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Research Article
Topical Analgesic and Anti-Inflammatory Properties of Bioengineered *Juglans regia* L. Silver Nanoparticles

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Abstract

Background and Objective: Research has demonstrated the antibacterial, anti-angiogenetic, antiviral, anti-inflammatory, anticancer and antioxidant properties of colloidal silver due to its biological, optical and electrical properties. The aim of this study was the anti-inflammatory effect of the silver bioengineered nanoparticles by using the acetonitrile-unripe fruit extract of *Juglans regia* L., on experimental animal model. **Materials and Methods:** The study uses various techniques to characterize nanoparticles, including ultraviolet spectra, dynamic light scattering and Fourier transform infrared. The study used carrageenan-induced rat paw edema as an induction model for inflammation and assessed its antinociceptive effects in mice using the formalin test. As well as evaluation of proinflammatory cytokines IL-6, TNF and IL-1. **Results:** The produced AgNPs were more compact and stable, according to physical characterization methods compared to chemical prepared nanoparticles. The formulation combining unripe fruit bio-reduced nanoparticles and extract, UF, shows a greater acute anti-inflammatory effect, while leaf extract has a better late anti-inflammatory effect. These bioengineered nanoparticles show efficient *in vivo* anti-acute inflammation, reducing skin inflammation through decreased cellular infiltrates and cytokine release. **Conclusion:** *Juglans regia* L., extract and silver nanoparticles show notable effects in both the early and late stages of the antinociceptive formalin test. While, bioengineered NP/UF and NP/LV can be used as topical analgesics. The potent topical anti-inflammatory and analgesic effects of these medications provide a sufficient basis for the use of this plant material in dermatological products.

Key words: *Juglans regia*, anti-inflammatory, bioengineering, pellicle, silver, metal nanoparticles

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Synthetic nanotechnology has created self-sufficient and environmentally friendly nanomaterials with precise sizes and shapes[1,2]. These materials can be created by engineering multiple or single molecules with various functional groups. Examples of biocompatible nanocarriers include peptides, customized antibodies, micelles, polymers and liposomes[3]. Nanomaterials can be used to deliver drugs to a dynamic tumor environment with great therapeutic efficacy and minimal toxicity[4]. Control of surface-to-volume ratio, shape, size, molecular charge and drug release is crucial for nanoparticles creation[5].

Nanoparticles, such as metallic nanoparticles, can link to cancer cell surface receptors and prevent blood removal[4]. These nanoparticles are considered potential treatments for various diseases due to their affordable synthetic processes and suitable physical and chemical properties[1]. Natural metal oxides are prevalent in nature, making them a new type of mineral particle with better adsorption power compared to other compounds containing metallic nanoparticles[6].

The biosynthesis of nanoparticles (NPs) is gaining attention due to the growing demand for nontoxic, clean, environmentally friendly and renewable solvents. Living species, including plants, have special uses in producing metallic nanoparticles, as they do not require maintaining cell cultures[7]. Studies have shown that plants are preferred over other biological processes for synthetic methods due to their ability to produce metallic nanoparticles without maintaining cell cultures. Extracellular extract has been found to be more effective in regulating the size, shape and dispersity of nanoparticles, according to various studies. Metallic nanoparticles (NPs) are produced using biological agents from plants, microbes and enzymes[5]. Plant-based NPs have shown significant antifungal, antioxidant and catalytic activity. Colloidal silver, with its biological, optical and electrical features, has been shown to have antibacterial, anti-angiogenetic, antiviral, anti-inflammatory, anticancer and antioxidant effects[1]. Studies have also described the antibacterial, anti-angiogenetic, antiviral, anti-inflammatory, anticancer and antioxidant effects of silver nanoparticles[7]. Plant-based synthesis of Silver Nanoparticles (AgNPs) offers several advantages over other methods, including reduced biohazards, cost savings and labor-free cell culture maintenance. These NPs are stable and do not aggregate. Plants, like animals, contain biomolecules like flavonoids and phenols that aid in the quick bioengineering of Ag^+ ions. They also contain anti-inflammatory compounds, primarily flavonoids and terpenoids, which are crucial in reducing diseases like atherosclerosis, arthritis, asthma, cardiovascular illnesses and cancer, which are influenced by inflammatory processes.

Inflammation is a major cause of various illnesses, including atherosclerosis, arthritis, asthma, cardiovascular diseases and cancer[8]. It is a key cause of oxidative stress, triggered by pro-inflammatory cytokines and enzymes. These reactions cause vasodilation, edema, cellular metabolic stress and tissue necrosis. The Nuclear Kappa B Factor (NF-κB) encodes these pro-inflammatory cytokines, chemokines and inducible enzymes, which contribute to various diseases[9]. The transcription factor NF-κB plays a crucial role in regulating these processes[10].

Anti-inflammatory cytokines like interferon, IL-4, IL-10 and IL-13 inhibit the pro-inflammatory response[9]. Steroids and non-steroidal anti-inflammatory medications are the primary treatments for inflammation. However, there is a need to find substitute pharmaceutical formulations with equivalent anti-inflammatory properties without harmful side effects, such as gastrointestinal problems and leukopenia[11]. Walnut, a deciduous tree, is a traditional medicine plant used for its antioxidant activity, which protects against oxidative stress-mediated diseases like cancer and cardiovascular disease. A daily aqueous decoction or alcoholic preparation from air-dried *Juglans regia* leaves is used to treat diabetes mellitus symptoms[12,13]. In the past, medicinal plants were applied topically to help heal wounds and ulcers. The tannin content of plants contributes to the benefits of topical treatments, while their anti-inflammatory and anti-microbial properties also contribute. Unripe fruit extract can cure wounds. Medicinal plant extracts are used in silver nanoparticle synthesis, with enzymes, proteins, polysaccharides and vitamins being biomolecules that decrease Ag^+ ions.

