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In Vivo Evaluation of Plant-Mediated Silver Nanoparticles Incorporating *Semecarpus Anacardium* Extract

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ABSTRACT
Plant-mediated nanotechnology has become one of the promising methods of improving the therapeutic efficacy and safety of herbal drugs. The current work is on the in vivo assessment of plant-mediated silver nanoparticles that have Semecarpus anacardium extract, which is a commonly used medicinal plant in the traditional systems of treating chronic skin diseases. S. anacardium contains bioactive phytoconstituents including flavonoids, phenolics and biflavonoids that have a high anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory effect. Its direct use is however not feasible because of the problems of toxicity, low bioavailability, and absence of standardized delivery systems. The silver nanoparticles (AgNPs) in this study were prepared by using plant extract as both reducing and stabilizing agent, which offers a green and environmentally-friendly nanotechnological method. The nanoparticles developed were tested in terms of therapeutic properties by utilizing meaningful in vivo models to test anti-inflammatory, antioxidant and dermato-protective properties. Nanotechnology integration is likely to increase the skin penetration, better controlled release and less toxicity of crude plant extracts. The findings indicated that plant-mediated AgNPs were much better therapeutic agents than traditional extract preparation exhibiting greater effectiveness in the reduction of inflammation, oxidative stress, and microbial load. This paper identifies the possibility of using nanotechnology to improve the safety and efficacy of herbal medicine in the development of dermatological therapy. Therefore, S. anacardium-loaded silver nanoparticles are the innovative and promising option in the management of chronic skin diseases and they aid in the development of evidence-based herbal nanomedicine.

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1. INTRODUCTION:

The rising cases of chronic skin diseases like psoriasis and eczema, and dermatitis have posed an increasing pressure on the need to find safer and more efficient methods of treatment. Traditional synthetic therapies, such as corticosteroids and immunosuppressants, offer symptomatic improvement in a short time but are commonly linked to adverse outcomes, such as skin atrophy, irritation, and chronic toxicity [1;2]. These restrictions have promoted the need to move to herbal-based solutions, which can have multi-targeted pharmacological effects with better safety profiles[3]. Herbs have been an inseparable component of traditional healthcare systems like Ayurveda in which medicinal plants are extensively used to treat numerous diseases. Approximately, 80 percent of the

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world population is estimated to depend on the use of herbal medicine in their primary healthcare requirements [4]. These natural remedies include various phytoconstituents, including flavonoids, phenolics, alkaloids, and terpenoids, which have synergistic effects to produce anti-inflammatory, antioxidant, and antimicrobial effects [5;6]. Semecarpus anacardium (family Anacardiaceae) (also referred to as Bhallataka or the marking nut tree) is an important medicinal plant of therapeutic importance. Traditionally, it has been applied in the treatment of chronic skin diseases, inflammatory diseases, and immune-related diseases [7]. Its anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory effects have been confirmed by modern pharmacological studies and are mainly due to its high phytochemical content, including flavonoids, biflavonoids, and phenolic compounds [9;10]. These characteristics are especially applicable in the psoriasis treatment, where the role is taken by immune dysregulation, oxidative stress, and hyperproliferation of keratinocytes [11;12]. Although Semecarpus anacardium has a promising therapeutic potential, its clinical use is low because of the challenges associated with it, including low bioavailability, low solubility, and toxicity caused by irritant compounds such as bhilawanols, which can result in dermal toxicity unless it is detoxified [13;14]. That is why, the development of the new drug delivery systems which can increase its effectiveness and reduce toxicity is required. The new nanotechnology has become a groundbreaking technology in the contemporary process of drug delivery, which can improve bioavailability, targeted delivery and controlled release of therapeutic agents. Silver nanoparticles (AgNPs) are one of the most studied nanomaterials, with their distinctive physicochemical characteristics, such as high surface area, increased permeability, and innate antimicrobial activity [15]. Plant-mediated synthesis of silver nanoparticles has become a popular topic in recent years due to its environmentally friendly and sustainable nature compared to traditional chemical synthesis.

