Research Article

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Iron indices and hemogram in renal anemia and the improvement with *Tribulus terrestris* green-formulated silver nanoparticles applied on rat model

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Abstract: The recent research was done to assess the hematological and biochemical disorders in nephrotoxicity induced by gentamicin (100 mg/kg) in Wistar rats. The green-formulated silver nanoparticles (AgNPs) by *Tribulus terrestris* leaf were analyzed by XRD, energy-dispersive X-ray spectrometer, and field emission scanning electron microscopy. The shape of the recent nanoparticles was spherical in size of 48 nm. In the *in vivo*, the classical and novel kidney injury parameters were used to assess the nephroprotective properties of AgNPs in animal model. Gentamicin decreased significantly ($P \leq 0.01$) the value of PCV, concentration of HB, and count of RBC. The value of serum iron, erythropoietin, Cr, and urea raised in the gentamicin group. Leucogram revealed thrombocytopenia, granulocytosis, and leukocytosis. AgNPs improved the iron, erythropoietin, thrombocytes, leukogram, and erythrogram. The levels of creatinine, urea, GGT, ALT, AST, and ALP significantly reduced and albumin and total protein increased in group treated with AgNPs. Also, AgNPs significantly raised the anti-inflammatory cytokines, i.e., IL3, TGFβ, IL10, IL5, and IL4, and decreased the pro-inflammatory cytokines, i.e., TNFα, IL18, IL12, IL6, and IL1. These findings may offer AgNPs as a nephroprotective agent that could be a suitable therapeutic supplement in blood disorders conditions.

Keywords: blood homeostatic, biochemical parameters, nephroprotective properties, renal anemia, silver nanoparticles, *Tribulus terrestris*

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1 Introduction

Nowadays, the medicinal plants used in disease treatments are expanding, and the investigation and extraction of the characteristics of herbal compounds and the mechanisms presentation of the compounds action in various diseases treatment have been considered [1–3]. The goal of traditional medicine and the use of medicinal plants is to consider local plant samples, modern uses as auxiliary drugs in chemical or antibiotic treatments, to understand the plants therapeutic value, and finally to discover new substances such as vitamins, antimicrobial substances, pseudohormones, anti-viral, and antitumor among plants [1,4–6]. Medicinal plants cultivation is considered currently as a main branch of the science of agriculture, which helps in useful compounds extracted from plants and used in medicine. Medicinal plants can be used in the treatment of common diseases such as anemia, diabetes, high blood pressure, blood lipids, and even cancer [1]. *Tribulus terrestris* is a botanical species that thrives primarily in Europe, India, Australia, and South Africa. Belonging to the Zygophyllaceae family, which encompasses a diverse range of 25 genera and approximately 250 species, *T. terrestris* is a creeping herbaceous plant that typically flourishes in dry climates and sandy soils, reaching a maximum height of 1 m. The widespread distribution of *T. terrestris*, its rich concentration of active compounds (specifically sterol saponins, alkaloids, phenol carboxylic acids, terpenoids, tannins, and flavonoids), and its frequent utilization in traditional medicine and dietary supplements emphasize the significance of assessing its pharmacological characteristics. The consumption of *T. terrestris* leads to an increase in testosterone secretion, an increase in muscle strength and volume, and an increase in the production of male and female sex cells [2]. Among athletes, especially bodybuilders, it is used along with nutritional supplements to strengthen muscle mass and increase muscle strength [3]. It is effective in cardiovascular diseases treatment such as anemia, high blood pressure, poor blood circulation, and angina pectoris. It is applied in traditional Indian and Chinese medicine to cure liver and kidney diseases and cardiovascular diseases [4]. In terms of traditional treatments, different properties have been considered for this plant. In old texts, the use of the extract of this plant for immune disorder treatments such as skin allergies and eczema, strengthening the liver, treating overeating, as well as treating food poisoning, treating inflammations and oral thrush, digestive disorders such as constipation and bloating, and relieving joint and back pain is mentioned [7–9].

Anemia abbreviation is used when the concentration of hemoglobin is less than 12 g/dL. Chronic kidney disease (CKD) anemia is a type of anemia caused by kidney disease. This type of anemia is often associated with outcomes of poor treatment and raised mortality [10–13]. The onset of anemia begins when the glomerular filtration rate falls below 60 mg/ml. Recent clinical trial studies show increased mortality and morbidity related to erythrocyte-stimulating agents [14–16]. CKD anemia often causes decreased life quality and raised risk of mortality, hospitalization, cognitive impairment, and cardiovascular disease. With the damage to the kidney, the levels of erythropoietin will reduce, fewer RBC will be produced, and low oxygen will reach the organs and tissues [15–18]. Recently, erythropoietin depletion has been associated with hypoxia-inducible factor. Iron deficiency, bleeding caused by dysfunction of platelets, and infection are other symptoms of renal anemia. CKD anemia treatment is aimed at improving kidney function and raising the production of red blood cells. Recently, nephrologists have focused on the treatment of renal anemia by nanotechnology [17–20].