This study investigated the *in vivo* anti-inflammatory and analgesic response characteristics of silver nanoparticles employing fruit extract from *Juglans regia* L., as a green novel synthesis approach. After producing inflammation *in vivo* utilizing the acute inflammation paradigm on Wistar rats, the anti-inflammatory effects of the phyto-synthesized AgNPs were assessed[14]. By measuring the amounts of pro-inflammatory cytokines (IL-6, TNF and IL-1), the assessment of experimental inflammation has been accomplished.

MATERIALS AND METHODS

Study area: The study was carried out at Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mutah University laboratory from May, 2022 to September, 2022.

Chemicals and plant materials: The Sigma-Aldrich Company was the source of all the chemicals and reagents used, all of which were of analytical reagent grade. The $AgNO_3$ (≥98%, powder, Sigma-Aldrich), acetonitrile (98%, Sigma-Aldrich). F carrageenan (Sigma Co., USA), methyl salicylate ointment 30% (The Mentholatum (Zhongshan) Pharmaceuticals Co., Piroxicam gel (0.5%, w/w; Pfizer; Centrifuge (Manufacturer B. herml AGD-7209 Gosheim). The UV-VIS spectrophotometer (Biotech Engineering Management Co. Ltd., UK). The FTIR (Model FTIR-8400S, Serial No. A21014000181 LP, Shimadzu, Japan). Zeta size analyser (Nano Series [Nano–ZS], Malvern Instrument, England).

The *Juglans regia* L. plant was obtained on May 10, 2022, from a private garden in Shmeisani, (Coordinates: 31°58'33"N, 35°53'24"E) Amman, Jordan (Dr. Shatat F., Faculty of Agriculture) and was verified by the University of Jordan. The voucher specimen was delivered to a drug research laboratory, which gave it the deposit specimen number R005. The plant's name has been confirmed on the websites of the plant list organization and World Flora Online. Earlier extraction methods have been published by Al-Nadaf *et al.*[12].

The plant samples (20 g) were extracted using the Soxhlet apparatus with acetonitrile as the solvent. The extraction procedures took place over the course of 4 hrs, with a solid-to-solvent ratio of 1:8 and the number of Soxhlet apparatus cycles taken into account in line with the component separation of the thin layer chromatography technique.

Biosynthesis of AgNPs with *Juglans regia* L. extract fractions: An Erlenmeyer flask holding 100 mL of one molar $AgNO_3$ and 2 g of the extracts P and L were added, swirled and heated to boiling[15]. The resulting solutions were heated and continuously mixed until an observable color shift (from light yellow/brown to deeper brown/green) took place. The resultant AgNPs (AgNP/L and AgNP/P) were centrifuged and resuspended in 1 mL of trisodium citrate 2.2 mM prior to sample characterization[16]. The creation of silver nanoparticles was monitored using a UV-VIS spectrophotometer, with spectrophotometric values acquired at various time intervals. As $AgNO_3$ was incorporated into the reaction at a fixed concentration (1 mM) and extracted in volume. The reaction was then conducted with extract at various time intervals, including 20 min, 1, 4, 24 and 48 hrs *Juglans regia* L., fruit extract to silver nitrate ratio was adjusted at 1:50, 1:60 and 1:80 concentrations of extract in a 1 mM silver nitrate solution[15].

Physiochemical characterization of AgNPs and AgNP/F: The AgNPs were identified and characterized using UV-visible spectroscopy, dynamic light scattering and transmission electron microscopy. The AgNPs were confirmed to have been created and an estimation of the particles' size and shape was made using UV-vis spectroscopy. The estimation of the hydrodynamic diameter and polydispersity index was performed using dynamic light scattering. Functional groups that may be in charge of regulating AgNP synthesis and capping protection were characterized using FTIR spectroscopy. The average diameter was determined by TEM imaging.

***In vivo* study:** The Research Center of Laboratory Animals at Applied Science University Amman, Jordan, provided BALB/c rats and ICR mice for the study using eight animals for each study group. The rodents were kept in a controlled environment, fed a conventional diet and given unlimited water. The study followed the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and was authorized by Mutah University's Scientific Research Committee.

Rat model of acute inflammation: The carrageenan-induced edema model was used in Muley *et al.*[17] techniques to evaluate the under-investigated substance's anti-inflammatory effects. There were four groups of eight rats each, to be more precise. The plantar surface of the left hind paw was treated with 0.2 g of the gel (carboxymethyl cellulose 5%) containing 1% of the test ingredient by gently rubbing 50 times with the index finger. Animals get 50 mL of 1% carrageenan solution injection within an hour of the dosing after being gently massaged 50 times with the index finger (SIGMA-Carrageenan Plant-Mucopolysaccharide, Sigma-Aldrich).

Rats in the control groups received only the gel base. Piroxicam gel (0.5%, w/w), delivered in the same way, served as the reference. There was either medicine or a placebo injection prior to the carrageenan injection. The volume of the paw was measured using a plethysmometer immediately after the injection of carrageenan and at intervals of 1, 2, 3, 4 and 5 hrs. Calculating the percentage of increase in paw volume allowed researchers to gauge the extent of the edema. A 100% initial paw volume assumption was made.

