injection, as the experiment specifies.

All animal groups were fasted overnight for the triton-induced hyperlipidemic model, and blood samples were taken from the retinal venous plexus, immediately centrifuged, and left to coagulate at room temperature. After that, the blood samples were centrifuged for 10 to 20 min at 2000 rpm. Following this method, ultra-pure serum was collected and cautiously transferred using a micropipette to a tiny microcentrifuge tube in order to determine serum parameters. With the use of conventional reagent kits, serum parameters including total cholesterol, triglyceride level, and HDL were assessed using an autoanalyzer (Espin Diagnostic Analyzer).

2.5 Statistical analysis

For each group at different time intervals, the means values and standard error of mean are determined, and the control group and each group are statistically compared.

3. Results

3.1 Hypolipidemic activity of AS

The hypolipidemic potential of the stem of AS was assessed. The saponins of AS were studied for their hypolipidemic activity in the experimental model. After the administration of triton (induces hyperlipidemia) doses to their respective groups; serum lipid levels were examined at a time interval of 0, 6, 24, and 48 h as described in the experimental model to assess the hypolipidemic activity of AS, respectively.

3.1.1 Effect of Triton WR 1339 on lipid parameters in albino rats

In several experimental models, Triton WR 1339, a non-ionic polyoxyethylene phenol, significantly enhances the lipid profile and is mostly utilized as a surfactant. It often results in a biphasic rise of plasma triglyceride and cholesterol levels. We displayed normal levels of lipid markers in the control group at intervals of 0, 6, 24, and 48 h, including total cholesterol, triglycerides, HDL-cholesterol, LDL, and VLDL, among others. When compared to the control, triton control Group II had a substantial rise in lipid parameters at the same time periods of 0, 6, 24, and 48 h, respectively (Tables 1-5, Figures 1-5).

3.1.2 Effect of ethanolic extract of AS on lipid parameters in albino rats

After 0, 6, 24, and 48 h, Group III, which received the conventional medication (Fenofibrate 50 mg/kg), had a substantial decline in lipid markers. After 6, 24, and 48 h, Group IV treated with (Triton 200 mg/kg body wt. + ethanolic extract of AS 1 mg/kg body wt.) showed a significant increase in HDL-cholesterol and a significant decrease in VLDL levels in comparison to the triton control group. However, Group IV treated with (Triton 200 mg/kg body wt. + ethanolic extract of AS 1 mg/kg body wt.) showed no reduction in total cholesterol, triglyceride and LDL levels $p<0.05$ (Tables 1-5, Figures 1-5).

When compared to the triton control group after 6 and 48 h, Group V treated with (Triton 200 mg/kg body wt. + ethanolic extract of AS 2 mg/kg body wt.) results in a notable decrease in lipid parameters such as total cholesterol level, triglycerides, LDL and VLDL profile with an increase in HDL-cholesterol (Tables 1-5, Figures 1-5).

3.1.3 Effect of aqueous extract of AS on lipid parameters in albino rats

Triton 200 mg/kg body wt. + aqueous extract of AS 1 mg/kg body wt. given to Group VI had no effect on triglyceride or HDL cholesterol levels at the appropriate time intervals, but had a significant impact on total cholesterol, LDL, and VLDL levels compared to the triton control group after 6 and 24 h (Tables 1-5, Figures 1-5).

After 6, 24, and 48 h, respectively, Group VII treated with (Triton 200 mg/kg body wt. + aqueous extract of AS 2 mg/kg body wt.) displayed a significant decrease in lipid parameters such as total cholesterol, triglycerides, LDL, and VLDL levels with an increase in HDL cholesterol in comparison to the triton control group. Drug levels in various dosages result in considerably lower levels of lipid parameters (Tables 1-5, Figures 1-5).