When transitioning from microparticles to nanoparticles (NPs), several alterations in physical properties occur. The primary changes involve a reduction in the volume to surface area ratio and the entry of particle size into the quantum effects realm [21–23]. Nanotechnology refers to the advancement in the manufacturing and utilization of materials and tools with dimensions ranging from 1 to 100 nm [23–25]. Silver nanoparticles (AgNPs) possess distinct and exceptional attributes. Noteworthy features include their capacity to be incorporated into polymer fibers and dyes, remarkable stability, compatibility with heat-resistant microorganisms, absence of resistance development, non-irritating nature to the human body, hydrophilicity, and rapid efficacy [24–27]. The utilization of AgNPs in various industries encompasses sanitary materials' production like soap, toothpaste, and toothbrushes and healing, wound treatment, and medicine. Additionally, they are employed in the manufacturing of household appliances such as food packaging containers, refrigerators, and plastic containers. Furthermore, AgNPs find application in agriculture [25–29]. In order to address these requirements, the nanomaterials toxicology field will assume a crucial role in facilitating the growth and advancement of secure and sustainable nanotechnology [26–28]. Despite the limited information currently available regarding the toxicological efficacies of NPs on humans and the environment, it is anticipated that these materials have the potential to interact with biological components. This interaction can significantly impact the behavior and characteristics of living macromolecules, cells, and organisms [27–30]. Extensive biomedical reports have shown that NPs enter various organs of the human body by inhalation, as evidenced by their effects on tissues and cells [29–31]. Despite the presence of unverified indications regarding the NPs transportation into the bloodstream, particularly when NPs reach the respiratory system and lungs and are assimilated into the bloodstream, it has been observed that these NPs are distributed to vital organs such as the liver, kidneys, and spleen in animals [30–32]. Given the extensive use of AgNPs for their extensive antibacterial properties, it becomes imperative to explore their compatibility with biological systems [28–30].

The recent research was done to assess the hematological and biochemical disorders in nephrotoxicity induced by gentamicin (100 mg/kg) in Wistar rats. The green-formulated AgNPs by *T. terrestris* leaf were analyzed by X-ray diffraction (XRD), energy-dispersive X-ray spectrometer (EDS), and field emission scanning electron microscopy (FE-SEM). In the *in vivo*, the classical and novel kidney injury parameters were used to assess the nephroprotective properties of AgNPs in animal model. AgNPs improved the iron, erythropoietin, thrombocytes, leukogram, erythrogram, creatinine, urea, GGT, ALT, AST, ALP, IL3, TGFβ, IL10, IL5, IL4, TNFα, IL18, IL12, IL6, and IL1, which are adversely affected by gentamycin.

2 Experimental

2.1 Preparation of extract

After collecting the *T. terrestris* leaf, they were dried for 2 weeks at 25°C and then at 35°C for 2 days. Before extraction, the dry materials were converted into smaller pieces (2–3 mm). The percolation (soaking) method was used for extraction. About

700 mL water was added to 100 g plant powder. After 2 days at 25°C, the collection was smoothed by Whatman filter paper. The extraction process was repeated three times and the total organic solvent was removed using a rotary evaporator to obtain raw solid extracts, then a freeze dryer was used to completely remove the solvent. The extraction efficiency was 34%.

2.2 Preparation of AgNPs

In the process of synthesizing AgNPs using a green method, we utilized an aqueous leaf extract of *T. terrestris* that had been prepared as per previous step. To achieve this, we combined 10 mL of the extract with 90 mL of a 1 mM aqueous $AgNO_3$ solution. The mixture was then heated at 80°C for 3 h while being continuously stirred. The formation of the AgNPs was initially identified by the change in color from yellow to dark brown. To separate the NPs, we employed centrifugation at a speed of 15,000×*g* for 20 min. This centrifugation process was repeated five times in order to eliminate any residual free silver that may have been associated with the AgNPs@*Tribulus terrestris*. The resulting green-synthesized AgNPs were designated as AgNPs@*Tribulus terrestris*. These NPs were subsequently freeze-dried and stored at a temperature of 4°C until they were ready for further use.

2.3 Experimental animals

In this particular experimental investigation, a total of 100 adult male rats from the Wistar breed, weighing between 260 and 280 g, were utilized. Each rat was housed individually in separate cages. The rats were maintained with a temperature ranging from 20 to 24°C and subjected to a 12 h light–dark cycle.

2.4 Method of inducing renal anemia

Rats were anesthetized using the anesthetic sodium thiopental (Nasdonal) at the rate of 40 mg/kg which was injected intraperitoneally. Subsequently, an injection of gentamicin (100 mg/kg, subcutaneously) was administered in the scruff area to induce renal anemia.

2.5 Conducting experiments and experimental groups

In this study, the rats were classified into six equal groups by random allocation. The initial group served as a sham control group and did not undergo any form of treatment. In the untreated group, the rats did not undergo any treatment process after inducing renal anemia. In the treatment group, the synthesized AgNPs were administrated with doses of 25, 50, and 100 µg/kg by gavage for 21 days after inducing renal anemia.

2.6 Measurement of biochemical and immunological parameters

After 21 days of treatment, the blood samples were extracted after anesthetizing the animals with sodium thiopental (Nasdonal) at the rate of 40 mg/kg. Serum immunological (pro-inflammatory [IL18, IL12, IL6, IL1, and TNFα] and anti-inflammatory [IL13, IL10, IL5, IL4, and IFNα] cytokines) and biochemical (GGT, ALT, AST, ALP, albumin, total protein, creatinine, urea, erythropoietin, and ferrous) parameter concentrations were determined by ELISA procedure kits (Ziest Chem Diagnostics).

2.7 Measurement of hematological parameters

The number of total WBC, basophil, eosinophil, neutrophil, monocyte, lymphocyte, RBC, and platelet, and the level of MCH, MCV, PCV, HB, and MCHC were checked by automatic hematology analyzer (Sysmex XS 800i).