Formalin test: The formalin test was carried out in accordance with the steps provided by Hunskaar and Hole[18]. By gently rubbing 50 times with the index finger, 0.1 g of the tested material in the gel was applied to the left hind paw's dorsal surface. The mice in the control group received nothing except the gel basis. The same technique was used to apply

30% methyl salicylate gel as a standard. Hunskaar and Hole[18] described the formalin test, which was used to measure the anti-nociceptive activity 15 min later. Twenty micro liters of 5% formalin were subcutaneously injected into the mice's left hind paw. The pain threshold was calculated by the length (in seconds) of the injected paw's licking and biting responses. Both the early (5 min) and late (30 min) phases of the reaction (5 min) were evaluated.

Enzyme-Linked Immunosorbent Assay (ELISA): To measure the proinflammatory cytokines, paw pieces (three from each group) were homogenized in cold PBS (phosphate buffered saline), treated with a protease inhibitor cocktail and centrifuged at 10,000 rpm for 20 min at 4°C (Sigma-Aldrich, St. Louis, Missouri, USA). On ELISA plates (Elabscience®), rat-specific antibodies for IL-6, TNF and IL-1 were pre-coated. After adding the samples or standards, each well received a sequential addition of a biotinylated detection antibody specific for rat IL-6, TNF and IL-1 and each well was then incubated. The IL-6, TNF and IL-1, coupled with the biotinylated detection antibody, were assessed using a plate reader with a 450 nm wavelength (BMG Labtech, 2004).

Statistical analysis: All statistical analyses were performed using the free trial version of the SPSS 22.0 program (SPSS Inc., USA). These experiments used eight mice in each group and the results were presented as Mean±SEM. Following a one-way ANOVA analysis of the data, a pair-wise comparison test utilizing Tukey's method was performed. The p-values of 0.05 or below were considered statistically significant.

RESULTS

Biosynthesis and characterization of AgNPs: Unripe fruit, pellicle and leaf extracts from *Juglans regia* L., were utilized to manufacture Silver Nanoparticles (AgNPs) using acetonitrile as the solvent. An aqueous silver nitrate solution's color changed when the extracts were added, demonstrating the production of AgNP. Similar color changes have been seen in earlier experiments, which attest to the conclusion of the reaction between extracts and $AgNO_3$.

The UV-vis spectra obtained 30, 45, 60, 24 and 48 hrs after the process began were shown in Fig. 1(a-b). The AgNP/P and AgNP/L show absorbance peaks in the ranges of 388-425 and 425-460 nm, respectively, in the spectra that were created.

According to Table 1, the polydispersity index (PI) value for NPs made from leaf extract was 0.336, whereas Fig. 2 shows the PI value for NPs made from pellicle extract, which had a mean size of 107 nm. The AgNP that has been chemically produced is smaller, measuring 65.8 nm with a PI of 0.68. The greater size, which is linked to the phytochemicals found in the pellicle and leaves of *Juglans regia* L., shows improved stability brought about by bioengineered.

The FTIR analysis utilized to identify the AgNPs derived from each component of the plant extract, such as the (a) leaves and (b) pellicles, was depicted in Fig. 3. At 1065, 1320, 1381, 1580, 1670, 2301, 3000 and 3350 cm^{-1}, the two spectra revealed substantial absorption bands. Ether connections, germinal methyl, alkene groups, hydroxy, aromatic rings, amine and alkyne bonds are peaks in the spectrum. These bands are vibrational and stretching bands that are present in substances like tannins, alkaloids, flavonoids and phenolics[13,19,20] and they can be attributed to the effective reduction of the obtained AgNPs[21]. The TEM images created by separately treating 2% of each kind of leaf extract with a solution containing 1 mM silver nitrate were shown in Fig. 2. A sphere was created out of each of the three prepared NPs.

Anti-inflammatory and antinociceptive activity: Following topical administration of nanoparticles (1%) and matching plant extracts, the effects of carrageenan-induced acute inflammation were presented in Table 2. According to statistical analysis, the preparations containing Piroxicam, UF, NP/UF, LV and NP/LV consistently inhibited edema in a way that was statistically different from the control group ($p<0.05$). Following the first hour, the following values were recorded: 67.5, 55.2, 45.5, 29.2 and 45.5%. The best piroxicam impact was at a value of roughly 60% after 5 hrs. The findings revealed that the formulation containing NP/UF and UF has a stronger anti-inflammatory impact than other formulations, whereas, LV has a better anti-inflammatory effect after 5 hrs (49.5%).

The pro-inflammatory cytokine-inhibitory activity was examined to evaluate the molecular effectiveness of topical application. After 5 hrs of challenge, topical administration of carrageenan significantly increased the production of IL-1β, IL-6 and TNF-β, as seen in Fig. 4. On the other hand, piroxicam NP, NP/UF and NP/LV therapy decreased TNF-α ($p<0.05$ vs control). Treatment with NP/LV, NP/UF and NP demonstrates a negligible decrease when compared to piroxicam, even though all therapies considerably lower IL6

Table 1: Parameters of prepared nanoparticles

Product	D (nm)	PI	Zp
NP/LV	116	0.336	-18.5
NP/UF	107	0.339	-18.3
NP	65.8	0.68	-33.3

NP: Silver nanoparticles, NP/LV: Silver nanoparticles bio-reduced using leaves extract, NP/UF: Silver nanoparticles bio-reduced using pellicle extract, D: Diameter, PI: Polydispersity index and Zp: Zeta potential

