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Impact Of Taraxacum Officinale Silver Nanoparticle On Lipid Profile In Hyperlipidemia Rats

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Abstract:
Hyperlipidemia is a significant modifiable risk factor for cardiovascular illnesses, which are still the main cause of death worldwide. In this work, rats with experimentally induced hyperlipidemia were used to test the therapeutic potential of biologically synthesized Taraxacum officinale silver nanoparticles (TOL-AgNPs). Taraxacum officinale leaf extract was employed in the production of the silver nanoparticles, which were subsequently analyzed by UV-Vis, FTIR, XRD, SEM, and EDX imagine. We randomly assigned thirty female Wistar albino rats (n = 6) to five groups: control group (normal diet), nanoparticle group (20 mg/kg TOL-AgNPs alone), hyperlipidemia group (cholesterol-enriched diet), antioxidant group (20 mg/kg TOL-AgNPs with cholesterol diet), and treated group received 2 weeks cholesterol-rich diet after that treated with 20 mg/kg TOL-AgNPs orally by gavage for four weeks .Upon evaluation of blood lipid profiles, liver function tests, oxidative stress biomarkers, kidney function tests, and histopathological investigations after 40 days, Low-density lipoprotein (LDL) and total cholesterol (TC) levels were found to be substantially reduced in the nanoparticle group than the hyperlipidemia group. Rats with hyperlipidemia had lower levels of high-density lipoprotein (HDL) than those that were treated ($P < 0.05$). Significant improvements were observed in liver function parameters, with ALT decreasing, AST, and ALP. Histopathological examination revealed complete prevention of hepatic steatosis, preservation of normal portal triads, and maintenance of sinusoidal architecture in TOL-AgNPs treated groups, contrasting with severe macrovesicular and microvesicular steatosis observed in hyperlipidemic controls.
Keywords: *Hyperlipidemia, silver nanoparticles ,cardiovascular disease, Taraxacum officinale*

INTRODUCTION

Hyperlipidemia, usually referred to as an excessive lipid status, is one of the main indicators of cardiovascular disease (CVD) risk. Two possible causes of hyperlipidemia, or elevated blood lipid levels: inherited conditions or acquired diseases that affect the body's lipid delivery mechanism (Mainieri et al., 2023) .This condition is usually quiet, and the individual is detected through the normal testing programs for the treatment of CVD. Feeling uncomfortable in the heart as a result of a coronary or cardiac event may appear as an outcome of a rise in cholesterol (Gour et al., 2023). Over the last few centuries, the prevalence of hyperlipidemia has been rising worldwide. The World Health Organization (WHO) pointed out that nearly forty percent of individuals worldwide had high serum total cholesterol levels in the year 2008. As of 2023, almost 3 million subjects in the US and Europe have been identified as having hyperlipidemia (Wei et al., 2024). Therefore, CVDs will overtake all other causes of mortality and disability worldwide.

For almost twenty years, nanotechnology has been created, and experts from all around the world have been interested in this novel technology. Particles smaller than 100 nm are known as nanoparticles, and their special qualities have garnered a lot of interest. Therapeutic and diagnostic nanomedicine has been made possible by recent developments in nanotechnology. The physical, chemical, and biological characteristics of silver nanoparticles (AgNPs) are different from those of their mass equivalents. During times past, metallic silver became widely employed in a variety of jewelry, currency, dentistry alloy, and photographic applications (Ahn et al., 2019). AgNPs may be synthesized using three main techniques such as chemical, physical, and biological. Both physical and chemical techniques are now less common because they require dangerous substances or are expensive. An acceptable alternative biosynthesis technique to the conventional physical and chemical methodologies has been offered by the biological method. An intriguing, environmentally friendly method with great effectiveness is the use of plant extract for reducing, stabilizing, and coating agents of AgNPs (Fahimirad et al., 2019). As well as, users and researchers are very interested in the idea of "functional diet" recently, as fruits, vegetables, and herbs contain bioactive substances. Herbal medicine encompasses a variety of approaches worldwide. Herbs are utilized in medicine in addition to being used for nourishment (Shaito et al., 2020).