2.8 Statistical analysis

The statistical analysis of the various experimental groups involved utilizing a one-way analysis of variance and the Duncan supplementary test. $P \leq 0.5$ was deemed acceptable. The data in all the graphs were presented as mean ± standard deviation.

3 Results and discussion

3.1 Characterization of AgNPs

FE-SEM was applied to analyze the morphology of the AgNPs that were biosynthesized. The AgNPs were observed to have various polymorphic shapes and were found to be aggregated. This aggregation could be attributed to the dehydration process that occurred during the preparation of the samples for SEM analysis, as shown in Figure 1. Also, the particles average size was measured by ImageJ software and it is about 48 nm.

In Figure 2, EDS was employed to follow the elemental composition of AgNPs that confirmed the O, N, C, and Ag elements within the prepared structure. The results presented the appearance of Ag (by the peak at 3.19 keV for AgLβ and peak at 3.02 keV for AgLα), carbon (by the peak around 0.3 keV for CLα), and oxygen (by the peak around 0.5 keV for OLα) in AgNPs.

XRD is a scientific method applied to investigate the arrangement of atoms in crystalline materials. It operates within the X-ray region of the electromagnetic spectrum, which lies between gamma and ultraviolet rays. By employing XRD, researchers can gather valuable insights into the structure of substances and identify the elements present. This technique has proven instrumental in analyzing crystal structures, including measuring the average distances between atomic and layer series, determining the individual crystals position and the atoms composition, as well as investigating crystal structures in unknown materials and measuring properties. The data reported in Figure 3 confirm the AgNP's presence, as the X-ray analysis spectrum aligns with the standard spectrum of NPs.

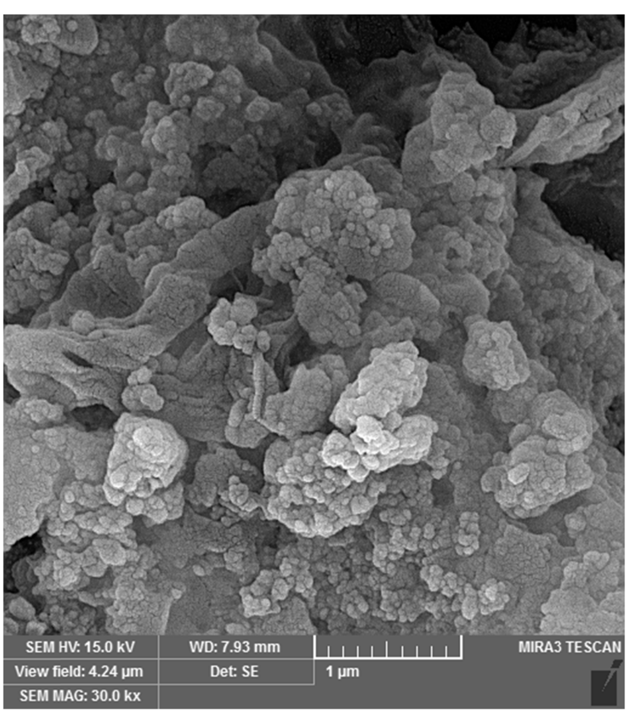

Figure 1: FE-SEM image of AgNPs synthesized with plant extract.

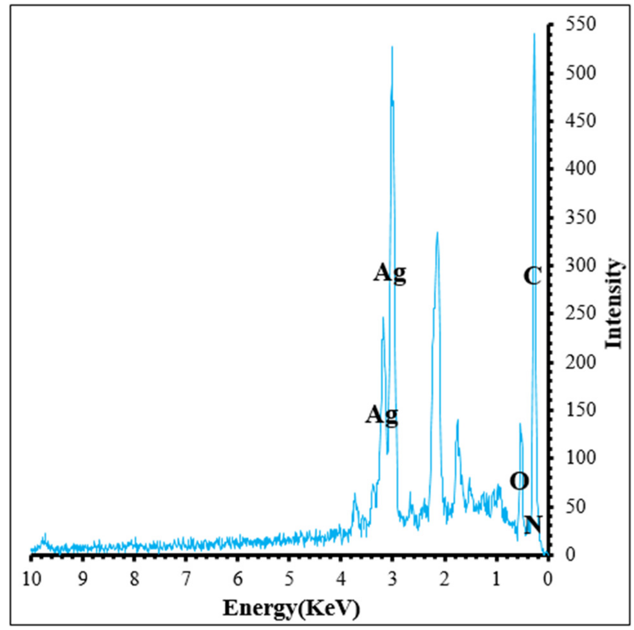

Figure 2: EDS pattern of AgNPs synthesized with plant extract.

The XRD method was utilized to confirm that the AgNPNPs were indeed AgNPs. The presence of (111), (200), and (220) peaks at 38°, 44°, and 63°, respectively, indicates the existence of AgNPs. This observation provides further evidence for the successful synthesis of AgNPs (Figure 3).