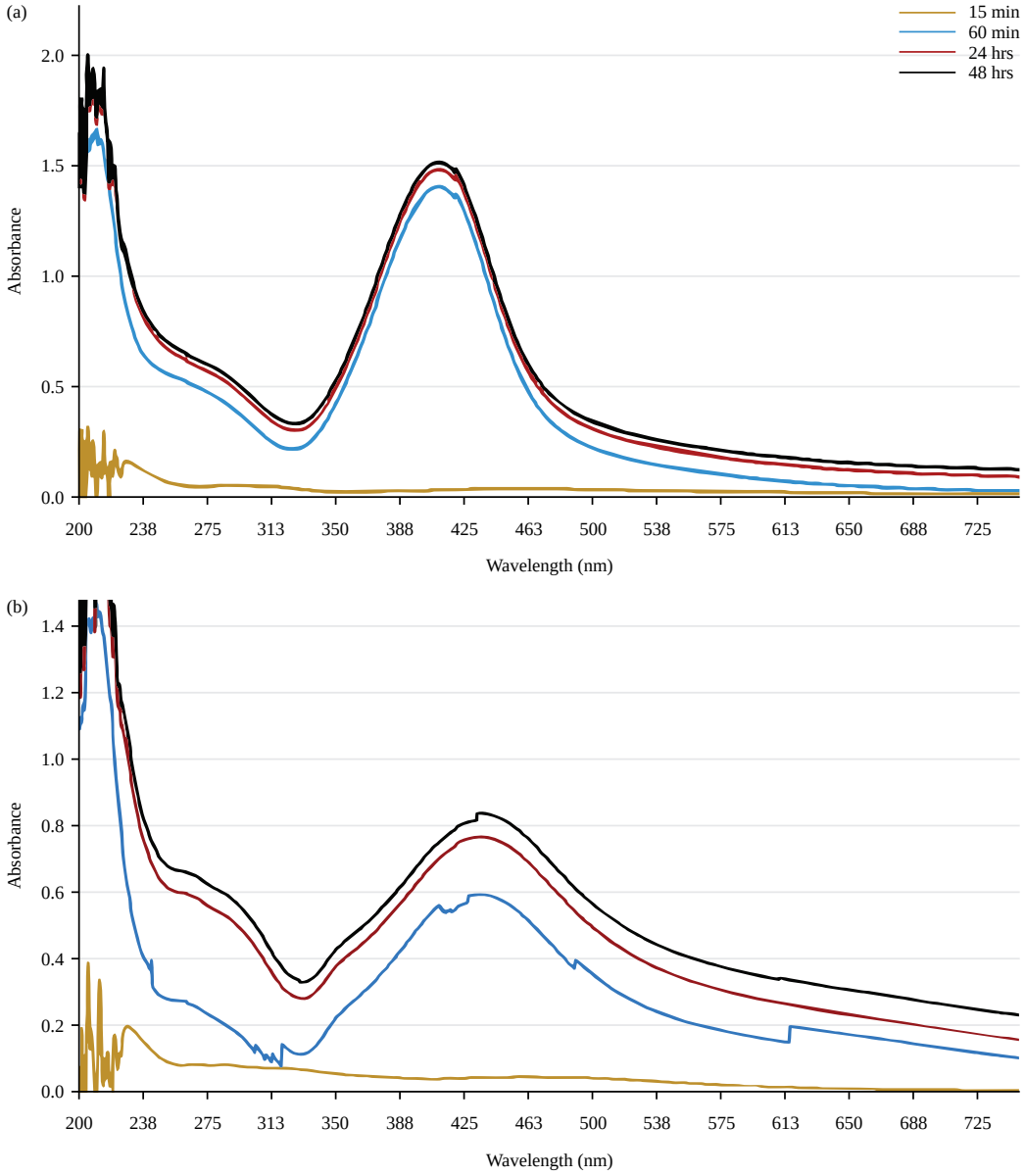

Fig. 1(a-b): UV-vis absorption spectrum and size distribution measured by DLS of silver nanoparticles, (a) UV-vis for AgNP synthesized using unripe fruit pellicle extract (NP/UF) and (b) UV-vis for AgNP synthesized using leaf extract (NP/LV)

Table 2: Effect of topical administration of plant extract and their nanoparticles preparation on carrageenan-induced paw edema in rats with time (hour)

	Paw volume at time (hrs) after carrageenan injection (mm)				
Treatment*	Initial	1st	3rd	4th	5th
Control	5.23±0.02	6.00±0.00	7.08±0.01	7.65±0.01	8.038±0.01
Piroxicam gel 0.5%	4.90±0.03	5.15±0.04 (67.5)	5.75±0.08 (54.1)	5.75±0.06 (59.7)	6.10±0.05 (60.85)
UF	4.93±0.03	5.27±0.09 (55.2)	6.47±0.07 (21.7)	6.82±0.07 (21.7)	6.87±0.06 (30.8)*
NP/UF	4.77±0.06	5.20±0.08 (45.5)	5.92±0.03 (23.2)	6.55±0.04 (26.9)	6.60±0.03 (35.2)*
LV	4.87±0.03	5.42±0.08 (29.2)*	6.12±0.08 (32.7)	6.35±0.08 (39.3)	6.30±0.08 (49.5)**
NP/LV	4.92±0.03	5.35±0.07 (45.5)	6.18±0.06 (32.0)	6.26±0.08 (44.9)	6.62±0.08 (39.7)*
NP	4.95±0.02	5.72±0.07 (0.7)	7.20±0.08 (2.2)	7.46±0.09 (3.8)	7.54±0.09 (0.0)