Table 1: Effect of different extracts of AS on total cholesterol level in triton-induced albino rats

Group/Time (h)	0 h	6 h	24 h	48 h
Control (I)	116.50 ± 3.81	109.70 ± 4.14	135.66 ± 3.63	118.00 ± 3.80
Triton WR 1339 control (200 mg/kg) (II)	135.60 ± 4.18	128.00 ± 2.12	162.32 ± 3.24	184.25 ± 3.12
Triton WR 1339 + standard drug Fenofibrate (200 + 50 mg/kg) (III)	95.70 ± 2.25	115.20 ± 4.25	126.05 ± 3.78	98.09 ± 2.07*
Triton WR 1339 + Ethanolic extract (200 + 1 mg/kg) (IV)	128.33 ± 4.23	167.50 ± 5.30	135.16 ± 3.72	122.50 ± 3.56
Triton WR 1339 + Ethanolic extract (200 + 2 mg/kg) (V)	136.15 ± 3.12	154.20 ± 4.42	143.06 ± 3.17	129.10 ± 2.09 **
Triton WR 1339 + Aqueous extract (200 + 1 mg/kg) (VI)	117.00 ± 3.33	134.33 ± 1.05 **	146.50 ± 1.87	116.00 ± 2.29
Triton WR1339 + Aqueous extract (200 + 2 mg/kg) (VII)	142.05 ± 2.83	164.12 ± 2.61	189.07 ± 3.84	143.00 ± 2.92 *

Values are expressed as mean ± SEM. (n = 6) for each group.

Value expressed in mg/dl serum (*= $p<0.05$, **= $p<0.01$, *= significant, **= more significant).

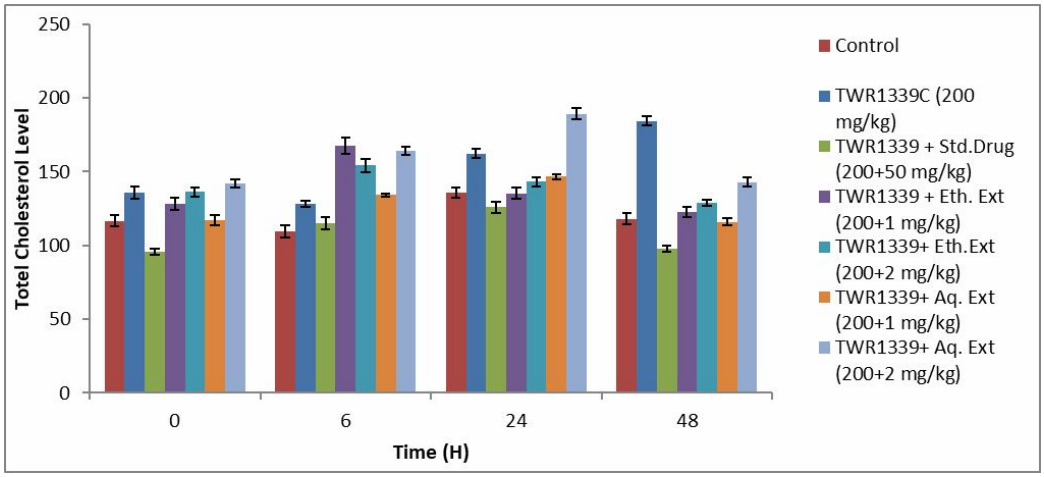

Figure 1: Effect of different extracts of AS on total cholesterol level in triton induced albino rats.

Table 2: Effect of different extracts of AS on total triglyceride level in triton induced albino rats

Group/Time (h)	0 h	6 h	24 h	48 h
Control (I)	75.50 ± 1.17	80.66 ± 0.71	80.08 ± 0.45	76.50 ± 1.02
Triton WR 1339 Control (200 mg/kg) (II)	62.30 ± 1.08	71.06 ± 1.07	88.04 ± 2.19	92.05 ± 3.02
Triton WR 1339 + Standard drug Fenofibrate (200 + 50 mg/kg) (III)	36.20 ± 1.05	53.10 ± 1.62	41.08 ± 1.52**	56.05 ± 1.78
Triton WR 1339 + Ethanolic extract (200 + 1 mg/kg) (IV)	74.02 ± 3.13	81.11 ± 3.14	76.31 ± 2.90	72.33 ± 4.03
Triton WR 1339 + Ethanolic extract (200 + 2 mg/kg) (V)	66.09 ± 2.86	90.06 ± 3.41	49.51 ± 2.39	57.16 ± 2.70*
Triton WR 1339 + Aqueous extract (200 + 1 mg/kg) (VI)	76.33 ± 1.85	90.50 ± 2.76	75.21 ± 1.29	75.40 ± 1.99
Triton WR1339 + Aqueous extract (200 + 2 mg/kg) (VII)	67.26 ± 1.98	86.15 ± 2.23	48.11 ± 1.18*	72.16 ± 2.49

Values are expressed as mean ± SEM. (n =6) for each group.