During the green synthesis of AgNPs, the synthesized solution undergoes a transition to a black color, indicating the stimulation and enhancement of surface plasmon

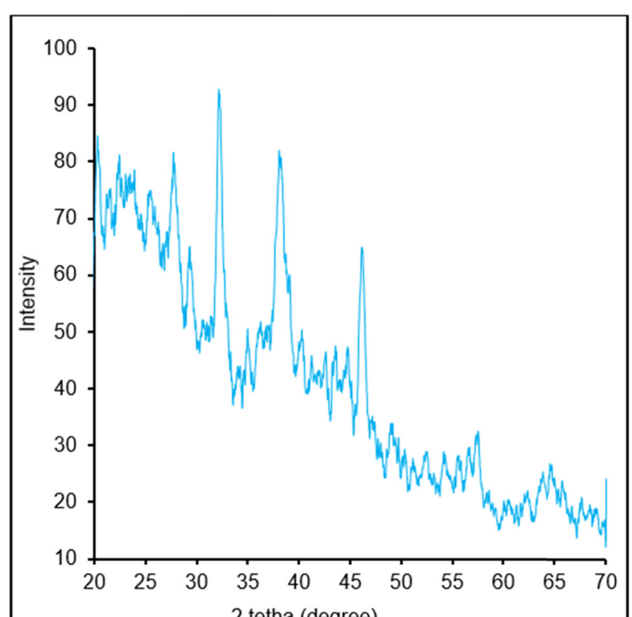

Figure 3: XRD pattern of AgNPs.

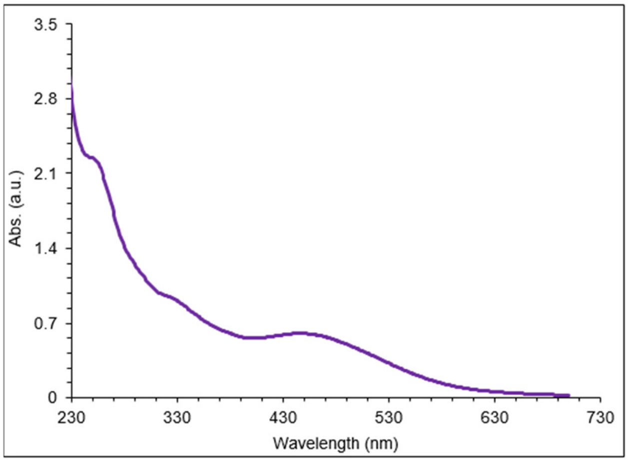

Figure 4: UV–vis analysis of AgNPs.

vibration in AgNPs. The ultraviolet–visible spectroscopy measurements showed that the spectra displayed the highest absorption at around 446 nm for the formulated NPs. This absorption peak gradually increased over time and eventually reached a stable state after 72 h, signifying the completion of the reaction (Figure 4).

3.2 Anti-renal anemia properties

As is seen in Figure 5, AgNPs significantly ($P \leq 0.5$) increased the numbers of platelet and RBC and reduced the WBC as compared with the untreated groups. The AgNPs significantly ($P \leq 0.5$) increased the RBC related parameters including the levels of PCV, MCV, MCH, HB, and MCHC (Figure 6). There is

