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Original Article

Methanolic extract of *S. securidaca* flowers, leaves, and seeds' antihyperlipidemic effects on high fat diet-induced hyperlipidemia in Wistar rats

Efeitos anti-hiperlipidémicos do extrato metanólico das flores, folhas e sementes de *S. securidaca* na hiperlipidemia induzida por uma dieta rica em gordura em ratos Wistar

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Abstract

Significant risk factors for atherosclerosis include hyperlipidemia and oxidative stress, which together rank as three of the most significant risk factors for cardiovascular diseases. *Securigera securidaca* lowers cholesterol levels in diabetic rats' blood. This investigation's objective was to determine how methanolic extracts affected the flowers, leaves, and seeds of plants in rats that were fed a high-fat diet (HFD). Five groups of animals were created (n = 5). A total of 35 days, divided into two intervals, were used for the study. Rats received HFD during the first 15-day interval, while during the second 20-day interval, they also received extracts or the Atorvastatin reference drug. The extract of seeds has a high phenol content as well as DPPH radical antioxidant activity. Extracts were given at a dose of 200 mg/kg; p.o. Methanolic treatment of *S. securidaca* flowers, leaves, and seeds in HFD-induced hyperlipidemic rats resulted in significant reductions in total cholesterol, triglycerides, LDLC, and VLDL-C levels. HDL-C levels increased significantly because of the leaves. While in hyperlipidemic rats, seeds significantly reduced the activities of the enzymes ALT and ALP. The findings showed that, to a certain extent, seeds, flowers, and leaves may have benefits in reducing hyperlipidemia brought on by HFD in terms of lipid profiles and liver function enzymes. The findings of this study indicate a promising application prospect, but more research is needed to determine the exact mechanism of these novel compounds as antihyperlipidemic agents and to clarify their potential combination effect with synthetic drugs such as Atorvastatin. Combinations can reduce the dose of chemical medications required, which lowers the risk of side effects.

Keywords: *S. securidaca,* seeds, leaves, flowers, hypolipidemia, methanol extraction, food rich diet.

Resumo

Fatores de risco significativos para aterosclerose incluem hiperlipidemia e estresse oxidativo. Esses três aspectos juntos classificam como três dos fatores de risco mais significativos para doenças cardiovasculares. *Securigera securidaca* reduz os níveis de colesterol no sangue de ratos diabéticos. O objetivo desta investigação foi determinar como os extratos metanólicos afetaram as flores, folhas e sementes de plantas em ratos que foram alimentados com uma dieta rica em gordura (HFD). Cinco grupos de animais foram criados (n = 5) e utilizados neste estudo, durante 35 dias, divididos em dois intervalos. Os ratos receberam HFD durante o primeiro intervalo de 15 dias, enquanto no segundo intervalo de 20 dias, eles também receberam extratos ou o medicamento de referência Atorvastatina. O extrato das sementes possui alto teor de fenóis, bem como atividade antioxidante do radical DPPH. Os extratos foram administrados na dose de 200 mg/kg; p.o. O tratamento metanólico de flores, folhas e sementes de *S. securidaca* em ratos hiperlipidêmicos induzidos por HFD resultou em reduções significativas nos níveis de colesterol total, triglicerídeos, LDLC e VLDL-C. Os níveis de HDL-C aumentaram significativamente por causa das folhas. Enquanto em ratos hiperlipidêmicos, as sementes reduziram significativamente as atividades das enzimas ALT e ALP. Os achados mostraram que, até certo ponto, sementes, flores e folhas podem ter benefícios na redução da hiperlipidemia provocada pela HFD em termos de perfis lipídicos e enzimas da função hepática. As descobertas deste estudo indicam uma perspectiva de aplicação promissora, mas são necessárias mais pesquisas para determinar o mecanismo exato desses novos compostos como agentes anti-hiperlipidêmicos e para esclarecer seu potencial efeito de combinação com drogas sintéticas como a atorvastatina. As combinações podem reduzir a dose de medicamentos químicos necessários, o que diminui o risco de efeitos colaterais.

Palavras-chave: *S. securidaca*, sementes, folhas, flores, hipolipidemia, extração de metanol, dieta rica em alimentos.

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1. Introduction

Atherosclerosis is significantly influenced by hyperlipidemia and oxidative stress, two of the most significant risk factors for cardiovascular diseases and conditions (Boden et al., 2020, Liu et al., 2022). In terms of global public health issues, cardiovascular diseases rank among the worst. They cause more than 17 million deaths each year, according to statistics from the World Health Organization (World Health Organization, 1992.). Heart disease is linked to several cardio-metabolic risk factors, including hypercholesterolemia, diabetes, high blood pressure, obesity, and a sedentary lifestyle. Triglycerides (TG), total cholesterol (CT), cholesterol of the low-density lipoprotein (LDL-C), and cholesterol of the high-density lipoprotein (HDL-C) all rise at higher rates in dyslipidemia, a metabolic disorder that is very common (Bakhtiyari et al., 2022; Kaur et al., 2021; Guilbert, 2003).

