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Research Article

The Preventive Effects of *Urtica dioica* Extract and Nanoparticle on Oxidative Stress and Lipid Profile in Hyperlipidemic Male Rats

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ABSTRACT

Cardiovascular diseases (CVDs) are the major factor in human premature death and disability and they are becoming. Oxidative stress is thought to be a major factor in the development of atherosclerosis. One of the CVDs risk factors are elevated lipid profile. Significant gaps in the treatment of CVDs still exist, despite making healthy lifestyle and using lipid-lowering medications as part of primary CVDs prevention. Natural extracts are a rich source of biomolecules that can be used as reagents for the biosynthesis of silver nanoparticles (AgNPs). The present study estimates the antioxidant and protective activity of *Urtica dioica* (UD) in induced hyperlipidemia rats divided into six groups; Group 1 fed on normal diet, Group 2 fed a high-fat diet, 200 mg/dl water extract, and ethanol extract, respectively, for Groups 3 and 4, Group 5 receives 15 mg/dl AgNPs, and Group 6 receives 20 mg/dl atorvastatin all fed on a high-fat diet and then evaluates lipid profile and malonaldehyde, superoxide dismutase and reduced glutathione of the serum after 40 days. The results of characterization test demonstrated the successful synthesis of AgNPs, also shows that the UD extract and AgNPs have a favorable impact on lipid profile significantly reduced the level of total cholesterol, low-density lipoprotein (LDL), and markedly decrease liver enzymes also can function as a good antioxidant that can be employed as a protective agent against rising lipids and oxidative stress. However, further research studies need to investigate the safety and activity of different doses of AgNPs of UD.

Keywords: Hyperlipidemia, antioxidant, *Urtica dioica*, silver nanoparticle, cardiovascular disease

INTRODUCTION

Hyperlipidemia is thought to be a very harmful factor contributing to cardiovascular and cerebrovascular diseases, in particular, atherosclerosis.[1] This happens as a result of a malfunction in the body's lipid metabolism, raising the serum lipid concentration over the usual range. The occurrence of hyperlipidemia has increased dramatically over time and is now one of the most prevalent pathological disorders in humans as a result of inheritance, diet, nutrition, medicine, and other variables.[2] The development of numerous diseases, including cancer, age-related disorders, neurodegenerative diseases, autoimmune disorders, and cardiovascular disease (CVD), is thought to be significantly influenced by oxidative stress. Oxidative stress can have an impact on CVD development in a variety of ways. According to the research on atherosclerosis, the formation of atherosclerotic plaques is said to begin with the oxidation of low dispersed lipid (LDL) particles in the arterial endothelium.[3]

Pharmaceutically dynamic bioactive substances found in medicinal plants have additive and synergistic therapeutic actions that are helpful in the treatment of metabolic diseases. Utilizing the knowledge of local populations, the majority of pharmaceutical medications are made by isolating the primary active ingredients from medicinal plants.[4] A significant advancement in modern medicine has resulted from the systematic research of medicinal plants and the investigation of their biologically active phytochemical components in the treatment of metabolic diseases.[5] Contrary to current medical systems, polyherbal formulations have drawn increased interest because of their ability to treat several conditions while having fewer negative effects.[6]

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Urtica dioica L. (UD) in North Europe and parts of Asia, the stinging nettle family, or Urticaceae, is widely distributed and is typically found in rural areas.[7] Traditional medicine has long used this herb to treat a variety of ailments, including dyslipidemia. *In vivo* studies have shown that UD has a beneficial effect against dyslipidemia.[8] More recently, it was demonstrated that a combination of aqueous extracts from *Peganum harmala*, *Rhus coriaria*, and UD improved metabolic and histological parameters in diabetic rats.[9] Another study demonstrates that methanol extracts from various common herbs, including UD, can be helpful in the formation of silver nanoparticles (AgNPs) verify their free radical scavenging activity and reducing power.[6]

This inspired us to examine the potential preventive benefits of UD ethanol, water, and NPs extracts on hyperlipidemia in a high-fat diet fed animal model with hyperlipidemia.