Value expressed in mg/dl serum (*= p<0.05, **= p<0.01, *= significant, **= more significant)

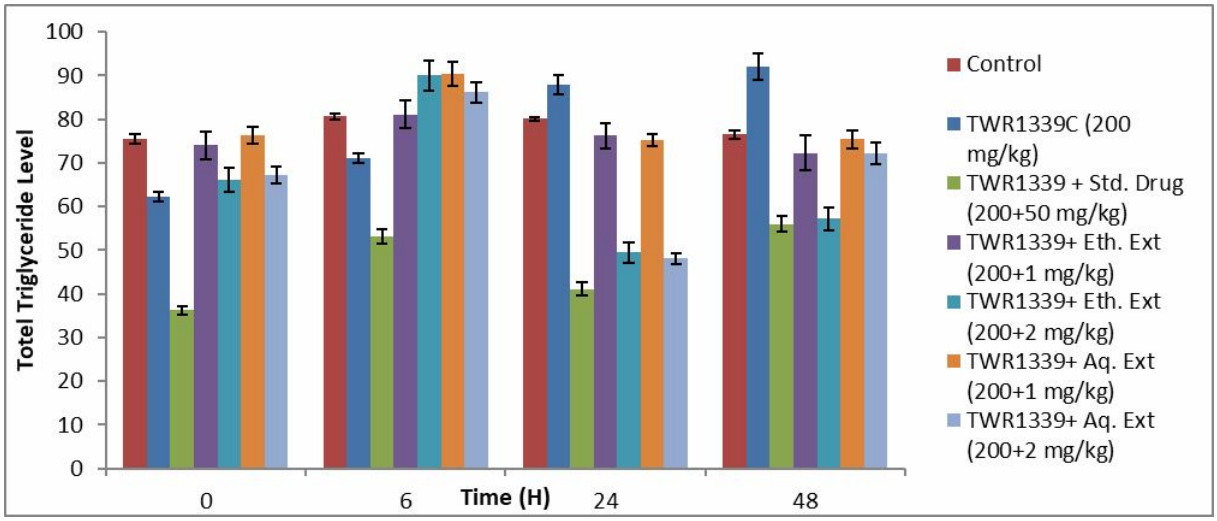

Figure 2: Effect of different extracts of AS on triglyceride level in triton induced albino rats.

Table 3: Effect of different extracts of AS on HDL cholesterol level in triton induced albino rats

Group/Time (h)	0 h	6 h	24 h	48 h
Control (I)	18.70 ± 0.63	25.33 ± 0.88	31.00 ± 1.06	22.83 ± 1.07
Triton WR 1339 Control (200 mg/kg) (II)	56.20 ± 1.23	77.10 ± 2.08	87.12 ± 3.36	108.06 ± 3.79
Triton WR 1339 + Standard drug Fenofibrate (200 + 50 mg/kg) (III)	49.50 ± 1.86	82.30 ± 1.19*	49.05 ± 1.29	55.20 ± 1.92
Triton WR 1339 + Ethanolic extract (200 + 1 mg/kg) (IV)	19.76 ± 2.12	30.91 ± 1.01*	25.58 ± 2.11	21.55 ± 1.81
Triton WR 1339 + Ethanolic extract (200 + 2 mg/kg) (V)	34.21 ± 3.71	52.09 ± 0.17**	59.06 ± 2.02	46.53 ± 1.66
Triton WR 1339 + Aqueous extract (200 + 1 mg/kg) (VI)	19.00 ± 1.93	24.00 ± 1.18	27.10 ± 0.58	19.98 ± 2.48
Triton WR1339 + Aqueous extract (200 + 2 mg/kg) (VII)	17.06 ± 1.53	38.14 ± 1.75	32.06 ± 1.21*	28.07 ± 1.81

Values are expressed as mean ± SEM. (n=6) for each group.