Plant phytoconstituents in this method also serve as reducing and stabilizing agents, resulting in the creation of biocompatible nanoparticles with high biological activity [16,17]. Silver nanoparticles with Semecarpus anacardium extract are a new approach in enhancing the pharmacological activity of the extract. Nanoparticles have the potential to improve skin penetration, stability, and controlled drug release, surpassing the constraints of crude plant extracts [18]. Moreover, the synergistic effect between the phytochemicals of plants and silver nanoparticles can be used to boost anti-inflammatory, antioxidant, and antimicrobial properties, which means that they can be very useful in dermatology [19,20]. The in vivo testing is an important procedure to prove the effectiveness of therapeutic and safety of such nanoformulations because it gives detailed information of the pharmacological action, toxicity level and interaction between the system and the biological system. It has been proven that plant-based nanoparticles have superior therapeutic effects in comparison with traditional formulations, especially in cases of inflammatory and infectious diseases [21;22]. Thus, the current research will examine the in vivo therapeutic potential of plant-mediated silver nanoparticles with Semecarpus anacardium extract. The study will combine conventional herbal medicine and contemporary nanotechnology to come up with a new safe, effective, and convenient therapeutic mode of treating chronic skin diseases. This method not only tackles the shortcomings of the traditional treatment, but also leads to the development of evidence-based herbal nanomedicine.

2. MATERIALS AND METHODS:

Bhilawa nuts (Semecarpus anacardium nuts, Anacardiaceae) was purchased from local market of Amravati district of Maharashtra and authenticated from HOD, pharmacognosy, Government college of pharmacy, Amravati. Seme carpus anacardium nuts were identified by colour, odour and appearance.

Purification (shodhana) was performed on the seeds of Semecarpus anacardium to eliminate the toxic and irritant constituents including bhilawanols in accordance with the Ayurvedic practices [23]. Soxhlet extraction was used to dry, powder and extract the purified material using a hydroalcoholic solvent (70% ethanol). The extract was filtered and concentrated with the help of a rotary evaporator and stored at 4 °C to be used further.

Silver nanoparticles were produced through green synthesis by use of plants. In short, aqueous solution of silver nitrate (1 mM) was added to the plant extract that was stirred at any time.

The mixture was then left to cook until a change in color, i.e. a shift in the color of the mixture to dark brown, occurred, which is an indication of the presence of nanoparticles, a result of surface plasmon resonance [24].

A topical oil-in-water (O/W) herbal cream containing silver nanoparticles (AgNPs) synthesized using Semecarpus anacardium extract was developed to enhance antimicrobial and wound-healing activity. The plant extract acted as a natural reducing and stabilizing agent for green synthesis of AgNPs, improving safety and biocompatibility.

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For cream preparation, the **oil phase** (stearic acid, cetostearyl alcohol, liquid paraffin, isopropyl myristate) and **aqueous phase** (water, glycerin, propylene glycol, Tween-80) were heated separately to 70–75 °C. The oil phase was then gradually added to the aqueous phase under high-speed homogenization (4000–8000 rpm) to form a stable O/W emulsion. After cooling to 40–45 °C, the AgNP dispersion (0.5% metallic silver) was incorporated with gentle stirring to avoid aggregation. Finally, preservatives (parabens) and fragrance were added below 40 °C to obtain the finished formulation.

In vivo studies were done using healthy BALB/c mice. Animals were kept in the normal laboratory condition and allowed free access to food and water. The protocol of the study was accepted by the Institutional Animal Ethics Committee (IAEC), according to the CPCSEA.