*Number of animals is 8 for each group, NP: Silver nanoparticles, NP/LV: Silver nanoparticles bio-reduced using leaves extract, NP/UF: Silver, LV: Leaf extract and UF: unripe fruit pellicle, 0.2 g of preparation were applied to the plantar surface of the right hind paw by gently rubbing 50 times with the index finger. Values are Mean±SEM (percent reduction), *p<0.05 vs piroxicam (significant difference) and **p>0.05 vs piroxicam (in-significant difference)

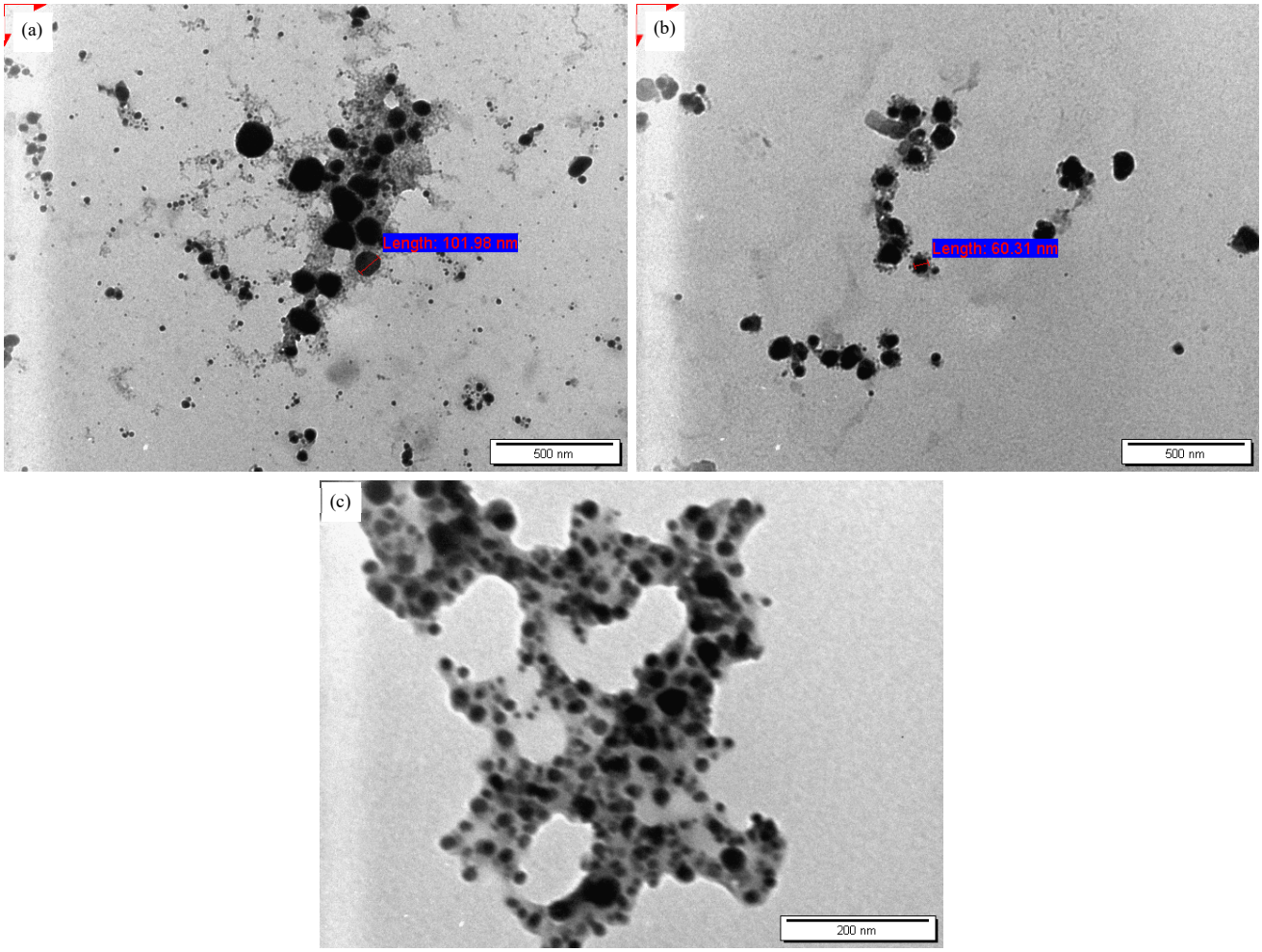

Fig. 2(a-c): Transmission electron microscopy (TEM) images, (a) NP/LV, (b) NP/UF and (c) NP

NP/LV: Synthesized using leaves extract, NP/UF: Nanoparticles synthesized using Unripe fruit pellicle extract and NP: Silver nanoparticles prepared via chemical reduction