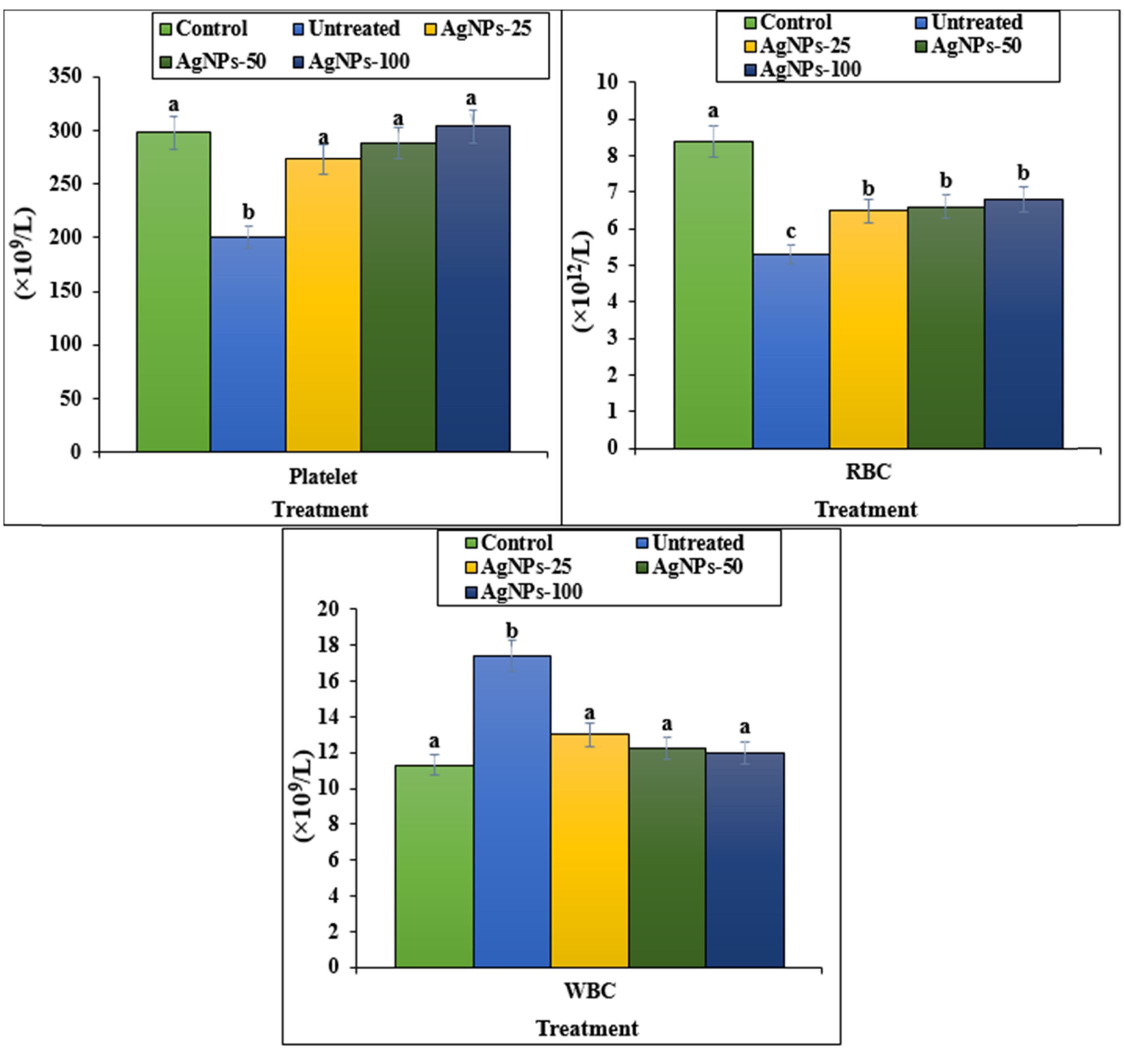

Figure 5: Effects of NPs on hematological parameters (RBC, WBC, and platelet) in a rat model of renal anemia ($P \leq 0.5$).

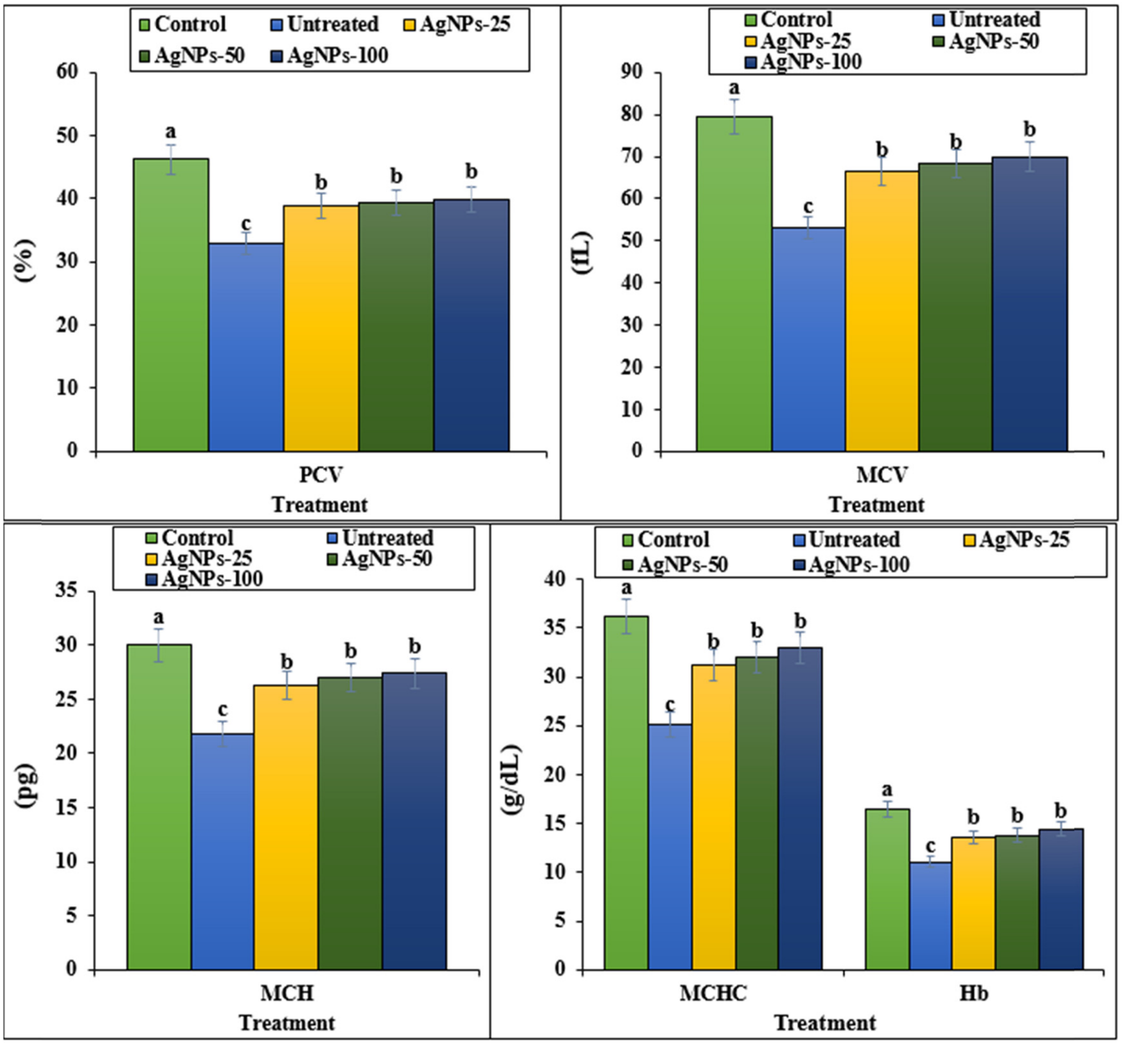

Figure 6: Effects of NPs on hematological parameters (PCV, MCV, MCH, MCHC, and Hb) in a rat model of renal anemia ($P \leq 0.5$).

no statistically significant distinction ($P \leq 0.5$) observed among various dosages of AgNPs in relation to the aforementioned parameters.