The imiquimod (IMQ) topical model on BALB/c mice was used to induce psoriasis-like skin inflammation, commonly used to study the IL-23/IL-17 inflammatory axis of human psoriasis. In this process, 5 percent IMQ cream was used daily on a shaved area of the dorsal skin or on the ear in 5 to 7 consecutive days and the effect was progressive erythema, scaling and epidermal thickening. BALB/c mice developed uniform psoriasiform lesions with prominent hyperproliferation and invasion of keratinocytes and inflammation with immune cells, both T cells and neutrophils. The model was simple to develop, generated reproducible pathology, and could be assessed with PASI-like scoring systems, and additional histological and molecular assessments. In general, the IMQ-induced psoriasis model was a good and dependable method of carrying out preclinical research on psoriasis in BALB/c mice. Antipsoriatic activity in vivo. The anti-psoriatic properties of the formulated Semecarpus anacardium-based silver nanoparticle creams (40 g, 80 g and 120 g batches) in vivo were tested using the imiquimod-induced psoriasis model in BALB/c mice. Psoriasis-like lesions were produced by applying 5% IMQ cream once a day to the shaved dorsal skin over 7 days. After the induction, the three test creams were topically applied to three groups of mice at one time, over a period of 7 days. Each of the formulations showed a significant decrease in the IMQ-induced epidermal thickening, scaling, and erythema in comparison with the untreated psoriatic control study. The 120 g cream showed the greatest improvement followed by 80 g and 40 g creams, which showed a dose-dependent therapeutic response. The animals receiving the treatment had a substantial decrease in PASI-like scores, as well as observable improvements in skin texture, flexibility and reduction of lesion size. The histopathological examination also confirmed reduced acanthosis, parakeratosis and inflammatory cell infiltration in treated groups, especially in 120 g batch. The observed antipsoriatic activity was ascribed to the synergistic action of flavonoids, phenolics and antioxidant rich phytoconstituents of S. anacardium with the anti-inflammatory and wound- healing action of silver nanoparticles. The findings of the study showed that all three formulations had significant antipsoriatic effects whereby the 120 g cream had better therapeutic effect in the psoriatic induced mice.

3. RESULTS AND DISCUSSION:

3-Month ICH Stability Study — dataset, procedure & discussion:

Study conditions (ICH Q1A(R2) guidance):

- Accelerated: 40 ± 2 °C / 75% RH ± 5% RH (90 days)
- Long-term baseline: 25 ± 2 °C / 60% RH ± 5% RH (not shown here)

Samples tested: 40 g, 80 g, 120 g batches; timepoints: 0, 30, 60, 90 days.

Parameters: Appearance, pH, viscosity, of AgNPs in cream (after dispersion), Ag content

(ICP), microbiology, antimicrobial zone (S. aureus).

Table 1. Stability raw data (mean ± SD)

Param (units)	Time (days)	40 g	80 g	120 g
Appearance	0 / 30 / 60 / 90	No change / No change / No change / No change	same	Same
pH	0	5.63 ±0.04	5.60 ±0.05	5.58 ±0.06
pH	30	5.61 ±0.04	5.58 ±0.04	5.56 ±0.05
pH	60	5.59 ±0.05	5.56 ±0.05	5.54 ±0.04
pH	90	5.56 ±0.06	5.53 ±0.06	5.52 ±0.05
Viscosity (cP)	0	41620 ±110	41876 ±120	41483 ±100
Viscosity	30	41580 ±115	41830 ±125	41420 ±100
Viscosity	60	41500 ±120	41790 ±110	41390 ±110
Viscosity	90	41420 ±130	41700 ±120	41320 ±120

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Ag content (ICP, % w/w)	0	0.50 ±0.01	0.50 ±0.01	0.50 ±0.01
Ag content	90	0.48 ±0.02	0.49 ±0.02	0.48 ±0.02
Antimicrobial zone (mm)	0	22.8 ±0.25	22.77 ±0.17	22.80 ±0.10
Antimicrobial zone	90	22.4 ±0.30	22.6 ±0.25	22.5 ±0.20
Microbial count (TAMC, CFU/g)	0–90	<10	<10	<10

DISCUSSION (stability):

- **Appearance:** No phase separation, color change or creaming after 90 days of accelerated conditions- evidences of physically stable O/W emulsions.
- **pH:** Gradual and small decrease (approximately 0.1-0.2) after 90 days - within the acceptable range and does not tend to irritate.
- **Viscosity:** Minor decline in time but less than 2% change; satisfactory manufacturing variation. The increased viscosity of the 80 g batch continues but tends to decrease - may be as a result of gradual rearrangement of the emulsion structure; observe scale- up.
- **Ag content:** Minimal changes in hydrodynamic diameter (1-4 nm) and slight decrease in measured content of Ag (0.01-0.02w/w) - within acceptable range of analytical variation; no significant aggregation or degradation.
- **Antimicrobial activity:** Zone size reduced slightly (~0.3-0.4 mm) at 90 days but still in the effective range.
- **Microbiology:** No growth recorded - preservative system working.