High levels of plasma LDL-C and total cholesterol significantly increase the risk of developing atherosclerosis and the associated arterial hypertension, according to a vast body of population-based and experimental evidence. Decreases in HDL-C and increases in triglycerides are two other alterations in lipid parameters linked to atherosclerosis. It is well known that having high cholesterol contributes to the development of atherosclerosis, hypertension, and renal failure (Etminan et al., 2021). The role of hypercholesterolemia in reducing mortality and the occurrence of cardiovascular and neurological events is assumed, and this is done by lowering the blood cholesterol levels associated with LDL-C. Reduced cardiovascular risks are correlated with decreased levels of LDL-C (Bakhtiyari et al., 2022).

Chemical drugs are currently used to treat hyperlipidemia, and traditional lipid-lowering drugs include niacin, fibrates, statins, and bile acid-binding sequestrants. However, long-term use of statins, like other chemical drugs, may result in several potential side effects, such as damage to liver and kidney function, gastrointestinal reactions, and other unfavorable reactions. Despite their clear clinical effects and obvious effects on lowering blood lipids (Boutari et al., 2021), due to the complexity of the regulation of lipid metabolism, which involves a variety of pathways and targets, it is also difficult to achieve the primary and secondary effects of the current single-target lipid-lowering drugs. To treat hyperlipidemia and its associated complications, safe and efficient alternatives are thus urgently needed (Juca et al., 2020; Zhang et al., 2020; Feldman et al., 2021).

Numerous research has revealed that combining AS with other therapeutic medications may be able to increase its effectiveness and lessen any associated side effects (Juca et al., 2020; Zhang et al., 2020; Feldman et al., 2021). Candidates for combination therapy with AS can vary greatly, but herbal remedies have tremendous promise because many of them have undergone systematic research, and combinations of western and herbal medications have lately gained more and more traction (Cheung et al., 2017).

Considering their safety and therapeutic potential, phytochemicals have drawn a lot of attention in this context. Natural active ingredients found in plants have been shown to have significant benefits in treating hyperlipidemia and preventing cardiovascular disease. Phytochemicals are multi-component, multi-targeted, and have only minor toxic effects (Zhang et al., 2020; Feldman et al., 2021). In Jordanian folk medicine, *Securigera securidaca* (L.) Degen & Dorfl (*S. securidaca*), an annual herb from the Fabaceae family, is used to treat diabetes (Idal'in et al., 2020; Ghorbani et al., 2014). According to experimental research, giving *S. securidaca* seeds to healthy and diabetic subjects lowers blood sugar levels (Jamshidzadeh et al., 2018; Ahmadi et al., 2016; Raesi et al., 2019). Furthermore, this plant lowers triglyceride and cholesterol levels in the serum of high-fat-fed rats (Ibrahim et al., 2015; Rajaei et al., 2015). It is frequently used in Jordan as a folk beverage, edible flavoring, and food. The flavonoid content of S. securidaca seed powder suspension has also demonstrated a protective effect against alloxan-induced hyperglycemia and oxidative stress in rats. The presence of numerous biologically active substances, such as phenolics, flavonoids, saponins, and tannins, as well as unsaturated fatty acids in the seed extract that have been identified by phytochemical analysis, is likely the cause of the observed positive effects (Adal'in et al., 2020).

Although several studies have confirmed the hypoglycemic and hypolipidemic effects of *S. securidaca* seed hydroalcoholic extract (Jamshidzadeh et al., 2018; Ahmadi et al., 2016; Raesi et al., 2019), no study has been done on the effects of comparing seeds, leaves, and flowers on hyperlipidemia in models that only investigate the high-fat diet. Previous research used a diabetic model to evaluate seed and flower extracts, not leaves. We believe that such research is necessary because there are cases of people becoming hyperlipidemic while not being diabetic. In addition to the importance of evaluating all parts using the same model for better conclusions. The goal of the study was to compare the outcomes with those of the standard drug Atorvastatin and assess the impact of methanolic extracts of flowers, leaves, and seeds on a rat model with high food-rich lipid levels (Venkateshan et al., 2016).

2. Material and Methods

2.1. Plant material collection and extraction

S. securidaca seeds were brought from a local herbal shop in Karak, Jordan, in the spring of 2021. The seeds were identified by Dr. Hammad K. Aldal'in (Department of Medical Support, Al-Balqa Applied University) and deposited with the herbarium code R006 for further references. A drug development lab received the voucher specimen. World Flora Online and the website of the plant list organization (Denkschr et al., 1897; The Plant List, 2023) have both been used to verify the plant name.

Plant seeds were planted until flowering, then leaves and flowers were collected. The aerial parts as well as the seeds dried in the shade and were ground into a fine powder by a coffee blender. The finely powdered seeds were stored in a special container until use. The extract was soaked in 750 ml of methanol solution for two days at room temperature, filtered and centrifuged for 15 minutes at 8000 rpm, then concentrated in vacuo at 50°C by a rotary evaporator. The resulting crude extract was air-dried and stored in a refrigerator at 4°C in a glass container until further use (Adal'in et al., 2020).

2.2. Preliminary Phytochemical analysis

To ascertain the presence of alkaloids, flavonoids, tannins, and saponins, Wagner, Kumar, ferric chloride, and the development of persistent frothing were used in the qualitative phytochemical analysis of the extracts.