In Figure 7, it is evident that the presence of AgNPs causes a significant ($P \leq 0.5$) rise in the anti-inflammatory cytokines concentration, while simultaneously reducing the pro-inflammatory cytokines concentration when compared to the untreated groups.

Based on the above data, the recent synthesized AgNPs may control the inflammation and be administrated as an anti-inflammatory supplement.

Antioxidant compounds are useful in the treatment and prevention of inflammatory disorders resulting from oxidative stress. The anti-inflammatory potentials of *T. terrestris* have been revealed in several research [33–44]. Ethanol extract of *T. terrestris* causes a decrease in the inflammatory enzymes expression such as 2-COX and INOS which play a role in many inflammations. This plant reduces the inflammatory cytokines expression including IL-4 and TNF-α [33]. Therefore, it inhibits the inflammatory cytokines expression, which has a positive activity on different inflammatory conditions. In research, the analgesic effect of the fruit has been proven in formalin and ethyl flake tests in mice [34]. Also, *T. terrestris* extract reduces chronic pain more than acute pain. Formalin test chronic phase inhibition by the extract can be due to inflammation that causes the compounds release, which can sensitize neurons in the central dose [35]. Tribulusamide D is one of the compounds in *T. terrestris*, which has an anti-inflammatory role and affects the lipopolysaccharide of RAW264.7 macrophages. By reducing the nitric oxide synthetase enzymes and cyclooxygenase 2 expression, this compound reduces prostaglandin E and nitric oxide induced by lipopolysaccharide, respectively. Also,

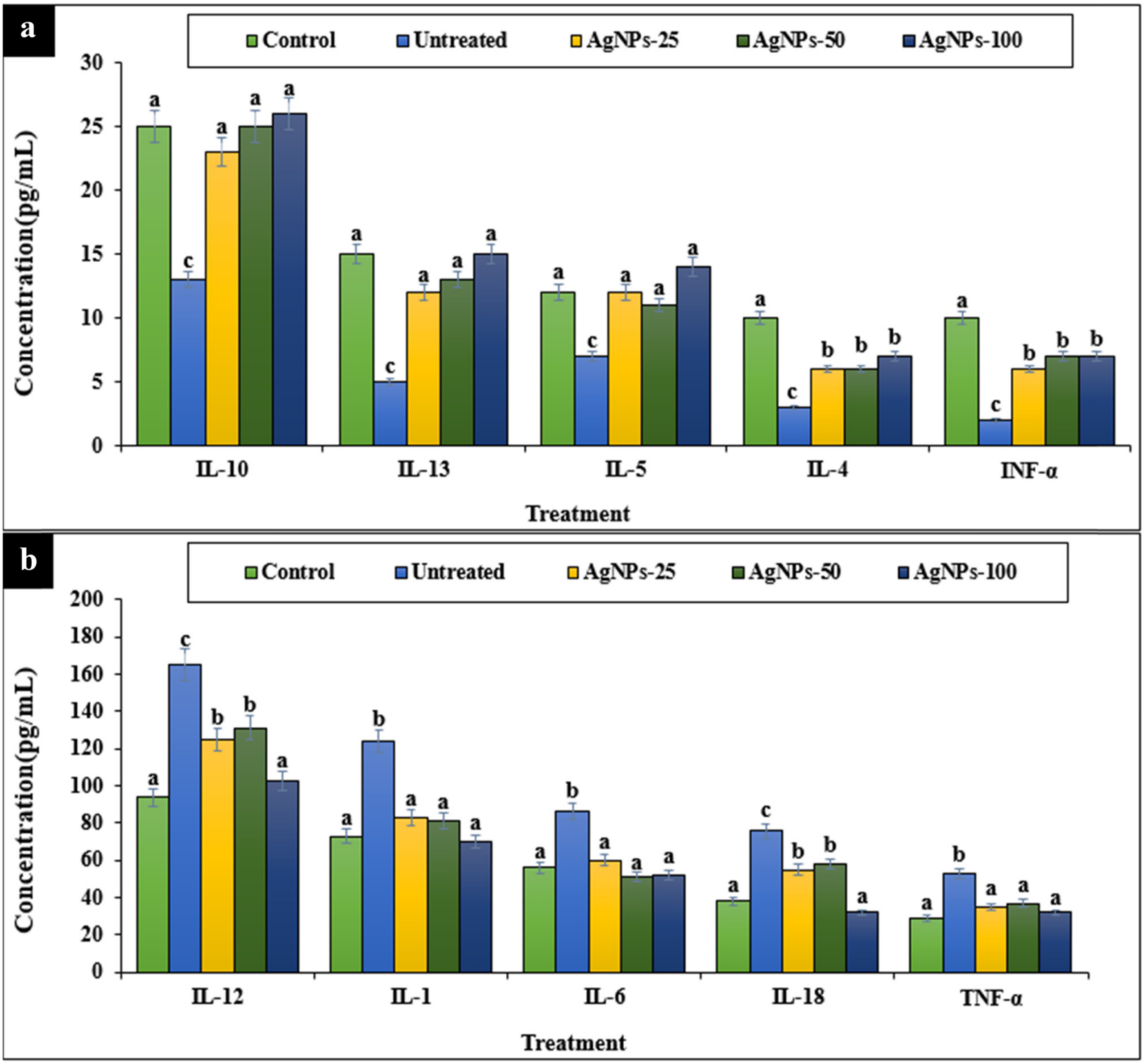

Figure 7: Effects of NPs on immunological parameters (pro-inflammatory cytokines (a) and anti-inflammatory cytokines (b)) in a rat model of renal anemia ($P \leq 0.5$).