A perennial member of the Asteraceae family, *Taraxacum officinale* is sometimes referred to as a dandelion(Carroll et al., 2022). Additionally, the name "*Taraxacum*" (remedy) comes from the Greek words "taraxos," which means disorder, and "akos." An "*offcinale*" plant has medicinal properties. The fact that this plant has been used as medicine for a long time is intriguing(Di Napoli and Zucchetti, 2021). Dandelion mostly acts as a diuretic while also cleansing the liver and blood. Dandelion's main component increases bile production, which lowers blood cholesterol and triglycerides (Jassim et al., 2012).Furthermore, dandelion contains pharmacological and phytochemical substances such as phenolic compounds, lactones, polysaccharides, and terpenoids (Hamza et al., 2020). In addition to fiber and vitamins B complex and C, dandelion is regarded as a natural source of minerals including iron, potassium, zinc, and calcium (Arafa et al., 2010).

MATERIALS AND METHODS:

Gathering and preparing plant and leaf extracts

Freshly picked *T. officinale* leaves (TOL) were dried in the shad for a week before being ground into a fine powder. The extract is made by thoroughly combining 100 milliliters of distilled water with 10 grams of leaf powder, boiling the mixture for 15 minutes at 60 degrees Celsius, letting it cool, and then filtering it using Whatman No. 1 filter paper. Using the filtered extract of TOL, AgNPs were produced (Ajitha et al., 2014).

Green Synthesis of Silver Nanoparticles

The magnetic stirrer was utilized to heat 1 Mm aqueous AgNo3 in 1000 mL for 60 minutes at 40°C after 100 mL of the extracted material was added. The generated AgNPs were cleaned after centrifugation and the TOL-AgNP result at 15,000 rpm for 15 minutes, and they were examined using UV-vis spectroscopy. Air-dried TOL-AgNPs that were still semisolid were collected as powder (Renganathan et al., 2021).

The Characterization of Synthesized TOL-AgNPs

A Perkin Elmer Spectrophotometer was used to record UV-Vis spectroscopy at wavelengths ranging from (200 – 700) nm. A SHIMADZU FTIR spectrometer (500–4000 cm-1) was used to perform Fourier-transform infrared (FTIR) spectroscopy. The TOL-AgNP's X-ray diffraction spectrum (XRD) was captured with an X-ray diffractometer (Rigaku, Japan). AgNPs were analyzed using a scanning electron microscope (SEM) to determine their size and surface shape(Konappa et al., 2025).

Experimental Animals and Ethics

Salahaddin University College of Education's Institutional Animal Ethics Committee accepted the experimental methodology, which included thirty female Wistar albino rats with body weights (185± 5)g. In the animal house at Kalar Private Technical Institute, the experiment was carried out on an animal housed during a light/dark cycle in a room that is 22 ± 2°C of 12:12 hours. To allow them to acclimatize, the rats were housed for seven days. Ethical number: (SUE2025AREC/20).

Hyperlipidemia Induction Protocol

According to a protocol by (Othman and Nanakali, 2022) the hyperlipidemic diet was composed of regular rat chow supplemented with 5% cholesterol powder (≥95% purity, Sigma-Aldrich) and 0.5% cholic acid sodium salt (≥95% purity, Sigma-Aldrich) to improve cholesterol absorption and prevent bile acid generation.

Experimental Design and Group Allocation

In this study, thirty female Wistar albino rats were randomly assigned to five groups, with six rats in each group. Group 1 (control) was given a normal diet and tap water, Group 2(nanoparticle) got 20 mg/kg TOL-AgNPs orally by gavage alone, Group 3 (hyperlipidemia) was given a cholesterol-enriched diet, while Group 4 (antioxidant) was given an (20 mg/kg TOL-AgNPs per day, administered by gavage with a cholesterol diet) for four weeks, Group 5 (treated) received 2 weeks cholesterol-enriched diet after that treated with 20 mg/kg TOL-AgNPs orally by gavage for four weeks. The entire experiment lasted forty days.

Biochemical analysis

On day 40 of the examination, the rats were given general anesthesia, allowed to fast for 12 hours, and then had their hearts punctured to obtain blood samples, which were then placed in centrifuge tubes and rotated at 3000 rpm for 15 minutes at 4°C. For tests, sera were collected and stored at −40°C.. Using commercial kits, sera were evaluated for lipid profiles (TC, TG, HDL, LDL, and VLDL) and activity of hepatic enzymes (ALT, AST, and ALP).

Histopathological Examination Protocol

Following the dissection of the animals in each group, the liver was extracted, the tissue was fixed in 10% neutral buffered formalin solution, and then slices were made, stained with hematoxylin and eosin, and then observed under a light microscope (Mustafa and Maulood, 2019).