2.3. Estimation of total phenolics

The total phenolic content of extracts was determined using the Folin-Ciocalteu method (AL-Nadaf et al., 2018). This method is based on the principle that phenolic substances reduce the reagent in the presence of sodium carbonate, resulting in a color change. Diluted samples were pipetted into test tubes and mixed for a minute before resting for 5 minutes in a dark place at room temperature for incubation. A UV/VIS spectrophotometer was used to measure absorbance at 760 nm (Thermo, USA). In terms of dry material weight (g), the results were expressed as mg gallic acid equivalent. The calibration curve was created using gallic acid solutions ranging in concentration from 0.0125 to 0.2 mg/ml. The average value was calculated from all the spectrometric measurements, which were all made in three replicates (AL-Nadaf et al., 2018).

2.4. Determination of antioxidant activity

S. securidaca extract antioxidant activity was assessed using the DPPH method. For this procedure, 0.025 g of the dark purple DPPH reagent must be dissolved in 1 L of methanol. 0.1 mL of each sample is diluted in 3.9 mL of this solution before being combined. The mixtures were incubated for 90 minutes at room temperature in a dark space before a UV/VIS spectrophotometer was used to measure the absorbance at 517 nm. In terms of IC_{50} g. ml^{-1}), the antiradical activity was expressed. All samples were analyzed in triplicate (Safi et al., 2022; Kayarohanam et al., 2019; Brand-Williams et al., 1995).

2.5. Animals

Healthy Wistar rats of either sex was provided by the Research Center of Laboratory Animals at the Applied Science University in Amman, Jordan (weight 150–200 g). All the animals were housed in a controlled environment with a 12-hour light-to-dark cycle, temperatures between 22 and 28, and an air humidity level between 60 and 70 percent. They were also given unlimited access to food and water. The Scientific Research Committee at Mutah University gave its approval to each experiment, which involved six animals. The animals were housed separately in their cages for a week before the study to help prevent biting. Before the experiment, they had unrestricted access to food and water pellets. All experiments were conducted following US guidelines (NIH publication, 1985) and were approved by the scientific committee of the faculty of pharmacy at Mutah University, Jordan (2/2020). All methods are reported following ARRIVE guidelines."

2.6. Acute toxicity

Male Wistar rats weighing 180–200g were divided into groups of 5 animals to test the acute toxicity. The crude extracts were administered intragastrically at doses of 200 and 1000 mg/kg, respectively. An intragastric dose of vehicle (1 mL/kg) was given to the control group. The mortality rate was observed within 72 hours. Chloral hydrate was used to sedate rats.

2.7. Effects of crude extracts on induced hyperlipidemia in rats

Five groups of animals (n = 5) were created. For the first fifteen days, all groups were given a high-fat diet (with a high goat fat content). Following that, the control group (Group I) was given a high-fat diet (HFD) (Mahmoud et al., 2024). The plant methanolic extract was given to Groups II, III, and IV along with HFD for a period of 20 days at a dose of 200 mg/kg p.o. Atorvastatin, the standard medication, was given to Group V along with HFD at a dose of 10 mg/kg p.o. At the beginning of each of the three treatment periods (zero, fifteen, and at the end of all treatments with atorvastatin and plant extract), blood samples were drawn, allowed to clot for 45 minutes, and the serum was centrifuged. A lipid profile was then performed using commercially available kits with the aid of an ELISA reader.

2.8. Biochemical tests

Serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C), very low-density lipoprotein (VLDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein and HDL/LDL, TC/HDL ratios were determined by enzymatic colorimetric methods using commercially available kits ("CHOD-PAP": enzymatic photometric test) by a biochemistry analyzer (DiaSys Diagnostic Systems GmbH, Germany/ DiaSys respons® 920).

2.9. Fecal cholesterol excretion

During the final three days of treatment, feces were collected. The fecal matter was extracted using CHCl3: MeOH after being dried and powered (2:1). The extracted substance was then examined for cholesterol content in a manner similar to how serum is examined. The amount of cholesterol excreted in feces (mg/g) was calculated (Liang et al., 2013; Jain et al., 2010).

2.10. Statistical analysis

Data were expressed as mean ± S.E.M. and evaluated by one-way ANOVA followed by the tuckey post hoc test. The data were considered significant at $p< 0.001$. Statistical analysis was done using the SPSS version 22.0 software package (SPSS Inc., USA).

3. Results

3.1. Preliminary phytochemical investigations

All extracts were subjected to semi-quantitative analysis to detect secondary metabolites such as flavonoids, tannins, alkaloids, and saponins, with flowers having the highest indicator for flavonoids and leaves and seeds having a higher possibility for alkaloids (Figure 1).

3.2. Total phenolic contents and DPPH free radical scavenging activity

The total phenolic content of the three extracts was determined by the Folin-Ciocalteu method, as shown in Figure 2. As seen in Figure 2, in terms of mg gallic acid equivalent per gram of dry material, all concentrations were expressed. Seed extract shows the highest phenolic content, which is 11.47 g%, with a significant difference compared to leaves (3.14 g%) or flowers (1.32 g) ($p < 0.001$). The antioxidant activity of *S. securidaca* extracts was assessed using spectrophotometry (DPPH radical) using samples with a concentration range of 0.0010–5.0 g/ml. As seen in Figure 2, the radical scavenging activity of the tested extracts is expressed as the percentage of deactivation of the DPPH free radicals. The quality of the antioxidants in the extracts was determined by the IC_{50} values. Seeds express a superior effect with an IC_{50} value of $4.62*10^{-2}$ mg/ml. This potency correlates with the total phenolic content.