this combination reduces cytokines such as IL6 and TNF-a [36]. Another anti-inflammatory compound found in *T. terrestris* is *N*-trans-*p*-caffel tyramine. This compound acts similarly to tribulusamide D and reduces nitric oxide and prostaglandin E2, thereby reducing inflammation in acute inflammatory diseases such as rheumatoid arthritis and asthma [37]. In the study of the properties of the plant extract on cerulein-induced pancreatitis, it was found that the extract of this plant can protect the pancreas in a dose-dependent manner. Cerulein acts as a cholecystokinin analog and leads to the release of free radicals, which subsequently produce NF-κB and lead to the creation of pathways for inflammatory responses. By inhibiting NF-KB signaling pathways, the *T. terrestris* extract leads to the reduction of inflammatory responses and ultimately pancreatitis. Considering the anti-inflammatory efficacies of *T. terrestris* extract, it can be used as an alternative anti-inflammatory herbal medicine with fewer properties than other anti-inflammatory medicines, including glucocorticoids [38–40]. The extract of this plant has been used in the improvement of induced dermatitis with the combination of oxalone. The extract of this plant reduces the inflammatory factors in the area of induced skin inflammation through the regulation of calcium channels and through

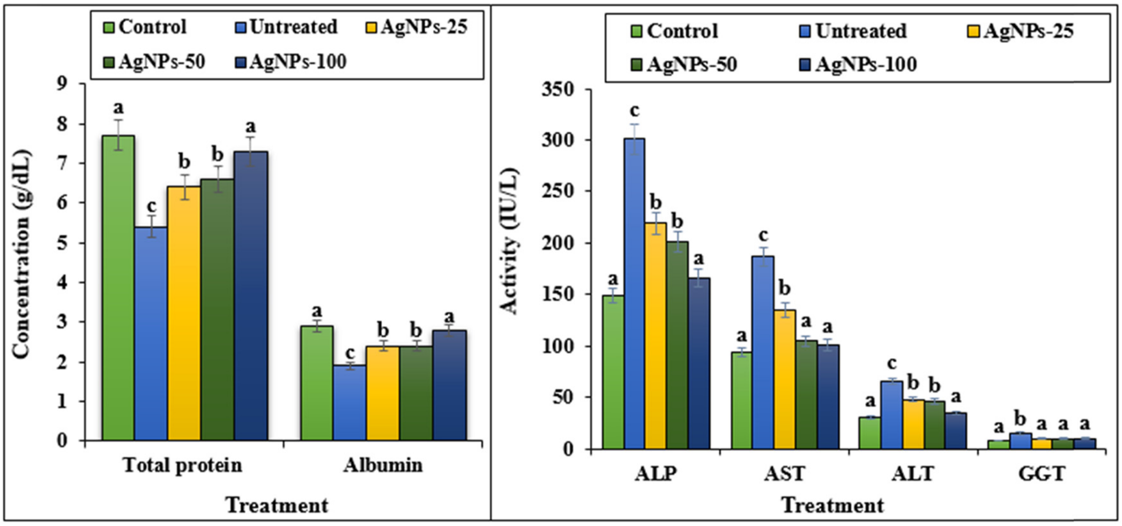

Figure 8: Effects of NPs on biochemical parameters (total protein, albumin, ALP, AST, ALT, and GGT) in a rat model of renal anemia ($P \leq 0.5$).