Value expressed in mg/dl serum (*= p<0.05, **= p<0.01, *= significant, **= more significant)

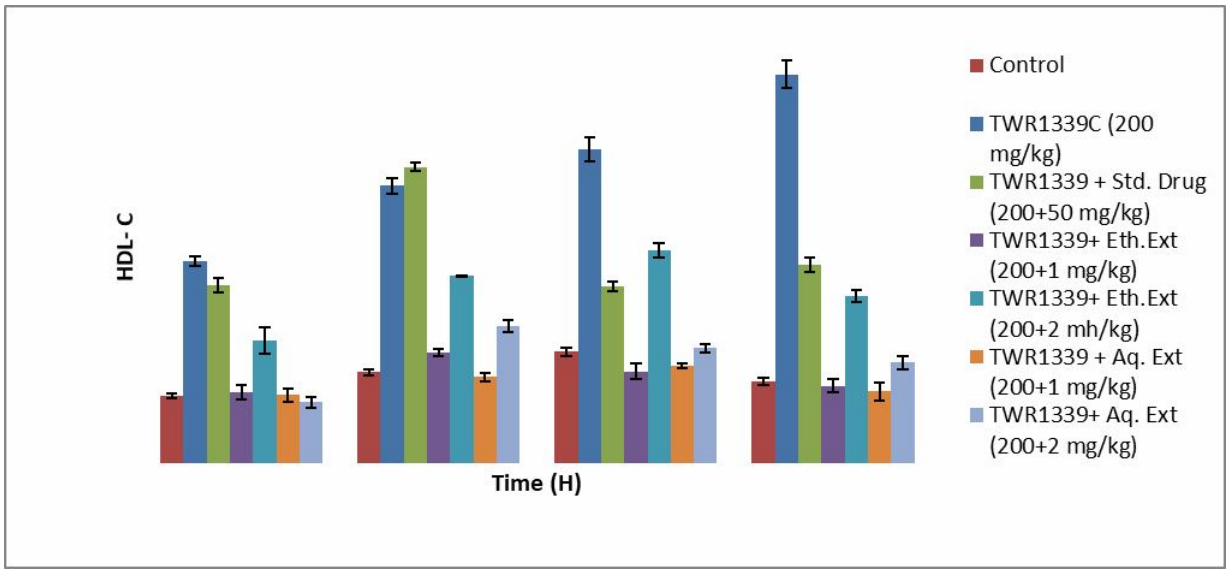

Figure 3: Effect of different extracts of AS on HDL cholesterol level in triton induced albino rats.

Table 4: Effect of different extracts of AS on LDL-cholesterol level in triton induced albino rats

Group/Time (h)	0 h	6 h	24 h	48 h
Control (I)	82.70 ± 4.15	115.53 ± 4.23	87.98 ± 2.68	79.86 ± 4.61
Triton WR 1339 Control (200 mg/kg) (II)	58.00 ± 2.36	91.06 ± 3.76	110.04 ± 3.21	108.00 ± 3.02
Triton WR 1339 + Standard drug Fenofibrate (200 + 50 mg/kg) (III)	28.90 ± 2.72	59.01 ± 1.26	38.08 ± 0.29**	36.03 ± 2.27
Triton WR 1339 + Extract (200 + 1 mg/kg) (IV)	89.80 ± 3.30	120.33 ± 4.18	93.15 ± 4.47	86.48 ± 2.67
Triton WR 1339 + Ethanolic extract (200 + 2 mg/kg) (V)	77.10 ± 2.81	96.50 ± 3.88**	84.31 ± 3.47	80.19 ± 2.96
Triton WR 1339 + Aqueous extract (200 + 1 mg/kg) (VI)	82.73 ± 5.00	92.23 ± 1.83**	104.36 ± 2.12**	80.93 ± 3.99
Triton WR1339 + aqueous extract (200 + 2 mg/kg) (VII)	106.01 ± 4.38	79.00 ± 2.11*	87.10 ± 2.32	96.15 ± 3.77

Values are expressed as mean ± SEM. (n =6) for each group.

Value expressed in mg/dl serum (*= $p<0.05$, **= $p<0.01$, *= significant, **= more significant)