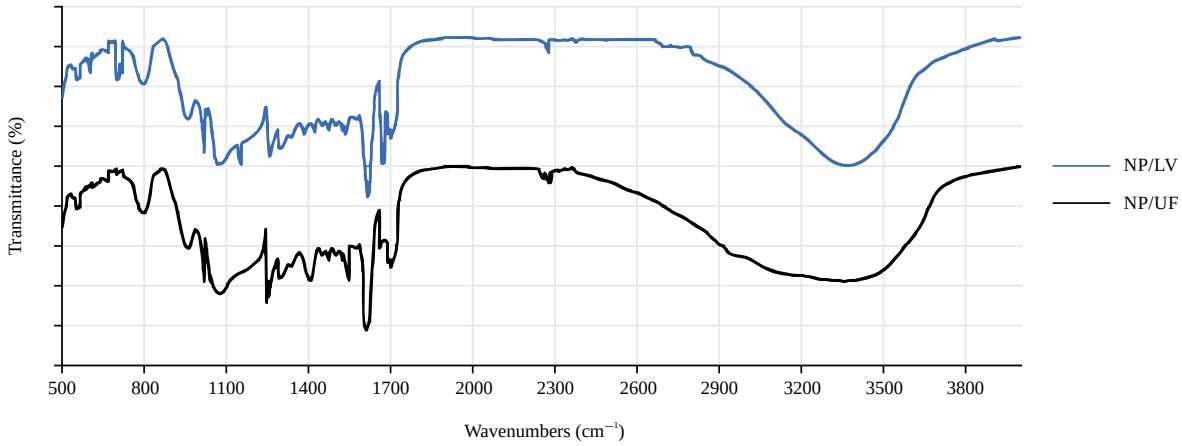

Fig. 3: Simulation for graphs obtained from FTIR analysis of AgNP

NP/UF: AgNP synthesized using unripe fruit pellicle extract and NP/LV: AgNP synthesized using synthesized using leaf extract

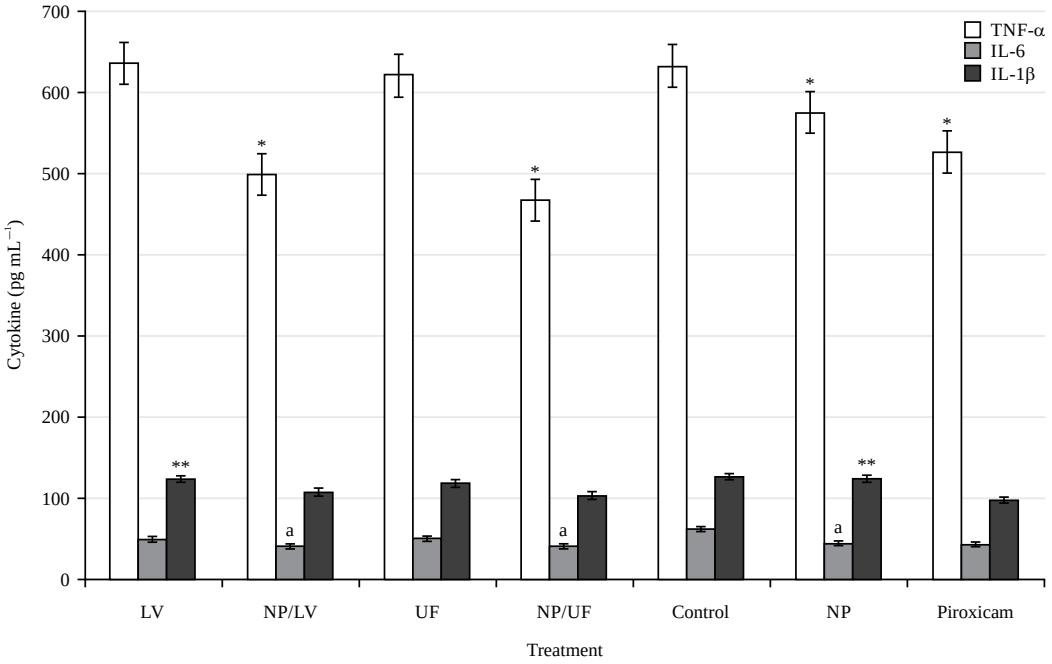

Fig. 4: Pro-inflammatory cytokine-inhibitory activity examination to evaluate the molecular effectiveness of topical applications: Control, piroxicam, UF, NP/UF, LV, NP/LV and NP

TNF-α, IL-6 and IL-1β levels in soft plantar tissue at 5 hrs after carrageenan injection, *p<0.05 vs control (significant difference), **p>0.05 vs control (no significant difference) and [a]p>0.05 vs piroxicam (no significant difference)

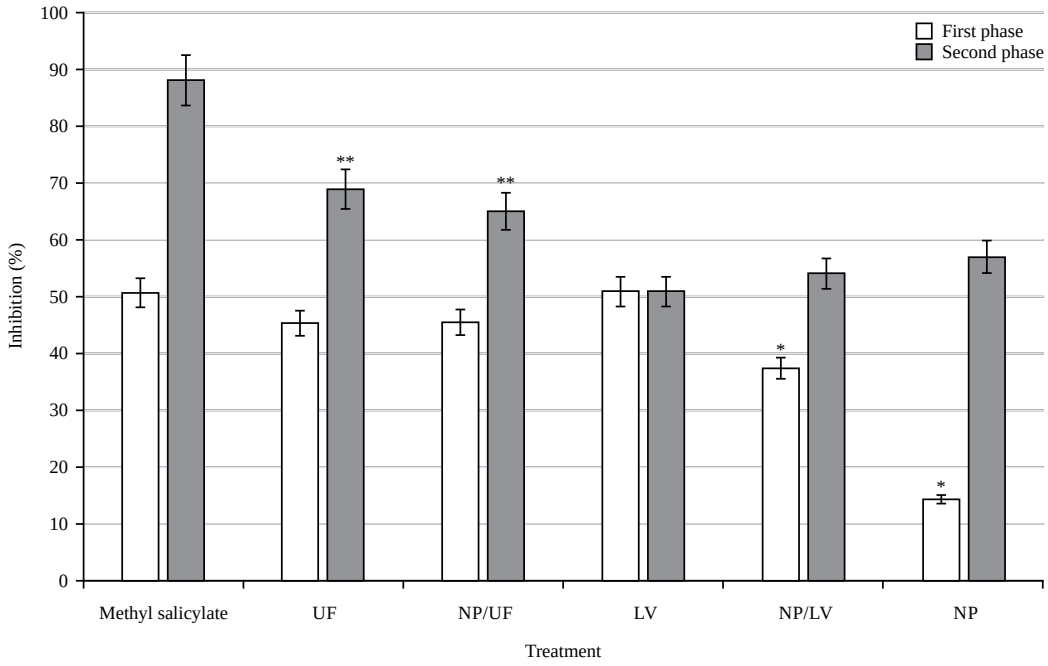

Fig. 5: Antinociceptive effect of topical administration of tested preparation gel on the first phase and the second phase of formalin-induced edema in mice