METHODOLOGY AND EXPERIMENTAL DESIGN

Preparation of the Plant Extracts

The dried leaves of the UD were gained from local herbal shop in Erbil city, which has been dried in shade at room temperature. The plant was identified by Professor Abdulla Shakur, Salahaddin University-Erbil, College of Education, Department of Biology. A 25 g of the powdered plant material was mixed with 250 ml of 70% ethanol 1 sample:10 solvent ratio(w/v) and sonicated 60 min Power Sonic 405/Lab Tech kept at 40°C. Following that, Whatman No. 1 filter paper with Buchner funnel was used to filter the obtained extract. The filtrate was concentrated in a rotary evaporator under a controlled vacuum. After air drying, the concentrated extract was preserved at 4°C in an airtight container.[10] Furthermore, another 25 g of the powdered plant material was mixed with 250 ml distilled water 1 sample:10 solvent sample ratio (w/v) then using the same producer as in ethanol extract.

AgNPs Synthesis

For the preparation of the nanoparticle, 10 g of dried plant powder was mixed with 300 ml of boiled distilled water then boiled for 10 min with a magnetic stirrer and filtered by Whatman No. 1 filter paper then stored at 4°C, 100 ml of prepared extract was added to 1000 ml of 1 Mm of aqueous $AgNo_3$ and put on a hot plate for 60 min at 40°C with magnetic stirrer up till the color turned dark brown and adjusting the pH to 11 by NaOH.

UV–Vis spectra were used to establish whether AgNPs were formed following the centrifuging of the wholly reduced solution at 5000 rpm for 30 min. The obtained pellet was dissolved in deionized water after the supernatant was discarded. To get rid of any materials that were adsorbed on the surface of the AgNPs, the same washing procedure was carried out 2 or 3 times by deionized water and ethanol.[11]

Characterization of AgNPs

The absorbency of all samples was measured using UV–Vis spectra for confirming the formation of AgNPs, Fourier-transform infrared (FTIR) spectroscopy was performed on SHIMADZU FTIR spectrometer to detect the possible functional groups in biomolecules present in the plant extract. The X-ray diffraction (XRD) measurement was carried out on X-ray diffractometer (Panalytical X'PERT-PRO PW3050/60) operated at 45 kV and 40 mA and the spectrum was recorded by Cu radiation with a wavelength of 1.54060 Å in the 2q range of 20e80. A scanning electron microscope (SEM) was used to investigate the AgNPs' size and surface shape.

Experimental Animals

The male albino rats were kept in a room with a temperature of 22 ± 2°C and a 12:12 h light/dark cycle. Each rat weighed between 250 and 300 g, the experiment on the animal was conducted in the animal house of pharmacy college/ Hawler Medical University, the rats were kept for 1 week for acclimatization.

Induction of Hyperlipidemia

For inducing hyperlipidemia, the standard pellet of the rat is mixed with 5% cholesterol and 0.5% cholic acid according to a method described by Mojarradgandoukmolla *et al*.[12] For determining whether the rats were induced for hyperlipidemia, a pretest was done after 30 days of feeding them a cholesterol-enriched diet, the rats were starved for 24 h before measuring their cholesterol serum level by enzymatically diagnostic kits.

Doses and Toxicity

According to a study done by Kedi *et al*.,[13] he concluded that silver NPs have advantage to treat inflammation in low doses (100, 200, and 400 mg/kg). Research on acute toxicity was conducted by Jacob *et al*.[14] on healthy female albino rats with one dose of 2000 mg/kg reduced by plant extract. The study revealed that the AgNPs synthesized by *Ficus carica* were relatively non-toxic. Another study uses 250 and 500 mg/kg of UD phenolic extract and 5–10 mg/kg AgNP of the same plant.[15]

Experimental Procedure

The animals were divided randomly into six groups of six each made up of six rats (n = 6). The experiment lasted for 40 total days.

- Group 1 rats were served a standard pellet diet and they were considered a negative control group
- Group 2 rats were served with cholesterol-enriched diet and they were considered as a positive control
- Group 3 rats were served with 200 mg/kg (UD) water extract with cholesterol-enriched diet
- Group 4 rats were served with 200 mg/kg (UD) ethanol extract with cholesterol-enriched diet
- Group 5 rats were served with 15 mg/kg (UD) AgNPs with cholesterol-enriched diet
- Group 6 rats were served with 10 mg/kg of atorvastatin with cholesterol-enriched diet.