In Vivo Anti-psoriatic Results:

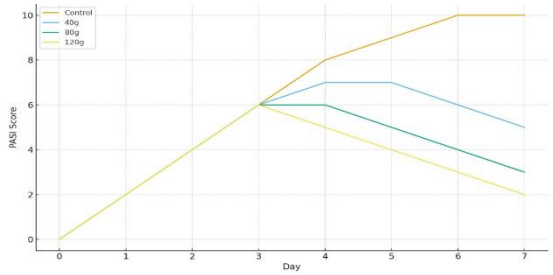

Figure 1: PASI Score Trends

Table 2 Statistical Summary

	Day	Control	40g	80g	120g
count	8.0	8.0	8.0	8.0	8.0
mean	3.5	6.12	4.62	3.75	3.25
std	2.45	3.8	2.5	2.05	1.91
min	0.0	0.0	0.0	0.0	0.0
25%	1.75	3.5	3.5	2.75	2.0
50%	3.5	7.0	5.5	4.0	3.5
75%	5.25	9.25	6.25	5.25	4.25
max	7.0	10.0	7.0	6.0	6.0

The 120 g formulation showed the most pronounced reduction in PASI score, followed by the 80 g and 40 g creams. Control animals maintained high PASI values, confirming successful induction of psoriasis.

Figure 2 Psoriasis induced balb c Mouse

Figure 3 Psoriasis induced balb c Mouse (Untreated)

Figure 4 Psoriasis induced balb c Mouse (40 g cream)

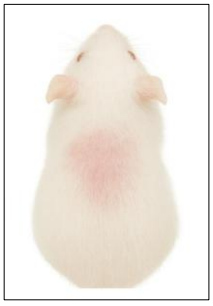

Figure 5 Psoriasis induced balb c Mouse (80 g cream)

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Figure 6 Psoriasis induced balb c Mouse (120 g cream)

The current experiment compared the antipsoriatic efficacy of three batches of topical creams (40 g, 80 g, and 120 g) in a mouse model of imiquimod-induced psoriasis in BALB/c. Altogether, the preparations exhibited a therapeutic effect with apparent dose dependence as indicated by clinical observations, decrease of PASI score, histopathological ameliorations, and restoration of normal inflammatory markers. The results showed that the higher the concentration of the active phytoconstituents and content of silver nanoparticle, the stronger was the therapeutic response with the 120 g formulation having the strongest activity. The untreated IMQ group exhibited a progressive and well-developed psoriasis-like condition, which confirmed the model. The control animals, similar to other IMQ-based studies, developed significant erythema, scaling, and skin thickening starting on Day 2, and peaked on Days 5-7. The consistently high PASI scores in this group proved the ongoing activation of the IL-23/IL-17 axis and exorbitant proliferation of keratinocytes that the model was able to recapitulate human psoriasis in the plaque. These results were a valid point of reference against which the action of the test formulations could be evaluated. The 40 g formulation had a relatively low therapeutic efficacy as indicated by a slow but partial decrease in PASI scores. Although erythema and scaling were decreasing at the end of the treatment period, they were moderate compared to the more concentrated groups. The 40 g formulation suppressed epidermal hyperplasia and inflammatory infiltration to a limited extent, indicating that there was only some suppression of the epidermal cytokine-mediated inflammatory cascade. It means that the concentration of active constituents of the 40 g batch was sufficient to stimulate recovery but was not sufficient to cause strong and rapid reversal of psoriatic pathology. The 80 g preparation resulted in a more therapeutic effect, with strong reductions in the PASI scores starting on Day 4. The treated skin was observed to have significantly better texture, less lesion propagation and observable improvement of surface smoothness. As observed histopathologically, there were significant decreases in acanthosis, parakeratosis, and leukocyte infiltration, with a stronger anti-inflammatory and antiproliferative effect than a 40 g batch. These findings imply that the middle range concentration of phytoconstituents and nanoparticles gave a more effective inhibition of psoriatic pathways, especially in the inhibition of the activation of keratinocytes through IL-17 and restrain the recruitment of immune cells. The most impressive therapeutic effect was found in the 120 g formulation, which led to a near linear decrease in PASI scores with the start of treatment. At the conclusion of the research, the erythema, scaling and thickness severity scores were significantly lower in comparison to all other groups and were close to normal values. The excellent performance of this formulation was also verified with the help of histopathological study that showed near