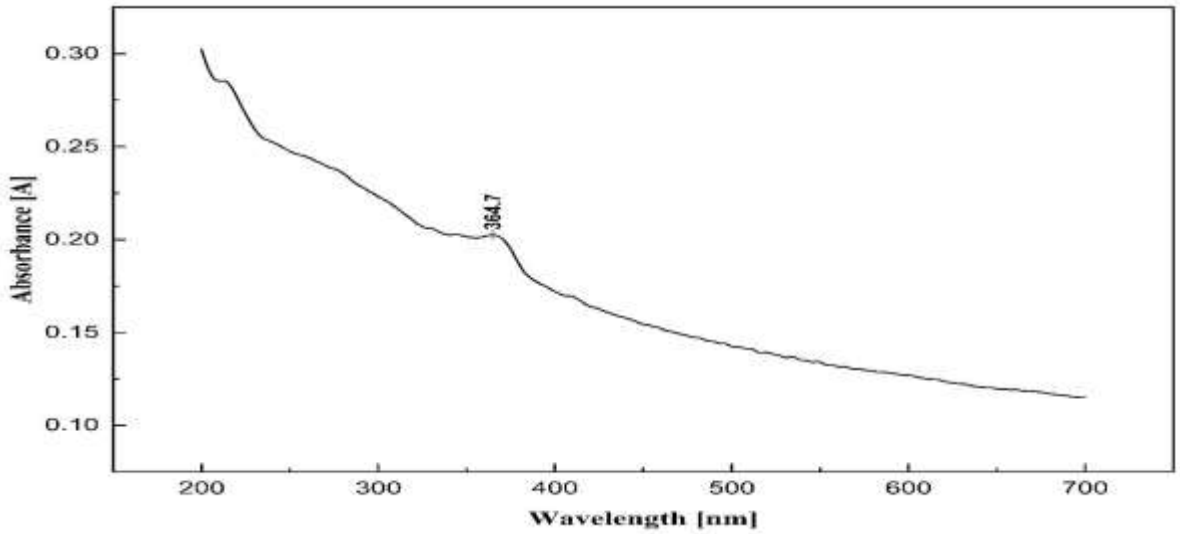
Statistical Analysis

Findings are shown as Mean ± SD. Software called SPSS/Version 23.0, or the Statistical Package for Social Science, was used for all analyses. For group comparison, the Duncan Alpha test was used after the one-way analysis of variance (ANOVA) was used to analyses the data. Statistically significant values were defined as probability values below 0.05.

RESULTS

The Formation of AgNPs

The silver nitrate (AgNO3) combination changed from a brilliant yellow tint to a deep brown color when TOL extract was added at room temperature. Using UV-visible spectroscopy to find out when TOL-AgNP is formed. Greater surface plasmon resonance (SPR) of around 364.7 nm was demonstrated by the produced nanomaterial, indicating the synthesis of TOL-AgNP (Fig.1) (Hamelian et al., 2020). (Fig. 2) illustrates the FT-IR spectral bands of the TOL extract together with the produced AgNPs derived from the leaf extract. Sharp



transmittance maxima were observed in the generated AgNPs at (3683, 2370, 1394, 1031, 870, and 565,455) cm-1. The size and form of the spherically shaped AgNPs were examined using SEM. (Fig. 3). The diffraction peaks can be observed seen at 38.06°, 44.25°, and 64.38° in the XRD result (Fig.4).

Figure 1: The outcomes of *Taraxacum officinale* leaf-silver nanoparticles' UV/visible spectrum