Body Weight

Rats were weighed once a week to observe changes in body weight throughout the duration of the 40-day study. When the experimental period ends the rats fasted overnight, the

next day anesthetizing of the rats was done by giving them a combination of ketamine 35 mg/kg with xylazine 5 mg/kg.[16] Then, the blood samples were drawn by puncture of the heart from each rat in each group and gathered in serum separating tubes, the blood was centrifuged at 3000 rpm for 15 min to prepare serum and determination of biochemical and liver function assay for antioxidant test the serum stored in −20°C.

Determination of Serum Lipid Profiles and Liver Function Test

Using a method where the color intensity of the dye formed is directly proportional to the concentration of the tested parameters, the measurement of serum total cholesterol (TC), serum LDL-C, serum triglyceride, and serum HDL cholesterol was performed using a Med Lab Reagent Kit/Cobas, USA.[17] Plasma concentrations of VLDL were determined by the method of Friedewald and Levy.[18] Liver function assay involving alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with gamma-glutamyl transferase (GGT) was determined by colorimetric method using Med Lab Reagent Kit/Cobas/USA.[19]

Oxidative Stress Evaluation

For determining the level of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) in the serum a Solarbio kit/China was used. MDA content is calculated by the difference between the absorbance at 532 nm, 450 nm, and 600 nm.[17] SOD can remove O□□ and inhibits the formation of methionine, SOD activity decreases with increasing darkness of the reaction solution's blue color, and the absorbency is detected at 560 nm.[20] GSH, 5,5-dithiobis-(2-nitrobenzoic acid), and GSH will react to form 2-nitro-5-mercaptobenzoic acid and GSH disulfide (GSSG). The yellow product 2-nitro-5-mercaptobenzoic acid has a maximum absorbance of 412 nm.[20,21] All absorbencies are detected by a microplate reader.

Statistical Analysis

The mean and standard error were used to express the study's data.[13] Analyzing of results performed by one-way analysis of variance to test the difference between the groups, Tukey multiple comparisons test in GraphPad Prism 8 version 8.0.1 for Windows 10 64X used. The analysis was conducted at a 95% confidence level and a significance level of 5%. Whenever $P = 0.05$ or less was regarded as statistically significant ($P \leq 0.05$).

RESULTS

The Formation of AgNPs

The formation of AgNPs was established by the color change observed visibly from bright green to a deep brownish color, further confirmation done by UV–Vis spectra, as shown in Figure 1. The result of absorbance of AgNPs from 200 to 800 nm gives the maximum absorbance between 440 and 450 nm and confirmed the presence of AgNPs. SEM was used to investigate the shape and size of AgNPs which was spherical in shape [Figure 2]. The result of XRD shows diffraction peaks at 37.6024°, 43.8755°, 64.0193°, and 76.9627° [Figure 3]. The