full recovery of normal epidermal architecture. Epidermal thickness was greatly reduced, keratinocyte proliferation was greatly reduced, and inflammatory cell infiltration was minimal- there was robust signaling pathway modulation of the psoriatic signaling pathways. These results vividly show that the increased bioactive content of the 120 g cream enabled to penetrate and inhibit cytokines implicated in psoriasis, including IL-17A, IL-23, TNF-a and IL-1b more effectively. The dose-dependent enhancement of the formulations is in line with the pharmacological properties of Semecarpus anacardium, whose flavonoids, phenolics, antioxidants, and immunomodulatory compounds have been shown to dampen oxidative stress, inhibit NF-Kb/STAT3 signaling and block proinflammatory cytokines. Moreover, addition of silver nanoparticles probably augmented skin permeation and added anti-inflammatory, antimicrobial, and wound-healing potential and hence, synergistically augmented therapeutic effectiveness. The higher activity of the 120 g formulation hence seems to be due to increased active constituent concentration as well as a better performance of the physicochemical formulation. Combined, the findings indicate that each of the three cream formulations had quantifiable antipsoriatic action, and the extent of therapeutic effect was related to the strength of the formulation. The 120 g cream was the most promising candidate with a high level of clinical, histological and inflammatory remission, and has a chance of being developed further as topical antipsoriatic agent. Subsequent investigations can involve the quantification of cytokine, characterization of the oxidative stress markers, the dermal absorption, and the long-term safety to further characterize the mechanism behind the observed effects.

The ongoing study managed to make Semecarpus anacardium (shodhit nut) extract a powerful, natural, and biocompatible reducing and stabilizing agent in

the green production of silver nanoparticles.s. Phytochemical studies identified the presence of phenolics, flavonoids, tannins, terpenoids and other bioactive compounds which not only supported high antioxidant and anti-inflammatory properties but also the formation of nanoparticle by eco-friendly bioreduction processes. Synthesized AgNPs with UV Vis spectroscopy, FTIR, zeta potential, and particle-size measurements exhibited nanoscale stability that could be used in the topical delivery. These nanoparticles were effectively integrated in an oil-in-water herbal cream base resulting in a pharmaceutically viable formulation with preferred pH, viscosity, spreadability, and stability. The therapeutic potential of the extract and its nanoformulation was proved as biological tests, such as antioxidant tests and mast cell stabilization, showed. The efficacy of the developed formulations was further confirmed in vivo by antipsoriatic studies in the imiquimod-induced BALB/c mouse model, with the 120 g cream showing almost full recovery of normal skin features and massive reduction of inflammatory responses. Altogether, the results provide a solid scientific base of Semecarpus anacardium- mediated green nanotechnology and prove the promising perspective of the developed herbal AgNP cream as a new, safe, and effective topical agent against psoriasis and other inflammatory skin diseases. Future Scope Although the research gives strong evidence of the therapeutic potential of the Semecarpus anacardium based silver nanoparticle cream, more research is needed to develop the formulation to clinical practice. The future research needs to investigate the detailed mechanistic pathways by cytokine profiling, measuring oxidative stress markers and keratinocyte proliferation to gain a clearer understanding of the mechanism underlying its antipsoriatic effect. The use of advanced characterization methods, dermal absorption experiments, and permeation tests will be useful to determine the deposition and distribution of nanoparticles in the skin layers. By further extending the research to other dosage systems like nano-gels, hydrogel, nanoemulsions and transdermal systems, research can improve therapeutic efficacy and patient acceptability. The transition between laboratory synthesis and commercial production will be assisted by long-term ICH stability studies, larger-scale formulation trials, and quality-by-design (QbD) optimization. Safety testing: Dermal toxicity, sensitization, repeated dose, and genotoxicity studies should be conducted to guarantee biocompatibility and compliance with regulations. Finally, to determine the effectiveness of therapeutic performance in a human subject, well-structured clinical trials will be needed. This herbal nano-cream can potentially transform into a safe, natural and commercially viable dermatological product with further optimization and scientific validation to treat psoriasis and other inflammatory skin disorders.

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CONFLICT OF INTEREST: None

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