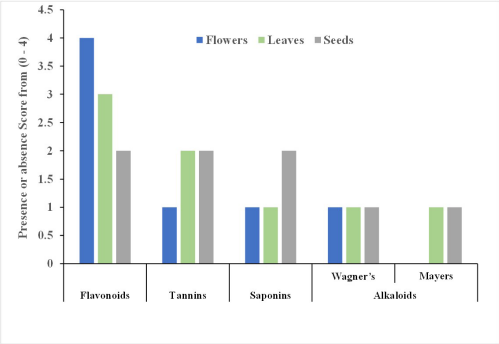

Figure 1. Nature and semi-quantitative analysis of phytoconstituents present in methanol extracts of *S. securidaca*: Flowers ; Leaves and Seeds.

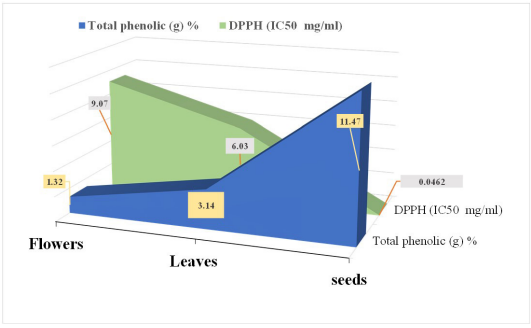

Figure 2. Total phenolic concentration and DPPH radical scavenging activity expressed as IC_{50} values (mg/ml) of crude methanolic extracts from *S. securidaca:* Flowers; leaves and seeds. For Total phenolic concentration: All the concentrations were expressed as mg gallic acid equivalent per (g) % of dry material.

3.3. Effect of S. securidaca extracts on serum lipid profile and atherogenic indices

There wasn't any mortality in groups of rats treated with crude extracts as an output for acute toxicity. The mean serum values for TC, TG, HDL-C, VLDL-C, LDL-C, HDL/LDL, and TC/HDL were monitored for the lipid profile analysis. The results are shown in Table 1. The default comparison was against control.

In comparison to their pretreatment values, the high-fat diet-fed (HFD) animals had significantly higher serum cholesterol, triacyl glycerol, and LDL levels, as well as lower levels of HDL. While HDL-C is cardioprotective, elevated blood cholesterol, particularly LDL-C, is a known major risk factor for coronary heart disease (CHD).

Atorvastatin and seed extract both significantly decreased cholesterol TC, while flower extract only had a marginally smaller impact ($p < 0.001$). Atorvastatin and seed extract had similar effects that were not statistically different ($p = 0.011$). All treatments significantly reduced TG ($p < 0.001$).

An increase of 33.3% and a value of 52.0 mg/dl ($p < 0.001$) were observed in HDL-C following the leaf extract. Atorvastatin reduced VLDL-C by 68.8% (p 0.001) and seed extract by 45.0% (p 0.001). LDL-C levels were reduced by 51.26% ($p < 0.001$) after treatment with seed extract (Table 1).

As shown in Table 2, the atherogenic index of plasma (AIP) was significantly reduced in all treated groups below zero compared to the control (0.23). Castelli's Risk Index II (CRI-II), a high HDL/LDL (3.76) ratio, was discovered to be a good marker for lipid profile in animals treated

Table 1. Serum lipid profile (Lipid parameters (mg/dL) of hyperlipidemic Wistar rats induced by high-fat diet (HFD) treated with CMC (control), Atorvastatin (20 mg·kg^{-1} of body weight) and methanolic extract of *S. securidaca*. Flowers, leaves, and seeds (200 mg·kg^{-1} of body weight).

	TC	TG	HDL-C	VLDL-C	LDL-C
Control	93.0±1.48	158.6±22.49	40.0±3.13	10.2±1.06	23.0±2.75
Flowers	81.4±1.91(12.5) *	48.6±0.50(66.8) *	42.0±31	8.0±0.31	25.0±0.31
Leaves	96.2±1.74	78.0±0.83(46.9) *	52.0±0.7(33.3) *	8.6±0.50	26.2±0.37
Seeds	58.6±0.89(36.9) *[a]	47.6±0.50(67.6) *	39.0±0.31	3.0±0.31(68.8) *[b]	10.4±0.24(51.26) *
Atorvastatin	66.0±1.0(29.0) *	61.0±0.70(58.7) *	37.2±0.96	5.2±0.37(45.0) *	17.6±0.50

Values are expressed as mean ± SEM (n = 5). Serum total cholesterol (TC); triglycerides (TG); high-density lipoprotein-cholesterol (HDL-C); very low-density lipoprotein (VLDL-C) and low-density lipoprotein cholesterol (LDL-C). *$p < 0.001$ considered statistically significant as compared to control group. [a]No significant difference compared to Atorvastatin p=0.0113. [b]seeds not sig differ from Atorvastatin as p= 0.10095.