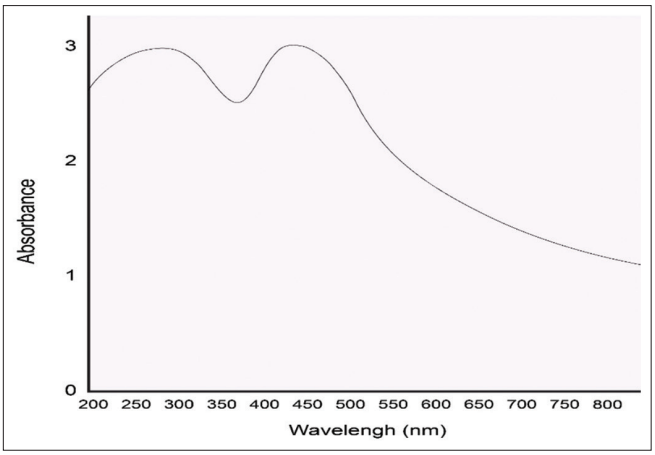

Figure 1: The result of UV–Vis spectra of *Urtica dioica* AgNPs

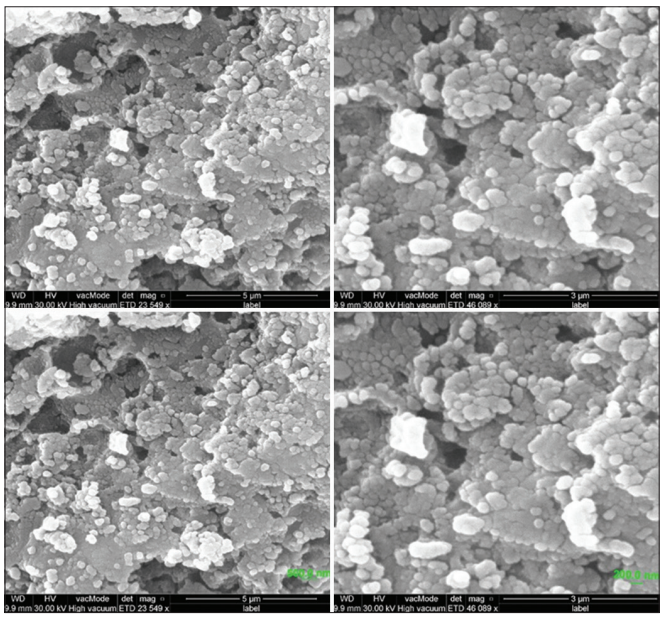

Figure 2: The result of SEM of *Urtica dioica* AgNPs

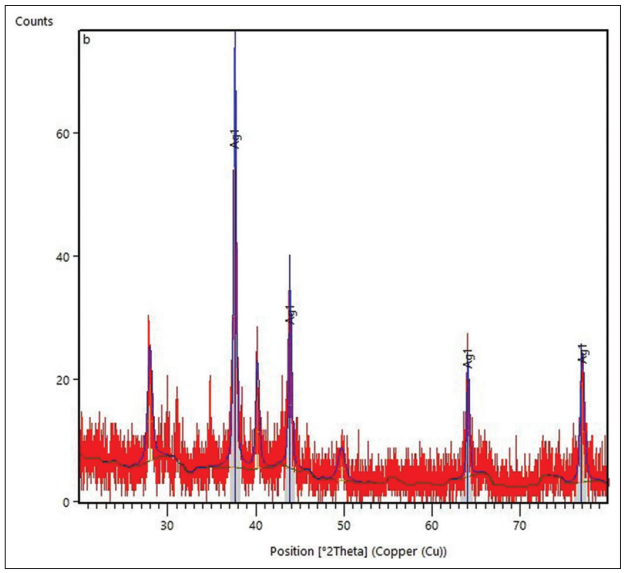

Figure 3: The result of XRD of *Urtica dioica* AgNPs

biosynthesis of AgNPs was investigated by FTIR spectroscopy and the result of the functional group is shown in Figure 4.

Lipid Profile

The total serum cholesterol in the positive control group was significantly ($P < 0.01$) increased (105 ± 6.776 mg/dl) when compared with the negative control group (65.90 ± 2.446 mg/dl), but in the hyperlipidemia rats treated with UD water extract (88.29 ± 1.523 mg/dl), ethanol extract (79.65 ± 2.677 mg/dl), nanoparticles NPs (83.82 ± 4.275 mg/dl), and atorvastatin (65.47 ± 2.827 mg/dl), the total serum cholesterol was decreased, these changes were significant ($P < 0.01$) as compared with positive group. The serum level of triglyceride in the positive control rats (116.2 ± 3.629 mg/dl) elevated significantly ($P < 0.01$) when compared with the negative control (71.67 ± 2.109 mg/dl), while this elevation was decreased significantly in the treated groups with UD water extract (80.4 ± 4.581mg/dl), ethanol extract (87.23 ± 2.639 mg/dl), NPs (77.73 ± 4.987 mg/dl), and atorvastatin (86.50 ± 3.221 mg/dl) when compared with positive control rats. The negative control (48.1 ± 2.65 mg/dl) and treated groups with UD water extract (40 ± 2.984 mg/dl) and ethanol extract (38.38 ± 1.257 mg/dl) and NPs (37.07 ± 1.711 mg/dl) are significantly ($P < 0.01$) improved in comparison with the positive control (23.23 ± 0.6712 mg/dl), while the atorvastatin group is non-significant with the positive control group. There is a significant difference in the level of LDL in the negative control and ethanol extract and NPs and atorvastatin group in comparison with the positive control, however, the result shows no significant change in the comparison between water extract and positive control rats. The obtained result shows a significant decrease in the level of serum VLDL in groups treated with UD water extract (16.08 ± 0.9163 mg/dl) and NPs (15.55 ± 0.9979 mg/dl) and atorvastatin (17.30 ± 0.6441 mg/dl) and negative control comparing with the positive control, also, comparing ethanol extract with the positive control, we found significant difference as shown in Table 1.