Values represent the Mean±SEM, *p<0.05 vs methyl salicylate value (significant difference) and **p>0.05 vs methyl salicylate value (insignificant difference)

levels ($p<0.05$ vs. control). The lowering of IL-1β is not significantly different between NP, LV and control ($p>0.05$ vs control). As a result, it could lower the quantities of activated cellular infiltrates and cytokine release, thereby lowering cutaneous inflammation.

Figure 5 shows the antinociceptive percentage of effects on the first and second phases for formulations including methyl salicylate (50.6, 88), UF (45.3, 68.8), NP/UF (45.4, 64.9), LV (50.8, 50.74), NP/LV (37.4, 54.05) and NP (14.3, 56.9).

DISCUSSION

Silver nanoparticles are employed in a variety of applications, including antibacterial agents, medication transporters, regeneration materials, biomaterial coaters and certain pharmacological actions, including anti-inflammatory and interoceptive. This work prepared bio-reduced silver nanoparticles from *Juglans regia* L. acetonitrile extracts from pellicle fragments and evaluated their anti-inflammatory and analgesic properties. Color change, UV-visible spectroscopy, FTIR, DLS and SEM were used to characterize silver nanoparticles. The initial sign of the creation of silver NPs was the color change from bright yellow to brown. Peaks of the silver properties were seen in the silver NPs solutions' UV-visible analyses between 380 and 460 nm (Fig. 1).

Peaks below 400 nm were visible in our data, which suggested the existence of organic substances. Silver NPs were formed, as evidenced by the highest peaks at 400 and 450 nm. The pellicle extract included a strong reductant that accelerated the bio-engineered process and promoted the creation of nanoparticles with sizes of around 107 nm. Most of the silver nanoparticles in green-synthesized AgNPs or AgNP/P, were spherical, but AgNP was aggregated and embedded in a dense, thick pattern (TEM images). In response to the $AgNO_3$ suspension's reaction with the metal nanoparticles of plant pellicle extracts, SPR was stimulated, which significantly altered the absorption maximum of biologically generated nanoparticles[22].

Wiley *et al.*[22] used this technique to investigate the size and shape of nanoparticles in liquid solutions. Both phytoextracts of *Juglans regia* L. converted silver into silver nanoparticles, according to UV-vis spectroscopic data (AgNPs). Peaks utilize the cubic shape and crystalline characteristics of biologically produced nanoscale silver nanoparticles[23]. The average pellicle extract size was 107 nm and the average PI value for AgNP was 0.68, as shown by the DLS size distribution histogram (Table 1).

The PI values of 0.339 with AgNP/P demonstrated the uniform distribution of bio-engineered NP and the lack of significant differences. The AgNP has lower stability and a higher propensity for agglomeration. It denotes improved stability caused by bio-engineering but a larger size caused by phytochemicals present in *Juglans regia* L., pellicles. The bio-engineered process is accelerated by the presence of a potent reductant in the pellicle extract, which also encourages the production of nanoparticles with an average diameter of 107 nm. The functional groups present in the AgNPs that have undergone bioengineering are clearly shown by the FTIR visible peaks at 3350 cm^{-1} with N-H and OH bond stretching. These bands indicate the stretched vibrational bands that give rise to substances such as tannins, alkaloids, flavonoids and phenolics[12]. These results supported previous research and show that a range of bioorganic components in extracts form long-lasting reducing agents on silver nanoparticles[23,24].

The FTIR analysis performed to identify the AgNPs derived from the plant extract was shown in Fig. 2. Significant absorbance bands can be seen in the spectra at 1065, 1320, 1381, 1580, 1670, 2301, 3000 and 3350 cm^{-1}. Peaks are ether connections, germinal methyl, alkene groups, hydroxy, aromatic rings, or amine and alkyne bonds. These bands are vibrational and stretching. Bands that are present in substances like tannins, alkaloids, flavonoids and phenolics and they may be linked to the effective reduction of the acquired AgNPs[12].

The TEM images created by independently reacting 2% of each kind of leaf extract with a solution containing 1 mM silver nitrate were shown in Fig. 3b-c. Spheres were created out of the two prepared NPs.

Bands of alcohol groups, carboxylic acids, flavonoids, phenols and tannin, were seen in the FTIR spectra of the nanoparticles. This was brought about by silver nitrate's interaction with these groups, which reduced silver, capped and stabilized. Based on the findings of the FTIR study, a peak at 3350 cm^{-1} was discovered. Alcohol stretching vibrations and H-bonded phenols may all have similar peaks. At a second inspection, at 1580 and 1670 cm^{-1}, the amide group for protein extension with carbonyl extension was found (Fig. 3). The presence of C-C showed CH3 stretching, as shown by peaks at 3000 and 1320 cm^{-1}, respectively. The presence of amine vibrations and anhydrides, ethers and carboxylic groups, respectively, was further shown by peaks at 1050 and 700 cm^{-1}. The carbonyl group of flavonoids or amino acid residues has the strongest capacity to bind silver and form a layer for capping the silver NPs, according to the FTIR spectra, which also verified that peaks at 1670 cm^{-1} indicated the expansion of C=O and C=C stretches. These findings were consistent with a prior study[13,23], which revealed the presence of terpenes, amines and tannins that wrapped the silver NPs, preventing aggregation and increasing their stability.