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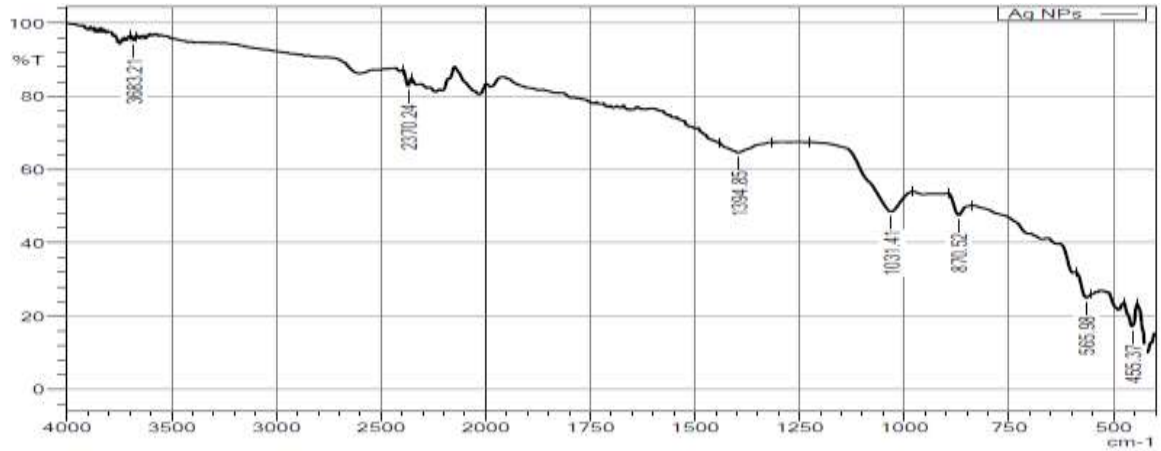

Figure 2: The findings of the *Taraxacum officinale* leaf-silver nanoparticle Fourier transform-infrared spectrum.

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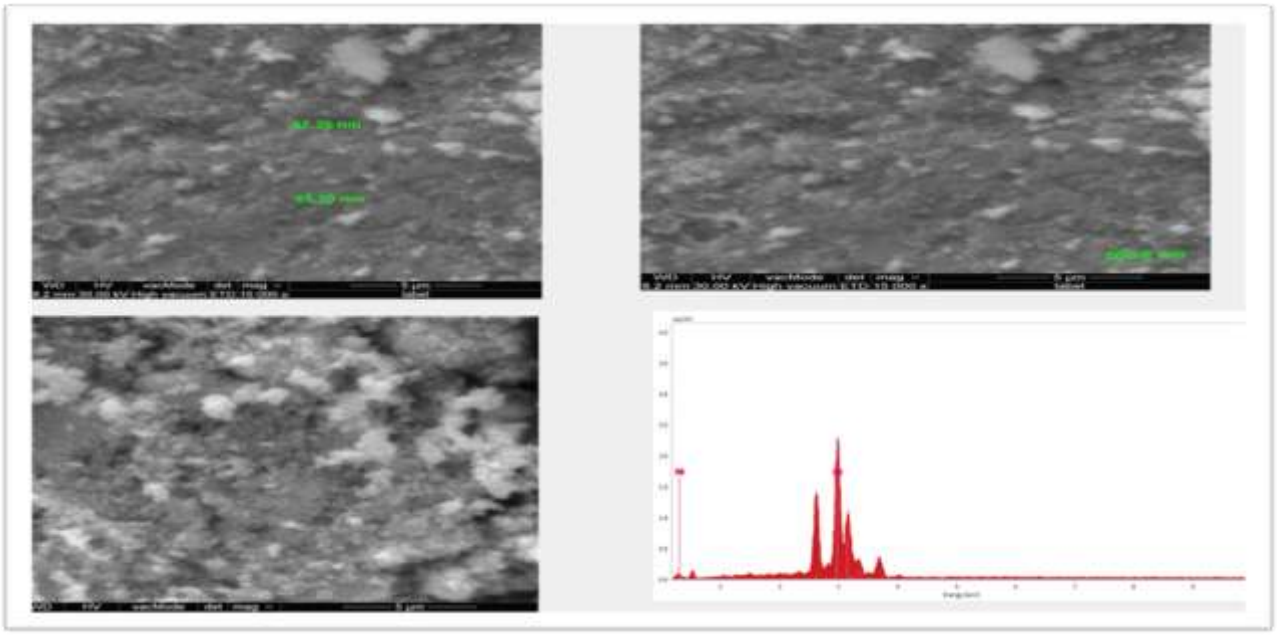

Figure 3: Images of the *Taraxacum officinale* leaf-silver nanoparticles taken with an energy dispersive X-ray and scanning electron microscopy.