Liver Function

Table 2 shows that a significant difference in serum ALT was observed between the positive control group (54.1 ± 2.652 IU/L) and ethanol extract (42.22 ± 1.756 IU/L) and NPs group (41.1 ± 2.473 IU/L). Whereas, the rats treated with water extract (46.25 ± 0.6994 IU/L) and atorvastatin (46.32 ± 2.42 IU/L) were not significant difference between positive control as well as the negative control (44.77 ± 2.683 IU/L). The serum level of AST in the negative control (119.3 ± 3.981 IU/L) was significantly different in comparison with the positive control (145.6 ± 7.471 IU/L), as well as the rats that were treated with water extract (117.9 ± 4.357 IU/L), ethanol extract (119.6 ± 6.491 IU/L), and NPs (122.5 ± 2.064 IU/L), but

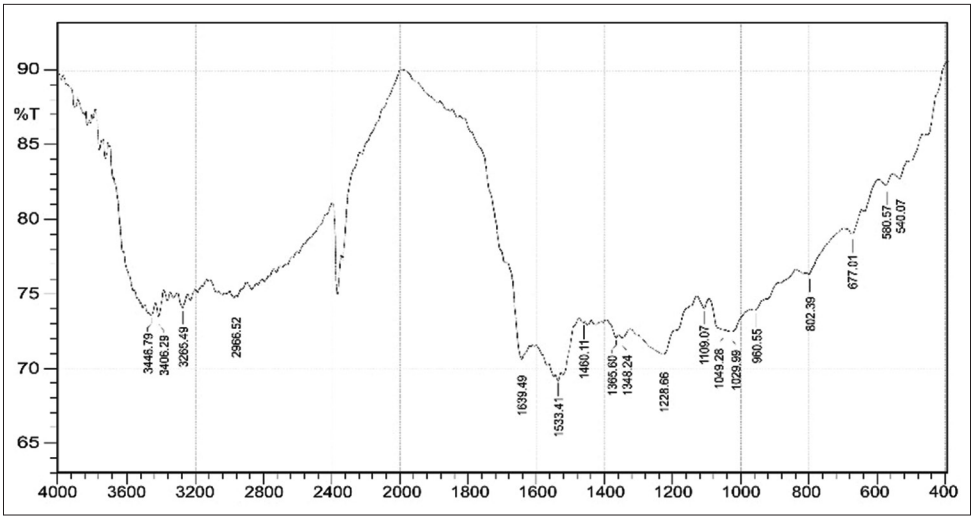

Figure 4: The result of Fourier-transform infrared spectroscopy

Table 1: Effect of *Urtica dioica* water and ethanol extract and its nanoparticle and atorvastatin on serum lipid profile in hyperlipidemic rats

Group/parameters	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL_C (mg/dl)	LDL_C (mg/dl)	VLDL_C (mg/dl)
Negative control	65.90±2.446****	71.67±2.109****	48.1±2.65****	26.85±1.805****	14.33±0.421****
Positive control	105±6.776	116.2±3.629	23.23±0.6712	57.97±5.682	23.25±0.725
Water extract	88.29±1.523*	80.4±4.581****	40±2.984****	45.85±3.460	16.08±0.916****
Ethanol extract	79.65±2.677***	87.23±2.639****	38.38±1.257****	37.43±3.681**	17.44±25.93****
NPs	83.82±4.275**	77.73±4.987****	37.07±1.711***	42.25±2.819*	15.55±0.997***
Atorvastatin	65.47±2.827****	86.50±3.221****	27.53±1.491	38.85±2.560**	17.30±0.644****

Significant differences between treatment groups were denoted by superscript asterisks symbols * significant at $P<0.05$, **$P>0.01$, ***$P>0.001$, and ****$P>0.0001$

the rats that treated with atorvastatin were non-significant different with the level of serum AST in positive control. Decreased levels of serum GGT were observed in the negative control, water extract, ethanol extract, NPs, and atorvastatin (0.81 ± 0.005164 U/L), (1.122 ± 0.1584 U/L), (1.178 ± 0.1712 U/L), (0.9483 ± 0.01721 U/L), and (1.263 ± 0.2177 U/L), respectively, and they are significantly different from positive control rats.