Often employed as an edematogenic agent, carrageenan is a sulfated polysaccharide that comes from plants and was isolated from the alga *Chondrus crispus*. Inducing paw edema by administering subplantar carrageenan is a typical method of simulating acute local inflammation that has a biphasic feature. The initial phase of edema, which occurs between 1 and 2 hrs after induction and is caused by increased prostaglandin production, is characterized by the release of histamine, serotonin and bradykinin. Prostaglandin, substance P, platelet activation factor (PAF), proteases, cytokines (IL-1, IL-6, IL-8 and TNF) and lysosomes are produced during the second phase of edema (3-4 hrs after induction)[25].

Since the carrageenan-induced paw edema model is biphasic, the *Juglans regia* L., silver NPs and UF extract may be able to reduce the inflammatory mediators produced in the first phase. Due to the phytochemical components, it contains, leaf extract LV works more effectively at a later stage. The cytokine study further supported this output. Reduction in the levels of active cellular infiltrates and cytokine production, hence reducing cutaneous inflammation. The TNF, IL-6 and IL-1 levels in bioengineered NP were significantly lower[26].

The formalin test is a reliable and accurate nociception model that might be responsive to different analgesic medication classes. In the early and late stages of the formalin test, which clearly demonstrated a biphasic response, various analgesics may respond differently[14,26]. Medicines that act peripherally, such as aspirin, indomethacin and dexamethasone, exclusively inhibit the late phase, but medicines that act centrally, such as opioids, equally inhibit both phases[27]. The late phase of inflammatory pain, which looks to be an inflammatory reaction, can be lessened by anti-inflammatory drugs.

The impact of *Juglans regia* L., extract and silver NPs on the early and more on the late phase of the formalin test shows that its activity may be caused by its peripheral action when compared to Piroxicam activity in this respect. *Juglans regia* L., extract improved the painkilling effects of silver NPs in the early phase more than in the late phase. Juglone is a flavonoid substance that is widely distributed in this plant. *Juglans regia* L., silver nanoparticles' antinociceptive and anti-inflammatory properties may benefit from this.

In line with findings from other studies, current study clearly shows that exposure to carrageenan increased the secretion of IL-1β, IL-6 and TNF-α, indicating that these cytokines mediate inflammatory signaling and are essential for the development of carrageenan-induced acute irritant contact dermatitis[9,11]. The findings of this study further implied that IL-1β, IL-6 and TNF-α inhibition at the site of inflammation may play a role in the inhibitory action of *Juglans regia* L., unripe fruit and leaf extract as well as bioengineered NP. Since many years ago[28], the adjuvant-induced arthritis model in rats has been used to assess the efficacy of anti-arthritic and anti-inflammatory drugs[29,30]. Experimental acute inflammation can be successfully treated by topically administering *Juglans regia* L. Additional research is required because the current study only used a crude extract and its NP equivalents; therefore, more studies will need to be done in the future to take the pure ingredient of this plant into consideration. Due to phytochemical studies showing this plant's constituents have a high concentration of tannins, flavonoids and polyphenols, we recommend using gel-base preparations of bioengineered silver nanoparticles made from acetonitrile extract from the unripe fruit and to a lesser extent, the leaf extract for topical application. The fact that bioengineered nanoparticles reduced topical pain and inflammation by roughly 1% at dosages of 0.2 g gel seems to validate this pattern, even though it has not been yet performed in-depth dose-response analyses. The potent topical anti-inflammatory and analgesic effects of these medications provide a sufficient basis for the use of this plant material in dermatological products.

CONCLUSION

According to the results of the current work, *Juglans regia* L., pellicle and leaf extracts were used to bioengineer AgNPs in a sustainable, regenerative manner. Silver nanoparticles might be made easily and affordably. Using TEM, DLS, UV-Vis and FTIR, NPs were characterized and generated from acetonitrile extracts. Physical characterization techniques revealed that the created AgNPs were more compact and stable than their chemically manufactured counterparts. Plant phyto-derivatives are used as capping agents for nanoparticles. By using green-synthesized NP/UF and NP/LV, the effectiveness of *in vivo* anti-acute inflammation was shown. In the early and late phases of the antinociceptive formalin test, the extract of *Juglans regia* L., and silver nanoparticles demonstrate significant effects. The anti-inflammatory and anti-nociceptive properties of metallic silver nanoparticles were examined, which was produced using environmentally friendly nanotechnology that is becoming more widely used. Considering the results of this study, bioengineered NP/UF and NP/LV might be employed as topical pain and inflammatory medication.

SIGNIFICANCE STATEMENT

Numerous disorders, such as atherosclerosis, arthritis, asthma, cardiovascular diseases and cancer, are significantly attributed to inflammation. The article discusses how silver nanoparticles bioengineered from *Juglans regia* have analgesic and anti-inflammatory effects. It is possible to use bioengineered NP/UF and NP/LV as topical analgesics. The study found that bioengineered AgNPs, combining unripe fruit bio-reduced nanoparticles and extract, have a greater acute anti-inflammatory effect, while leaf extract has a better late anti-inflammatory effect. These nanoparticles effectively reduce skin inflammation through decreased cellular infiltrates and cytokine release, making them suitable for use as topical analgesics in dermatological products.

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