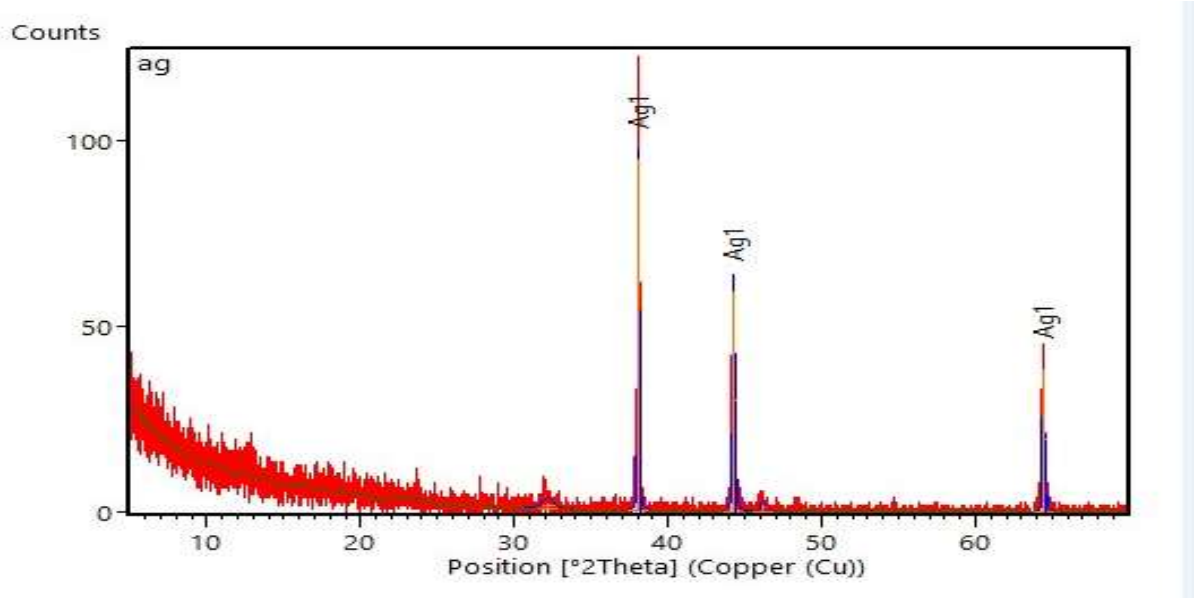

Figure 4: Analysis of the *Taraxacum officinale* leaf-silver nanoparticle using X-ray diffraction

Lipid Profile

The rats in the hyperlipidemia group had considerably ($P < 0.05$) higher blood levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL) than the control and nanoparticle groups. Comparing the therapy group to the hyperlipidemia group, however, revealed a marked decrease in the levels of blood triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL). Additionally, the total blood levels of HDL in the control and nanoparticle groups increased significantly in comparison to the antioxidant and hyperlipidemia groups. The results show a significant rise in the treatment group's serum Very Low-density lipoprotein (VLDL) level as compared to the other four groups (Table 1).

Table 1: Showed (Mean ± SD) effect of TOL AgNPs on serum lipid profile in hyperlipidemia rats.

parameters / Groups	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	64.17 ± 4.49a	40.20 ±4.14b	46.77 ±3.55 b	7.53 ±1.04 a	8.53 ±1.41 a
Nanoparticle	67.05 ±7.28 a	34.31 ±4.67 a	52.93 ±9.28 bc	6.36 ±1.15 a	7.23 ±1.34 a
Hyperlipidemia	262.75 ±89.99 b	41.40 ±2.70 b	32.82 ±4.52 a	198.49 ±62.79 b	8.40 ±0.56 a
Antioxidant	345.20 ±23.09 c	38.00 ±4.63 ab	31.89 ±2.26 a	263.22 ±26.50 c	7.50 ±0.86 a
Treatment	73.50 ±5.89 a	39.16 ±3.06 ab	55.56 ±6.09 c	7.70 ±2.07 a	40.66 ±0.89 b

No significant differences are shown by the same letters in a column, whereas substantial differences are indicated by different letters.

Liver Function

The hyperlipidemia group's aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) activations were considerably higher ($P<0.05$) than those of the control group. The treatment group's serum levels of AST, ALP, and ALT were noticeably ($P<0.05$) reduced than those of the hyperlipidemia and antioxidant groups. Furthermore, the serum levels of ALP and AST in the antioxidant group substantially rose ($P<0.05$) in comparison to the nanoparticle group, but there was no significant in the serum level of ALT (Table 2) .