In the present study, rats in the negative control show a reduction in the level of blood glucose (220.3 ± 11.72 mg/dl) compared with the positive control as in the treated groups also observe a significant difference in the level of blood glucose for water extract (146.7 ± 5.735 mg/dl), nanoparticle (133 ± 11.94 mg/dl), and atorvastatin (230.8 ± 15.58 mg/dl) in comparison with the positive control (320.4 ± 15.23 mg/dl), but in rats that were treated with ethanol extract, we observe no significant difference. Results show that serum levels of total protein in treated groups with water extract (6.803 ± 0.09646 g/dl) and ethanol extract (6.937 ± 0.1768 g/dl) increased significantly when compared with positive control rats (6.175 ± 0.09139 g/dl). Furthermore, the treatments effect of hyperlipidemic rats with nanoparticles (6.623 ± 0.1273 g/dl) and atorvastatin (6.407 ± 0.1177 g/dl) was not significant when compared with untreated hyperlipidemic rats, also the rats in the negative control (6.46 ± 0.1497 g/dl) have no significant difference with the positive control (6.175 ± 0.09139 g/dl).

As shown in Table 3, no significant variations existed between untreated and treated animals in the level of serum amylase. While the level of blood urea in negative control and water extract and ethanol extract and nanoparticles decreased which is significantly different from the level of blood urea in the positive control, but when we compare the level of blood urea of atorvastatin (54.33 ± 1.745 mg/dl) with the positive control (56.53 ± 2.122 mg/dl), there was a non-significant difference.

There was an insignificant ($P > 0.05$) decrease in the MDA in the negative control group (1.135 ± 0.1453 nmol/mL), water extract (1.115 ± 0.3712 nmol/mL), ethanol extract (1.076 ± 0.3645 nmol/mL), and NPs (1.024 ± 0.1353 nmol/mL) compared with the positive control group (2.722 ± 0.2852 nmol/mL), and also, the MDA was not changed significantly in atorvastatin group when compared with the rats of the control positive. Significant difference in SOD was observed between rats in the water extract group (54.57 ± 1.390 U/mL), ethanol extract group (53.23 ± 0.768 U/mL), and NPs group (54.09 ± 1.629 U/mL) with the positive control (61.48 ± 0.8206 U/mL). Furthermore, the hyperlipidemic rats treated with atorvastatin (60.96 ± 1.601 U/mL) did not change SOD significantly when compared with the positive control rats as well as the result of the negative control was non-significant. Regarding the GSH, results of the ethanol extract (36.83 ± 2.216 mg/mL) and NPs treated rats (42.59 ± 6.71 mg/mL) showed a significant rise ($P < 0.01$) in the GSH as compared to the positive control rats (21.06 ± 0.7582 mg/mL). However, a non-significant variation ($P > 0.05$) was detected among the water extract (34.24 ± 1.191 mg/mL), atorvastatin (30.69 ± 4.475 mg/mL) group, and the positive control group in GSH [Figure 5].

DISCUSSION

If the serum levels of TC, LDL, and TG in the hyperlipidemic rats are taken into consideration, our results revealed that the

Table 2: Effect of *Urtica dioica* water and ethanol extract and its NPs and atorvastatin on liver function tests in hyperlipidemic rats

Group/parameters	ALT (IU/L)	AST (IU/L)	GGT (U/L)
Negative control	44.77±2.683	119.3±3.981*	0.81±0.005****
Positive control	54.1±2.652	145.6±7.471	3.833±0.600
Water extract	46.25±0.699	117.9±4.357*	1.122±0.158****
Ethanol extract	42.22±1.756**	119.6±6.491*	1.178±0.171****
NPs	41.1±2.473**	122.5±2.064*	0.9483±0.017****
Atorvastatin	46.32±2.42	144.3±5.673	1.263±0.217****

Significant differences between treatment groups were denoted by superscript asterisks symbols *significant at $P<0.05$, **$P>0.01$, ***$P>0.001$, and ****$P>0.0001$

Table 3: Effect of *Urtica dioica* water and ethanol extract and its nanoparticle and atorvastatin on blood glucose, total protein, amylase, and blood urea in hyperlipidemic rats