Table 2. Atherogenic indices: atherogenic index of plasma (AIP), Castelli Risk Index I and II (CRI), atherogenic coefficient (AC), and non-high density lipoprotein cholesterol (HDLc) (NHC).

Treatment	AIP	CRI-I	CRI-II	AC	NHC	Fecal cholesterol(mg/g)
Control	0.23	2.33	1.74	1.33	1.37	7.79
Flowers	-0.30*	1.94[a]	1.68	0.94[b]	1.02*	9.94
Leaves	-0.18*	1.85*	1.98	0.85*	1.14*	17.09
Seeds	-0.27*	1.50*	3.75*	0.50*	0.51*	4.45
Atorvastatin	-0.15*	1.77*	2.11	0.77*	0.74*	15.30

Atherogenic index of plasma (AIP) = log triglyceride/high-density lipoprotein cholesterol (HDLc), Castelli's Risk Index (CRI-I) = Total cholesterol/HDLc, CRI-II = Low density lipoprotein cholesterol/HDLc, Atherogenic coefficient (AC) = (Total cholesterol−HDLC)/HDLC, Non-high density lipoprotein cholesterol (HDLC) (NHC). *p<0.001; [a] p=0.0052 [b]p=0.0055

Table 3. Hepatic parameters (aspartate aminotransferase – AST, alanine aminotransferase – ALT, alkaline phosphatase – ALP) I.U. for hyperlipidemic Wistar rats induced by HFD treated with CMC (control), Atorvastatin (20 mg·kg^{-1} of body weight) and methanolic extract of *S. securidaca*. flowers, leaves, and seeds (200 mg·kg^{-1} of body weight).

	ALT	AST	ALP	Total Protein
Control	84.5±10.38	115.7±13.15	315.2±57.20	8.3±0.19
Flowers	118.0±0.31(48.59) *	143.5±0.24	435.0±0.24	6.9±0.24
Leaves	157.3±0.31(98.06) *	135.5±0.40	522.3±0.63(103.86) *	7.6±0.40
Seeds	79.1±0.33	115.4±0.40	254.2±0.27	7.9±0.25
Atorvastatin	15.8±0.58(80.22) *	142.5±0.67	673.2±3.31(162.82) *	7.3±0.37

Values are expressed as mean ± SEM (n = 5). *p < 0.001 considered statistically significant as compared to control group.

with seed extract. Finally, all extracts except flowers had a lower Castelli's Risk Index I (CRI-I) (TC/HDL) compared to control (p<0.001), with values of 1.85, 1.5, and 1.78 for leaves, seeds, and Atorvastatin, respectively, while flowers had a value of 1.94 with no sig difference as the p-value is 0.0052 compared to control. The CRI-I for seeds did not differ significantly from that of atorvastatin (p = 0.100). Higher CRI-II values were observed for seeds and atorvastatin (p <0.001). Except for flowers, where the AC value did not significantly differ from the control (p <0.001), all treatments had higher AC and NHC indices than the control (Table 2).

Clinical biochemistry levels such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured to determine the enzymatic activities of the livers. Serum AST, ALT, and ALP are enzyme biomarkers that help in the clinical diagnosis of liver toxicity conditions by monitoring the structural integrity and damage of the liver. Table 3 depicts the effects of *S.securidaca* extracts on the levels of AST, ALT, and ALP.

When the organs are injured for any reason, these enzymes are released into the bloodstream. We, therefore, looked at the levels of AST, ALT, and ALP in rat serum. There are no discernible differences between any of the groups in the serum. AST levels are shown in Table 3. The control group had a mean ALT enzyme level of 84.46±10.38 I.U., flowers 118.0±0.31 (48.59%), and leaves 157.3±0.31 (98.06%) were significantly higher than controls (p > 0.001). Atorvastatin, however, was significantly less than the control at 15.8±0.58 (80.22%) (Table 3).

Table 4. Effect of repeated daily oral treatments with Atorvastatin, control and crude methanolic extracts from *S. securidaca*: Flowers ; Leaves and Seeds in the average body weight of rats on day 1 and end of treatments.

	Day 1	End day	Increment weight %*
Control	212.5±2.07	273.8±1.36	28.8
Flowers	216.3±1.62	243.8±1.62	12.7
Leaves	212.5±2.25	248.8±1.84	17.1
Seeds	208.8±1.84	238.8±1.04	14.4
Atorvastatin	210.0±1.97	240.0±1.25	14.3

*All significantly differ compared to control p<0.001.

The ALP levels were 315.2±57.20 I.U (mean ± S.E.M) in the control group and 522.3±0.63 I.U (103.86%) in the leaf extract fed group, which is significantly higher than the control (p<0.001) while they were 673.2±3.31 I.U (162.82%) in the Atorvastatin fed group, which is significantly higher than the control (p<0.001). There is no discernible difference between the other groups and the control (p>0.001) (Table 4). In contrast to the untreated control rats (28.8% increase in body weight), daily oral treatments of rats with Atorvastatin (20 mg/kg) and the crude flowers, leaves, and seeds methanolic extract (200 mg/kg) with HFD did not significantly (p<0.001) increase their average body weight (Table 4). Leaf methanolic extract (200 mg/kg) and Atorvastatin show a significant increase in cholesterol

excretion compared to control (p<0.001). Leaf extract is not significantly different compared to Atorvastatin (p = 0.128), while seed methanolic extract (200 mg/kg) shows a significant reduction in cholesterol excretion compared to control (p<0.001).