Table 2: Rats with hyperlipidaemia and the Impact of TOL AgNPs on Hepatic Function Tests

parameters / Groups	ALT (IU/L)	ALP (IU/L)	AST (IU/L)
Control	55.16 ±11.40 b	133.68 ±28.96 ab	153.74 ±34.85 a
Nanoparticle	50.86 ±6.13 ab	152.36 ±26.13 ab	148.52 ±25.44 a
Hyperlipidemia	67.06 ±14.54 c	229.86 ±12.15 c	205.10 ±31.87 c
Antioxidant	60.16 ±1.36 ab	161.80 ±42.87 b	211.10 ±40.07 c
Treatment	42.11 ±8.31 a	112.90 ±23.02 a	164.66 ±32.63 a

No significant differences are shown by the same letters in a column, whereas substantial differences are indicated by different letters. ALP: Alkaline phosphatase, TOL, ALT: Alanine aminotransferase, and AST: Aspartate aminotransferase.

DISCUSSION:

Elevated blood TG, TC, and LDL-c levels are indicative of hypercholesterolemia, an aberrant state that may lead to the development of cardiovascular illnesses. Because of its flexible planar shape and several other properties, cholesterol, which is synthesized by animal tissues, is essential for maintaining the balance of membrane structures. Nonetheless, a rise in cholesterol is often a reason for fear (Karuppannan et al., 2021). It's interesting to note that reducing LDL was linked to raising HDL, which is thought to play a crucial part in lipid metabolism by moving cholesterol ester from HDL to LDL (Kaabia et al., 2018). This study showed that TOL AgNPs markedly reduced blood levels of total cholesterol, HDL, and LDL that were raised as a result of cholesterol therapy, which was confirmed by (Choi et al., 2010), who reported that dandelion extract prevented atherosclerosis by lowering the lipid profiles of animals given cholesterol. Additionally, a different study found that dandelion fractions might lower total cholesterol and TG by improving the lipid profile. It was proposed to lower cyclooxygenase synthesis, induce vasodilation, and then adjust heart rate and blood pressure (Majewski et al., 2020). In additions, a more recent study showed that dandelion aqueous extract actually decreased the lipid profile. This might be because it increased adenosine monophosphate activation, which in turn decreased fat formation in the liver (Ikram et al., 2021).

The most notable result was the significant 72% drop in total cholesterol levels in the hyperlipidemic group compared to the therapy group, which was close to normal control values. This remarkable enhancement is consistent with recent findings by (El-Baz et al., 2023) , who showed that in diabetic rats with concomitant hyperlipidemia problems, green-synthesized silver nanoparticles dramatically lowered cholesterol levels. The scientists ascribed this result to the biologically synthesized nanoparticles' improved cellular absorption and metabolic regulation capabilities. Considering that LDL is the main atherogenic lipoprotein, the 96% reduction in LDL cholesterol is quite noteworthy. This result supports the findings of (Shanker et al., 2017), who showed promise as a nanomedicine for the treatment of hyperlipidemia by reporting comparable LDL-lowering results with biologically produced silver nanoparticles at 400 mg/kg. Recent studies conducted by(Bhale et al., 2024) has clarified the complex mechanics of cholesterol transport, highlighting the diverse roles that HDL apolipoproteins play beyond basic cholesterol export, which may help to explain the various lipid benefits seen with therapies including nanoparticles. As evidenced by studies demonstrating that nanoparticles may modify macrophage cholesterol content through surface binding and internalization processes, the mechanism most likely entails better cellular cholesterol efflux and enhanced cholesterol metabolism.

In contrast to the treated animals, hyperlipidemic rats exhibited a positive rise in HDL cholesterol that exceeded even control values. Since HDL is the main conduit for reverse cholesterol transport, which makes it easier to transfer extra cholesterol from peripheral tissues to the liver for elimination, this increase has therapeutic significance. HDL increase and LDL lowering work together to provide the ideal lipid profile for cardiovascular protection. Recent developments in HDL studies have shown that these naturally occurring nanoparticles have other uses beyond lowering cholesterol, such as anti-inflammatory and antioxidant effects (Kuai et al., 2016) . This discovery is consistent with new theories of HDL mimicking nanotherapeutics, which may help explain the reported therapeutic advantages by controlling monocyte recruitment and macrophage polarization towards anti-inflammatory phenotypes (Zhen et al., 2024).

Curiously, VLDL cholesterol unexpectedly increased in the treatment group compared to hyperlipidemic rats, although triglyceride levels were mostly constant across groups. It is important to interpret this data carefully since VLDL rise may cancel out certain cardiovascular advantages. The improvement in the lipid profile overall, especially the sharp decline in LDL and the rise in HDL, probably exceeds this worry.