Group/parameters	Glucose (mg/dl)	Total protein (g/dl)	Amylase (u/dl)	Blood urea (mg/dl)
Negative control	220.3±11.72****	6.46±0.149	21.95±0.956	32.42±1.593****
Positive control	320.4±15.23	6.175±0.091	24.26±0.619	56.53±2.122
Water extract	146.7±5.735****	6.803±0.096*	23.72±1.144	43.63±1.907**
Ethanol extract	312.9±9.531	6.937±0.176**	25.55±0.979	44.7±3.344**
NPs	133±11.94****	6.623±0.127	23.87±1.517	36.43±1.819****
Atorvastatin	230.8±15.58***	6.407±0.1177	23.63±0.785	54.33±1.745

Significant differences between treatment groups were denoted by superscript asterisks symbols *significant at $P<0.05$, **$P>0.01$, ***$P>0.001$, and ****$P>0.0001$

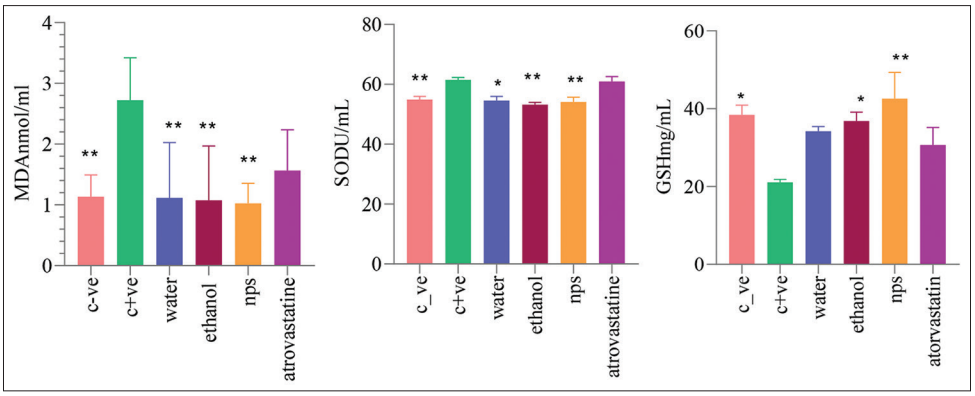

Figure 5: Effect of *Urtica dioica* extracts and AgNPs on MDA, SOD, and GSH biomarkers

water, ethanol, and NPs extracts of UD hold a favorable effect in controlling these parameters and ultimately preventing the risk of atherosclerosis and related CVDs. Stinging nettles have been shown to be richer in individual polyphenols than other wild plants.[22] Many studied found that the phenolic compound has the ability to normalize hyperlipidemic disturbance.[23,24] Another study showed that the UD extract lowers the level of lipid profile due to a significant decrease of apolipoprotein B (apoB) levels irrespective of the fat in the diet.[25] ApoB is a surface marker of VLDV and LDL and TC.[26]

The findings of this research indicated that the effect of ethanol extract is greater than the water extract which is similar to the studies done by Avci *et al*.,[8] Nassiri-Asl *et al*.[27] and also greater than the NPs. The nettle leaves contain sterols: Campesterol and sitosterol.[28] Plant sterols resemble cholesterol in structure, they may replace cholesterol from mixed micelles because compared to cholesterol, they are more hydrophobic, this replacement causes a decrease in micellar cholesterol concentrations and consequently lowers cholesterol absorption.[8] In research by Rau *et al*. to determine which of two ethanolic herbal extracts activated the peroxisome proliferator-activated receptors, which play a crucial role in lipid homeostasis, the ethanolic extract of stinging nettle was one of the most active.[29] The results of this study showed a significant effect of NPs on hyperlipidemic rat's lipid profile, which also may be due to the presence of phenolic acids in the plant which have a high reducing power for NPs synthesis.[6] HDL also increased significantly as shown in the result of this study and has in agreement with the result of Nassiri-Asl *et al*.[27] It was hypothesized that the extract may directly affect the production and metabolism of lipoproteins.

Several serum parameters were examined in the current investigation, other than lipid profile to evaluate the inhibitory effect of the UD on hepatic marker enzymes such as ALT, AST, and GGT which give a significant decrease except those in atorvastatin, and elevation of AST, ALT, and GGT observed in hyperlipidemic rats which have an agreement with the study done for the effect of some plants; one of them was UD as an antioxidant and they concluded that the ethanolic extract of UD has antihepatotoxic potential and hypercholesterolemia causes fatty liver and eventually elevation of the liver enzyme.[30] Furthermore, in two other studies, their results demonstrate the hepatoprotective effect of stinging nettle.[31,32]