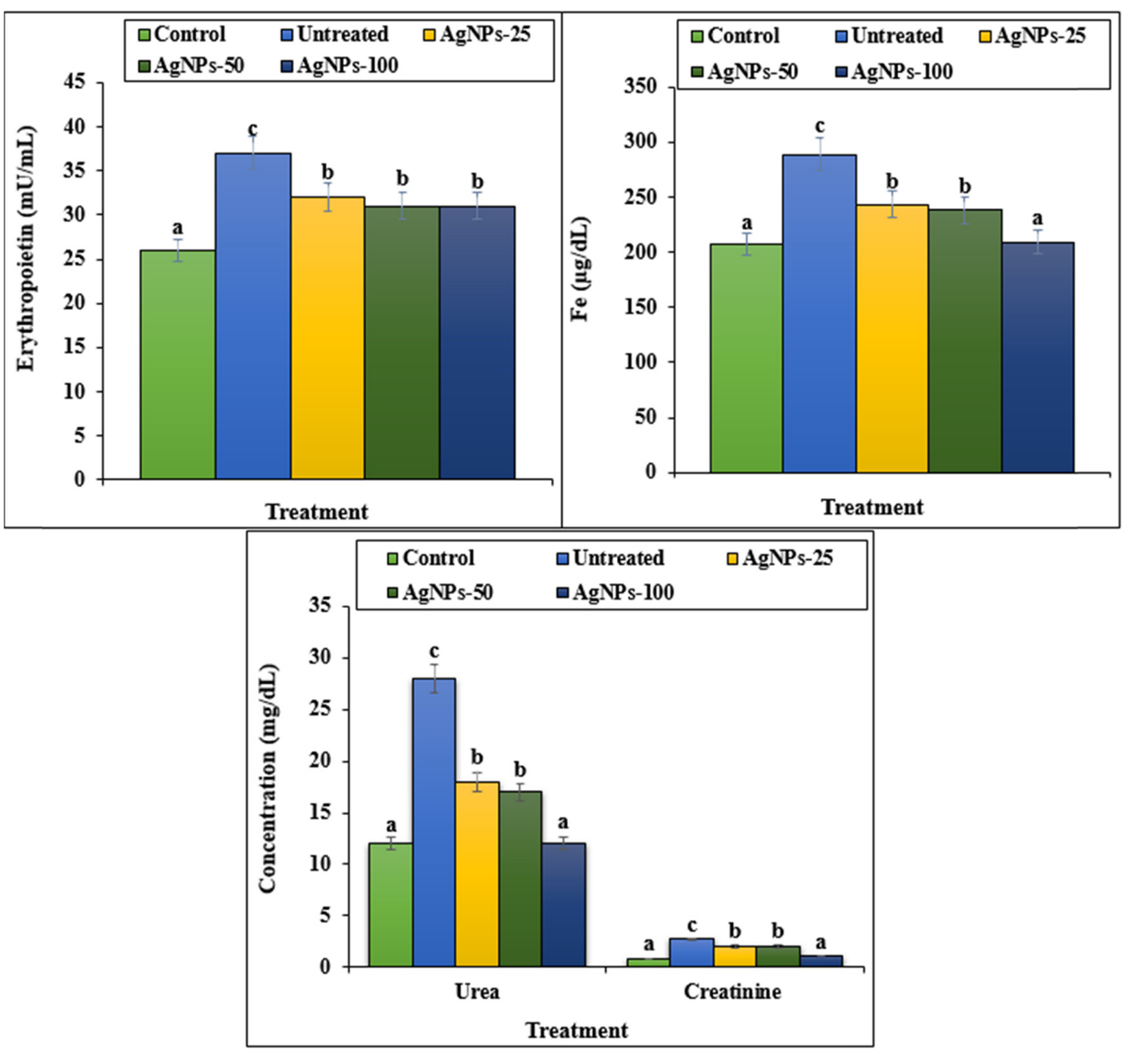

Figure 9: Effects of NPs on biochemical parameters (erythropoietin, iron, urea, and creatinine) in a rat model of renal anemia ($P \leq 0.5$).

the expression of Orai-1 and TRPV3 genes and the activity of mast cells, which ultimately helps in its recovery [42,43]. The dose of 100 mg/kg is considered as an effective pain-reducing dose, whose analgesic mechanism is not related to opioid receptors [44].

As seen in Figures 8 and 9, AgNPs significantly ($P \leq 0.5$) reduced the concentration of hepatic enzymes such as ALP, AST, ALT, GGT, serum Fe, erythropoietin, urea, and creatinine, and increased the concentration of total protein and albumin. The recent results indicate the hepatoprotective, hematoprotective, and immunoprotective characteristics of the recent synthesized NPs.

The liver has a main role in controlling physiological activities and breaking down drugs in the body. *T. terrestris* extract has protective properties following liver damage. In a study, *T. terrestris* against hepatotoxicity caused by acetaminophen in Mozambican tilapia fish (*Oreochromis mossambicus*) increases enzymes involved in antioxidant processes [45]. Non-steroidal drugs such as gelofen cause pathological changes with degeneration and necrosis of liver tissue lobules by creating free radicals; also, it increases the serum enzymes of AST, ALT, and ALP, while the extract of *T. terrestris* inhibits the effect of gelofen in a dose-dependent manner. Probably, this plant, having strong antioxidant compounds and preventing the destructive properties of oxidant compounds resulting from the metabolism of gelophane, causes a decrease in liver transaminases [46]. The hepatoprotective properties of the extract of this plant have been investigated in liver damage induced by non-alcoholic compounds including fructose [47].

4 Conclusion

Previous research has established that AgNPs have significant implications for drug delivery and the treatment of anemia. Because of their small size and large surface area, these particles can be easily targeted to specific tissue sites. Consequently, AgNPs can serve as carriers for therapeutic agents. Additionally, the application of a magnetic field externally can concentrate drugs at abnormal tissue sites. In general, AgNPs are loaded with drugs and employed in the various diseases treatment. The findings indicate that silver NPs possess potent therapeutic properties. In this study, a green formulation approach was effectively employed to create nanomaterials using an herbal extract. The chemical techniques of EDS, FE-SEM, and XRD were employed to identify the NPs. The particles average size was about 48 nm. The presence of (111), (200), and (220) peaks at the positions of 38°, 44°, and 63° reveal the AgNP's presence, which approves the AgNPs correct synthesis. AgNPs (25, 50, and 100 µg/kg) significantly raised the anti-inflammatory cytokines, i.e., IL3, TGFβ, IL10, IL5, and IL4, and decreased the pro-inflammatory cytokines, i.e., TNFα, IL18, IL12, IL6, and IL1. The levels of creatinine, urea, GGT, ALT, AST, and ALP significantly reduced and albumin and TP raised in the groups treated with NPs. AgNPs improved the iron, erythropoietin, thrombocytes, leukogram, and erythrogram.

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Conflict of interest: Authors state no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: Derived data supporting the findings of this study are available from the corresponding author on request.

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