Hepatic Safety Profile

The evaluation of liver function showed promising outcomes, with the therapy group outperforming the hyperlipidemic and antioxidant groups in terms of hepatic markers. In contrast to normal control values, ALT levels in treated rats dropped dramatically, suggesting improved hepatic integrity instead of toxicity. This result is in opposition to other research that has documented the hepatotoxic effects of silver nanoparticles. For

example,(Heydrnejad et al., 2015)found that when mice were given silver nanoparticles, their ALT, AST, and ALP levels significantly increased. However, more current studies by (Olugbodi et al., 2023)revealed increased liver enzymes and dose-dependent hepatic damage. Nonetheless, there is growing evidence that silver nanoparticles generated from plants have hepatoprotective properties..(Zhang et al., 2019)shown that Rhizophora apiculata leaf extract-synthesised silver nanoparticles had notable hepatoprotective benefits against carbon tetrachloride-induced liver injury, as evidenced by enhanced histological parameters and normalized liver enzyme levels.Similarly, (Hamouda and Aljohani, 2024) showed that by preventing oxidative damage in hepatocytes and regulating ROS production, silver nanoparticles made from Sargassum vulgare had hepatoprotective benefits. The difference might be explained by the current study's dosage schedule, particle size, surface changes, or biological production technique.

Histopathological Examination

According to the results of our histological analysis, the liver tissue of the rats in the control group had normal liver architecture, including a normal central vein and hepatocyte arrangement Figures [6A and 6B] as shown in the Tables (1 and 2), which led to normal all parameters in the lipid profile and liver function tests, respectively. The TOL-AgNP group's tissue sections had a typical structure that was comparable to that of the control groups Figures[6C and 6D] also reported that in the Tables (1 and 2) has normal all parameters in the lipid profile because the dandelion is a rich source of phytosterols with sitosterol being the predominant component can inhibit the absorption of cholesterol, promote its degradation and metabolism, and inhibit the biosynthesis of cholesterol and liver function tests also showed moderate improvements in liver enzymes. Green-synthesized AgNPs have been shown to mitigate liver injury by scavenging free radicals. Downregulation of pro-inflammatory cytokines, and improving hepatic enzyme balance so close to the control group (Yan et al., 2024).Sections of the liver from rats given cholesterol showed significant pathological changes that are typical of diet-induced hepatic steatosis. Large cytoplasmic lipid vacuoles were seen to push hepatocyte nuclei to the periphery, causing extensive macrovascular steatosis. Severe lipid accumulation caused a considerable distortion of the normal hepatic architecture, demonstrating the effective production of experimental hyperlipidemia Figures [6E and 6F] , Tables (1 and 2), which showed increased all parameters in the lipid profile, resulting from taking cholesterol , leading to accumulation of the lipid and steatosis, and indicating hepatic stress or damage. Elevated levels of these enzymes are commonly associated with liver inflammation, hepatocellular injury, and impaired membrane permeability due to lipid accumulation and oxidative stress. The antioxidant group had modest microvascular steatosis but overall hepatoprotection, with the hepatic plate layout remaining mostly intact and the central hepatic veins retaining their normal shape. Sinusoids had a patent shape with typical interactions among hepatocytes and sinusoids Figures [6G and 6H] as shown in the Tables (1 and 2) also increased all parameters in the lipid profile due to intake cholesterol enriched diet, also presented intermediate enzyme levels , Still, the enzyme levels remained elevated, particularly AST, indicating persistent liver stress.

The treatment group showed the best possible therapeutic response in the current investigation. There was no congestion in the central veins. In general, the hepatic architecture was close to normal control values Figures [6I and 6J] as well as, Tables (1 and 2) showed that significant improvements in lipid profiles and near to the control group which due to administrated TOL-Ag nanoparticle caused by reduce lipid profiles and showed the most significant improvement in liver enzyme levels values were significantly reduced these results suggest a hepatoprotective effect, likely due to the synergistic action of the biosynthesized silver nanoparticles. One of the biggest health problems and a worldwide public health burden is liver disease (Pimpin et al., 2018). As previously reported by (Karkos et al., 2011) , TOL was the most effective hepatoprotective species based on histological data.