The majority of prior investigations demonstrated that harmful chemicals adsorbed on the surface of chemically produced AgNPs at higher dosages may induce liver or kidney damage.[33,34] Some investigations showed that modest concentrations of AgNPs had no harmful impact on hepatic and renal functioning.[33,35] Similarly, the levels of liver enzymes were not affected by the injection of lesser doses of 13–40 nm sized AgNPs (5 or 10 mg/kg), according to research by Dhermendra *et al*.,[25] while intact rats exposed to greater dosages of AgNPs (13–40 nm, 20–40 mg/kg) had impaired liver function.[36] However, the results of a different study showed that, although both chemically and eco-friendly AgNPs at a dose of 2.5 mg/kg were able to lower blood sugar levels in diabetic rats, green AgNPs were more protective than chemically made AgNPs at the same dose in terms of regulating and enhancing liver function.[7] The results of the present study showed that a 15 mg/kg dose of green AgNPs synthesized using UD for about 6 weeks significantly reduced the serum concentration of liver enzyme levels including ALT, AST, and GGT compared with the hyperlipidemic rats. To learn more about the potential processes behind the beneficial benefits of green produced AgNPs utilizing UD extract on liver function in hyperlipidemic rats, more research is required.

The glucose level in the hyperlipidemia rats in this study was elevated which is agree with the previous study done by Mojarradgandoukmolla *et al*.[12] The result shows a significant decrease in the level of treated rats with UD. The findings demonstrate that the UD extract's ability to reduce blood glucose was caused by Langerhans islets' increased insulin production.[37] Coumarins derivative has also been identified in nettle roots.[38] A review of several studies on the effect of coumarins on blood glucose concluded that numerous *in vivo* and *in vitro* studies show that coumarins help reduce blood sugar levels in people with diabetes by inhibiting protein-tyrosine phosphatase 1B (PTP1B), reducing inflammation and oxidative stress, enhancing pancreatic function, and correcting abnormal insulin signaling. As a result, they are useful and need more study and development. In addition, several coumarins, including osthole and psoralen, have the ability to activate adenosine monophosphate-activated protein kinase (AMPK), enhancing the translocation of glucose transporter type 4 (GLUT4) to the plasma membrane, and facilitating glucose uptake. An important justification for creating insulin mimics

is the fact that AMPK may increase GLUT4 translocation in the absence of insulin.[39]

It has been hypothesized that hypercholesterolemia raises the amount of lipid peroxidation in the serum of affected guinea pigs and rabbits.[40] Hence, we evaluate the level of MDA, SOD, and GSH, our result shows the increasing level of MDA, SOD and decreasing GSH in hyperlipidemic rats which agrees with Das *et al*.[40] but in the treated rats, this level was significantly different from the hyperlipidemic rats, and according to the findings of a study, there is a positive linear association between total phenolic and total flavonoid content and antioxidant activity. According to Stanojević *et al*., nettle leaf extracts in aqueous methanol might serve as an alternative to synthetic additions as a source of natural antioxidants.[41]

Our results also have an agreement with two other studies; the hydroalcoholic extract of UD plant has shown significant results for antioxidant activity with half inhibitory concentration (IC50) value of 88.33 ± 2.88 μg/ml[42] and the aqueous extract has significant reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities.[43] In another study, it has been shown that the ethyl acetate fraction of UD has potent antioxidant activities as compared to other fractions.[44] As a result, the antioxidant capabilities of UD may have been impacted by the extraction technique or the tissue or location examined. The activity of the UD ethanol extract increased noticeably.[8] This proves the result of GSH level of ethanol extract and NPs which was significant while the rats in water extract are not significant. Due to the protective capping of AgNPs by polyphenols and the corresponding size of the AgNPs, biosynthesized AgNPs utilizing *Pongamia pinnata* leaf extract have been demonstrated to have a better antioxidant radical scavenging capability than chemically synthesized AgNPs.[7]

CONCLUSION

Our results show that ethanol, water extracts, and AgNPs of UD reduced TC, LDL, and TG levels while raising HDL levels. Further research should be done on this plant to use as an active medicinal agent that bears fewer side effects on drug-based people suffering from CVD. In addition, the biosynthesis of AgNPs of the UD plant occurred, giving spherical 44–48 nm in diameter that has a positive effect on lipid profiles and acts as antioxidants and it was safe in the dose of 15 mg/kg for the rats.

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