4. Discussion

One of the primary factors that seriously compromise human health is hyperlipidemia. The prevalence of hyperlipidemia was 20–40% in the general population, and it was growing at an estimated rate of 10,000 per day (Liu et al., 2022). The primary risk factors for vascular disease are elevated levels of TC, TG, and LDL-C. High HDL-C levels protect against its development. Although synthetic hypolipidemic drugs currently dominate the market, their toxic components cannot be eliminated. Because of the negative effects of synthetic drugs and chemicals, herbal medicines are being researched and used more to maintain our basic health. Previous studies demonstrated that this plant's hydroalcoholic seed extract lowers serum cholesterol in diabetic rats. This work's purpose is to investigate the antihyperlipidemic effect of *S. securidaca* methanolic extract for flowers, leaves, and seeds against the HFD rat model (Watroly et al., 2021).

Phytochemical studies have revealed the presence of several classes of bioactive compounds in the seeds of *S. securidaca*. More emphasis on seeds, with few research findings evaluating the flower effect and no previous study assessing the leaf effect (Jamshidzadeh et al., 2018; Ahmadi et al., 2016; Raesi et al., 2019). Flavonoids, alkaloids, steroids, saponins, fatty acids, and pentacyclic triterpenoids are among the compounds with pharmacological effects. Phytochemical analysis revealed the presence of flavonoids, tannins, alkaloids, and saponins in both seeds and leaves (Singh et al., 2021). This is consistent with previous findings in the hydroalcoholic portion of seeds by Garjani and coworkers (Garjani et al., 2009).

Flavonoids are abundant in flowers. The phenolic content of seeds methanolic extract (11.47 g%) is significantly higher than that of leaves (3.14 g%) or flowers (1.32 g%) (p 0.001). The hydroethanolic phenol content (17.88%) of flowers was reported by Ahmadi et al., (2016) to extract weight and is now reported as mg gallic acid equivalent per gram of dry material. Ibrahim et al. (2015) revealed that flowers total phenolics and flavonoids estimated as gallic acid equivalents were 82.39 mg/g.

The low DPPH radical IC50 ($4.62*10^{-2}$ mg/ml) of seeds is explained by their high phenol content (Figure 2). The radical scavenging activity does not solely result from phenolic content. It is the cooperation of all reactive components that accounts for the lowest DPPH activity contributed by leaves with intermediate phenol content (Figure 2). The previously reported DPPH IC_{50} for seeds hydroalcoholic extract (19.2 g/ml) (Ahmadi et al., 2016). However, there have been no previous reports of DPPH activity in flowers or leaves.

Some of the metabolic abnormalities that contribute to atherogenic dyslipidemia include the production of triglyceride-rich lipoproteins, HDL catabolism, and increased cholesterol synthesis. Higher hepatic production of lipoproteins is thought to be the key factor in these abnormalities. The availability of triglycerides within hepatocytes has a significant impact on how much VLDL is produced. Extrahepatic fatty acids, or de novo synthesized fatty acids, are used to make triglycerides. The most prevalent extrahepatic source of free fatty acids for triglyceride synthesis is adipose tissue. The tightly regulated process of lipolysis results in the release of fatty acids from adipose tissue (Sahoo et al., 2021; Zuraini et al., 2021; Niroumand et al., 2015).

In this study, adding goat fat (50%) to rats' diet for 15 days resulted in hypercholesterolemia as indicated by a significant increase in serum total cholesterol (32%; p < 0.001), TG (60.97%, p < 0.001), LDL cholesterol (66.55%; p < 0.001), VLDL (65.2%, p <0.001), and a significant reduction in HDL (22.35%, p < 0.001). With a few exceptions, these findings are consistent with other studies in which an increase in rat dietary cholesterol intake increased plasma or serum cholesterol (Garjani et al., 2009; Firdous et al., 2021). HFD is associated with abnormalities in lipid profile indices and metabolism and is thought to be the primary cause of atherosclerosis progression.

A rise in TC and LDL cholesterol, which is a key factor in the generation of reactive oxygen species, which then causes oxidative stress and stimulates lipid peroxidation, is reflected in hyperlipidemia (Brand-Williams et al., 1995). Atorvastatin, seed extract, and only to a lesser extent flower extract significantly decreased the TC (p< 0.001). Atorvastatin's effect was not significantly different from that of seed extract (p=0.011). A significant reduction in TG was also seen across all treatments (p<0.001) (Table 3). This provides evidence for the advantageous impact of seeds' bioactive components, which may be attributed to their high antioxidant potential, as shown by DPPH activity. Although they come in lower percentages of flower extract. The beneficial HDL-C, which is increased by a value of 33.3% (p< 0.001) is the only kind of blood that the bioactive components of leaves significantly affect. The diverse collection of bioactive substances found in leaves results in this behavior (Figure 1).