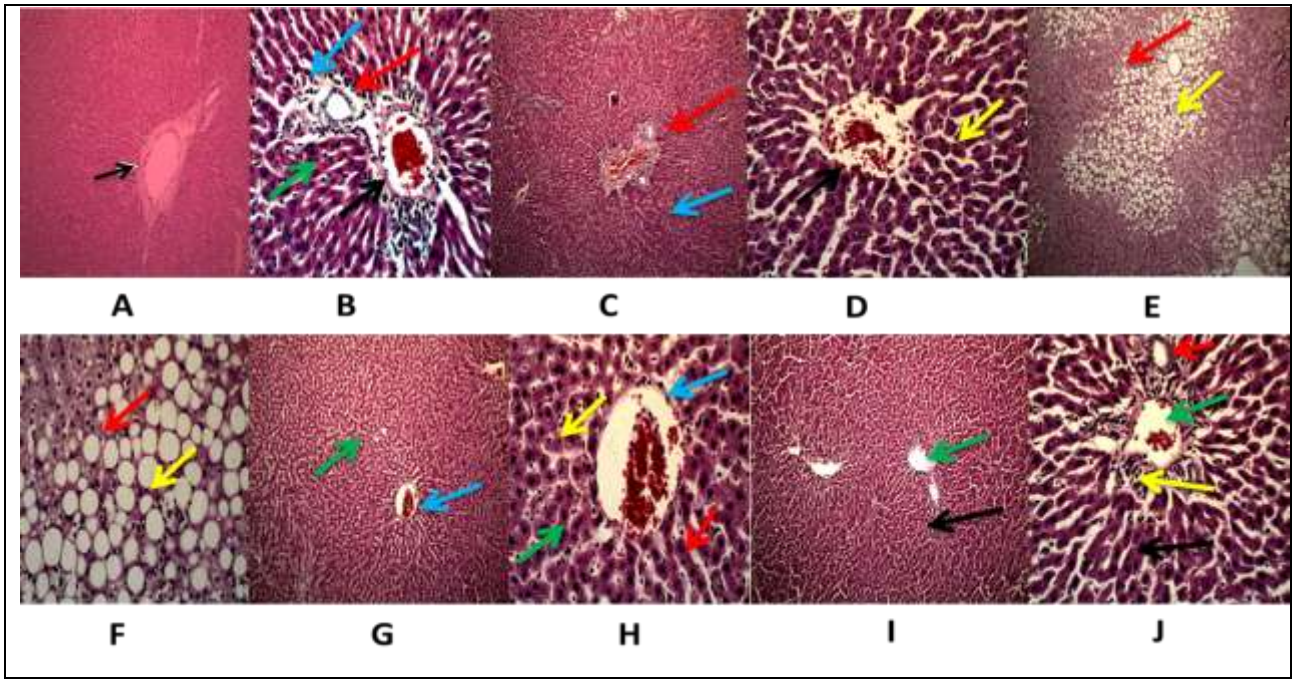

Figure 5.The impact of *Taraxacum officinale* leaf-silver nanoparticles (TOL-AgNPs) on the lipid profile in rats with hyperlipidemia are demonstrated by histopathological sections of the liver's tissue stained with H&E. (A, B) Normal histological structure was displayed by the control group, which included the bile duct (red arrow), hepatic artery (blue arrow), hepatocytes and sinusoids (green arrow), and central vein (black arrow), (C, D) TOL-AgNPs group: Healthy hepatocytes and sinusoids (yellow arrow), the portal tract (red arrow), the hepatic parenchyma (blue arrow), and the central vein (black arrow),. (E, F) hyperlipidemia group: Microvascular steatosis (red arrow) and macrovascular steatosis (fatty change) are both present. (G, H) antioxidant group: Hepatocytes and sinusoids (green arrow), the central vein (blue arrow), modest microvascular steatosis (yellow arrow), and the hepatic plate (red arrow) are identified. (G, H) treatment group: Hepatocyte (black arrow), portal vein (green arrow), bile duct (red arrow), and a few inflammatory cells (yellow arrow) are magnified as follows: A, C, E, G, I (×100); B, D, F, H, and J (×400).

CONCLUSION:

Through hepatoprotection, antioxidant enhancement, and complete lipid profile normalization, *Taraxacum officinale* silver nanoparticles show great therapeutic potential for the therapy of hyperlipidemia. A viable strategy for preventing cardiovascular disease is the combination of traditional herbal medicine and nanotechnology, which may help overcome the drawbacks of existing treatment approaches. The development of TOL-AgNPs as a new, naturally generated therapeutic agent is supported by our findings; nevertheless, before translation to human applications, more safety assessment and clinical research are necessary.

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