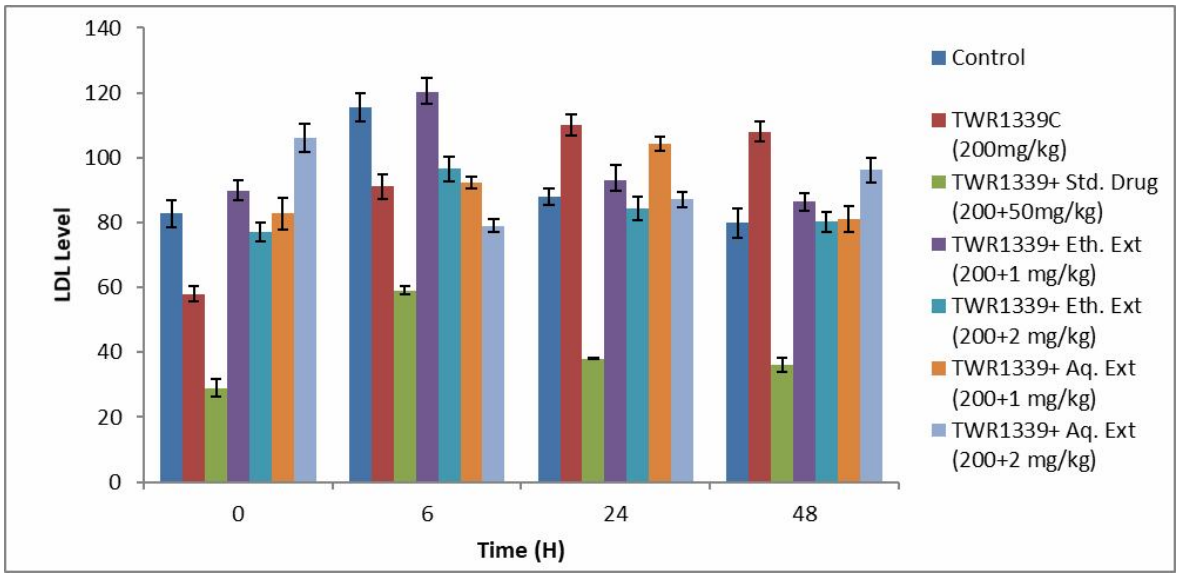

Figure 4: Effect of different extracts of AS on LDL level in triton induced albino rats.

Table 5: Effect of different extracts of AS on VLDL- cholesterol level in triton induced albino rats

Group/Time (h)	0 h	6 h	24 h	48 h
Control (I)	15.10 ± 0.23	16.13 ± 0.14	16.68 ± 0.62	15.30 ± 0.20
Triton WR 1339 Control (200 mg/kg) (II)	32.09 ± 1.02	46.00 ± 1.15	87.06 ± 2.01	89.02 ± 2.21
Triton WR 1339 + Standard drug Fenofibrate (200 + 50 mg/kg) (III)	40.00 ± 1.66	36.22 ± 1.19*	66.75 ± 1.98	51.85 ± 1.75
Triton WR 1339 + Ethanolic extract (200 + 1 mg/kg) (IV)	14.90 ± 0.58	16.25 ± 0.62	14.62 ± 0.56*	14.46 ± 0.80
Triton WR 1339 + Ethanolic extract (200 + 2 mg/kg) (V)	18.50 ± 1.27	28.05 ± 2.11	23.62 ± 1.73	24.06 ± 1.39*
Triton WR 1339 + Aqueous extract (200 + 1 mg/kg) (VI)	15.26 ± 0.37	18.10 ± 0.15**	15.01 ± 0.05	15.06 ± 0.39
Triton WR1339 + Aqueous extract (200 + 2 mg/kg) (VII)	38.41 ± 0.79	62.12 ± 1.19	41.06 ± 0.82*	59.07 ± 1.89

Values are expressed as mean ± SEM. (n =6) for each group.

Value expressed in mg/dl serum (*= $p<0.05$, **= $p<0.01$, *= significant, **= more significant)