Ibrahim et al. (2015) reported that the ethanolic extract of the flowers had a hypolipidemic effect on elevated serum triacylglycerides and cholesterol levels in hyperglycemic rats, while the methanolic flower extract reduced TC and TG by 12.5% and 66.8%, respectively. These variations are reflected by differences in phytoconstituents, which resulted in a big difference in total phenol content from 82.39 to 1.32 g% (Table 1) (Chinnasamy et al., 2019).

Several lipoprotein ratios, or "atherogenic indices," have been defined to optimize the predictive capacity of the lipid profile. Atherogenic index of plasma (AIP), Castelli Risk Index I and II (CRI), atherogenic coefficient (AC), and non-high density lipoprotein cholesterol (HDLc) are the various atherogenic indices (NHC). Higher atherogenic indices are more likely to cause oxidative damage. According to previous studies, AIP can reliably predict the risk of atherosclerosis and coronary heart disease (Dobiásová, 2004). AIP is correlated with pre- and anti-atherogenic lipoprotein particle size, reflecting the true correlation between protective and atherogenic

lipoproteins. To calculate AIP, one uses the formula log (TG/HDL-C). AIP values between 0.11 and 0.21 and greater than 0.21 have been associated with intermediate and increased risks of CVD, respectively. It has been proposed that an AIP value of less than 0.11 is associated with a low risk of CVD (Niroumand et al., 2015; Dobiásová, 2004).

As shown in Table 2, AIP was significantly decreased in all treated groups below zero compared to the control (0.23). After receiving the extract treatment, the LDL-C level was significantly reduced by 51.26% ($p< 0.001$). Animals given seed extract showed improved lipid profiles as measured by Castelli's Risk Index II (CRI-II), which has a high HDL/LDL 3.76 ($p< 0.001$) ratio.

Finally, except for flowers, all extracts had lower CRI-I (TC/HDL) values than the control ($p<0.001$), with values of 1.85, 1.50, and 1.78 for leaves, seeds, and Atorvastatin, respectively. Flowers, however, receive 1.94 with no significant difference as the p-value is 0.0052 compared to the control (Table 2). The CRI-I for seeds and Atorvastatin did not differ significantly ($p=0.100$). CRI-II levels were higher in the cases of seeds and Atorvastatin ($p<0.001$). Except for flowers, the AC and NHC indices were significantly lower in all treatments compared to the control ($p<0.001$). Flowers AC did not significantly differ from control ($p = 0.0055$) (Table 2) (Gnanaraj et al., 2022; Lum et al., 2022). These outputs correlate with Jamshidzadeh et al. (2018). The significant rise in plasma TC and TG levels following HFD administration can be attributed primarily to the increased absorption of a high-fat diet, which helps to explain these findings. With the breakdown and metabolism of VLDL and LDL, the proportion of TG in VLDL, which is several times higher than the proportion of cholesterol, rapidly decreases. HDL accelerated the mobilization of TG and TC from plasma to the liver, causing them to break down and be secreted as bile acids. This can help clarify the effect of leaves on high HDL and low TG levels.

Although flowers and leaves have some good effects on increasing HDL-C levels, seed extract is more efficient in reducing hyperlipidemia. This is explained by the distribution and profile of phytochemical constituents such as phenols, saponins, tannins, and flavonoids throughout plant development.

These findings show an antiatherogenic lipidic profile and allow for the suggestion that the extract has a protective effect against hypercholesterolemia brought on by the mode of metabolism enriched in cholesterol. Phenols, saponins, tannins, and flavonoids, particularly saponins, appear to be responsible for the reduction in total cholesterol in hyperlipidemic rats. All the cases had lower LDL cholesterol levels and occasionally higher HDL levels. Tannins and saponins would either form a complex with cholesterol or have an immediate effect on cholesterol metabolism (Francis et al., 2002).

Saponins are mostly surface-active glycosides derived from plants, and they can be triterpenoid or steroid saponins. Studies have shown that saponins from various sources lower serum cholesterol levels. When saponin-rich foods like soybean, lucerne, and chickpea are consumed, it is hypothesized that the interaction of saponins with bile acids causes the bile acids to be excreted in greater amounts (Baum et al., 1998; Francis et al., 2002; Han et al., 2000).

Serum levels of cholesterol may decrease because of the liver's accelerated metabolism (Francis et al., 2002). According to Han et al. (2000), pancreatic lipase (PL) activity is suppressed by the saponins found in the aqueous extract of Platycodi radix, which prevents intestinal absorption of dietary fat. The extract's hypolipidemic and advantageous properties are probably to some extent due to the saponins, anthocyanins, or sterols found in *S. securidaca* seeds and leaves (Akhlaq et al., 2021). However, the precise way they work to lower hyperlipidemia is yet unknown, necessitating more study.

Fecal cholesterol levels provide additional support for the serum-lowering effect. When compared to the control group, in the treatment groups with leaves and Atorvastatin, cholesterol excretion in the feces was higher, which expresses the mode of action attributed to Atorvastatin and supports the marginal impact of leaf extract on TG reduction. Intriguingly, the seeds exhibit a significant hypolipidemic effect when compared to Atorvastatin, while fecal cholesterol excretion is reduced when compared to the control (Table 1). These outcomes could be explained by a rise in fecal bile acid excretion or a fall in sterol absorption. Similar findings were made regarding blueberry anthocyanins by Liang et al. (2013), as well as the hypolipidemic effect of Moringa oleifera by Jain et al. (2010). The reduction in reabsorption of cholesterol from endogenous sources along with a concurrent rise in its excretion into feces as neutral steroids could be the reason for the effect on cholesterol.

Through the stimulation of antioxidant enzymes, flavonoids, phenols, and fatty acid content have been shown to protect liver tissue. Flavonoids are antioxidants that reduce LDL cholesterol oxidation, implying a role in preventing hyperlipidemia damage in cardiovascular disease (Baum et al., 1998). It is also claimed that flavonoids increase the number of LDL receptors in the liver, increasing the liver's uptake of lipids from the blood (Garjani et al., 2009). This can boost the hypolipidemic action of *S. securidaca* extracts such as seeds, leaves, and flowers (Subramaniyan et al., 2018).

Treatments had no effect on AST levels, but flowers and leaves had elevated ALT levels. Atorvastatin has a beneficial effect against ALT but a negative effect on high ALP levels (Table 3). There was no significant effect on total serum protein (Table 3). All enzymes are found at normal levels in seeds (Owu et al., 1998). This suggests liver dysfunction brought on by ingesting extracts of leaves as well as, to a lesser extent, Atorvastatin. The liver's cellular membranes are shielded from oxidative stress by the antioxidant and free radical scavenger properties of seed extract, so experimental methods used to study liver enzymes yield adequate results. However, the active ingredient present in extracts can harm the liver. Additionally, LDL-C oxidation in the body may cause hepatotoxicity (Xu et al., 2019). According to the evidence, LDL-C oxidation occurs in vivo, and this modified LDL-C is cytotoxic to various organs, including the liver (Subramaniyan et al., 2021).

In this study, we showed that rats fed leaf extract had elevated plasma levels of LDL-C and TC. The main organs vulnerable to oxidative damage are the testes, liver, and kidneys, according to earlier research. There is a possibility

that enzymes will leak out of damaged liver cells and accumulate in the blood at high levels (Dobiásová, 2004). With leaf extracts being the least safe, this may make it clearer how safe seed extracts are compared to those from flowers. In contrast to seeds, which have a much higher level of hepatoprotective activity, atorvastatin produces significant amounts of ALP. The serum ALP is sensitive for detecting early intrahepatic and extrahepatic bile obstruction as well as the presence of infiltrative liver diseases (Owu et al., 1998). As a result, the elevated serum ALP level in the current study is most likely due to increased TG and LDL-C oxidation.

These thorough results provide evidence for *S. securidaca* extracts' multipurpose properties and raise the idea that taking atorvastatin and *S. securidaca* extracts together may be helpful for complicated illnesses like hyperlipidemia. The current study's objectives were to confirm the effectiveness of *S. securidaca* extracts in comparison to atorvastatin. Additional research is required to ascertain the potential for employing *S. securidaca* extracts in conjunction with atorvastatin in the treatment of hyperlipidemia and to lessen its side effects.

We investigated the hypolipidemic and antioxidative effects of a methanolic extract of *S. securidaca* flowers, leaves, and seeds in hypercholesterolemic rats. The oral administration of *S. securidaca* seed extract to hyperlipidemic rats for a brief period (20 days) significantly reduced serum triglyceride levels, bringing them back to those of control rats with normal TG levels. The extracts also reduced serum total cholesterol levels while significantly lowering LDL cholesterol.

Treatment with *S. securidaca* extracts—seeds, leaves, and, to a lesser extent, flowers—resulted in a transitory or temporary reduction in serum lipid profile, confirming a protective potential against hyperlipidemia. Combining the findings, *S. securidaca* extracts have strong hepatoprotective and antihyperlipidemic effects. Though flowers and leaves also reflect a favorable scenario regarding the effect of HDL elevation, seed extract was still the best. The findings of this study indicate a promising application prospect, but more research is needed to determine the exact mechanism of these novel compounds as antihyperlipidemic agents and to clarify their potential combination effect with synthetic drugs such as Atorvastatin. Combinations can reduce the number of chemical drugs needed, which reduces the likelihood of side effects.

5. Conclusion

We investigated the hypolipidemic and antioxidative effects of methanolic extract of *S. securidaca* flowers, leaves, and seeds in hypercholesterolemic rats. Treatment with *S. securidaca* extracts: seeds, leaves, and, to a lesser extent, flowers, resulted in a transitory or temporary reduction in serum lipid profile, confirming a protective potential against hyperlipidemia. Though flowers and leaves also reflect a favorable scenario regarding the effect of HDL elevation, seed extract was still the best. The findings of this study indicate a promising application prospect, but more research is needed to determine the exact mechanism of these novel compounds as antihyperlipidemic agents and to clarify their potential combination effect with synthetic drugs such as Atorvastatin. Combinations can lessen the number of chemical drugs needed, which lessens the likelihood of side effects.

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