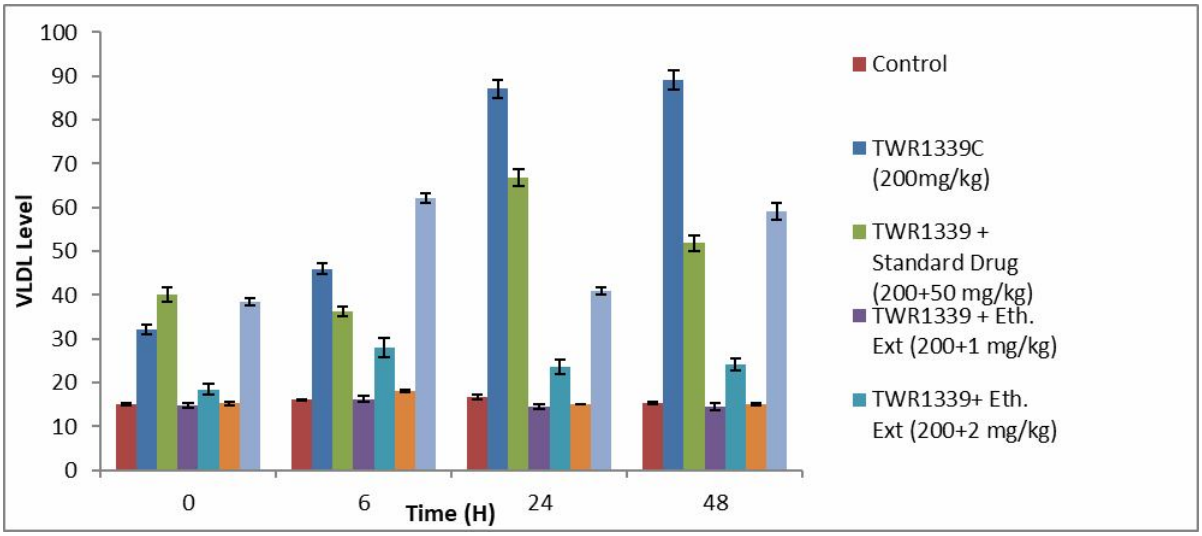

Figure 5: Effect of different extracts of AS on VLDL level in triton induced albino rats.

4. Discussion

One of the main risk factors for coronary heart disease is high total cholesterol. Diabetes is also known to exacerbate hyperlipidemia and the prevalence of atherosclerosis. Numerous herbs and plant-based products have hypolipidemic and hyperglycemic-lowering effects. The goal of the current investigation was to test the antihyperlipidemic effects of the AS bulb powder's ethanolic and aqueous extracts in a model of hyperlipidemia brought on by triton (Mahmoodi *et al.,* 2006), which, in hypercholesterolemic male albino rats, has hypocholesterolemic and antioxidant effects. Various lipid profile indicators rise in response to activation of triton WR 1339 (Beyegue *et al.,* 2012). Triton WR 1339 averts the catabolism of triacylglycerol-rich lipoprotein by lipoprotein lipase (LPL), thereby preventing the removal of triacylglycerol from plasma (Shrivastava *et al.,* 2013). Consuming extra fat might result in a rise in VLDL synthesis, which produces LDL in the highest possible concentration that can adhere to blood vessel walls and create a typical blockage of blood flow (Igweh *et al.,* 2005). When fenofibrate, a common medication, is used to treat an underlying condition, weight gain is successfully prevented; there is a clear correlation between the risk of coronary artery diseases, high LDL, and low HDL that has been well documented (Yoon *et al.,* 2002). While both 1 mg/kg and 2 mg/kg of the aqueous extract of AS only slightly decreased the blood lipid profile, administering isolated saponin from the ethanolic extract of AS significantly reduced the serum lipid profile.

Therefore, the phytosterol and saponin content of AS root may be responsible for the decrease in cholesterol levels in hyperlipidemic rats treated with ethanolic extract and aqueous extract. Intestinal bile acid micelles are totally replaced by phytosterol, which lowers circulating cholesterol (Quilez *et al.,* 2003), and saponin precipitates cholesterol from micelles and prevents bile acids from flowing *via* the enterohepatic circulation, making it hard for the intestinal tract to absorb cholesterol, which lowers plasma cholesterol (Harwood *et al.,* 1993). Triton causes a rise in oxidative stress and LDL- cholesterol levels, which results in an increase in oxidized LDL levels and the development of atherosclerotic plaque. The presence of saponin in the AS root, which might boost hepatic absorption of LDL-cholesterol and accelerate bile acid catabolism, may be responsible for the considerable reduction in plasma LDL-cholesterol in the treated groups.

5. Conclusion

The disease known as hyperlipidemia affects people of all ages and is a global issue. Traditional medicine has a wide variety of herbal treatments available to help many individuals with this significant condition.

The plant medicines were identified, shade-dried, and then ground into a rough powder. The herbal medicine [*A. sativum*] was ground into a medium-coarse powder and extracted with ethanol and distilled water.

The aqueous extract of *A. sativum* is less effective than the ethanolic extract at reducing lipid parameters like total cholesterol level, triglycerides level, LDL and VLDL level with an increase in HDL-cholesterol in terms of hypolipidemic activity in triton induced hyperlipidemic model experiments, according to the above conclusion.

The main issue connected to hyperlipidemia, which causes fatty layer to deposit in the physiological system, is coronary heart disease and its consequences as well as atherosclerosis. So, in order to stop this terrible condition, we need to discover additional natural medicines.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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