EVALUATION OF ANALGESIC PROPERTY OF BIO-
SYNTHESIZED AGNPS USING LEAF METHANOL EXTRACT
OF Brugmansia suaveolens

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ABSTRACT

The plant Brugmansia suaveolens (Bercht. & Presl) belongs to the family Solanaceae and is endemic to the Malnad region (Shimoga) of the Western Ghats. Traditionally leaf extract has been applied externally to treat cut wounds, pain-killer swellings, scalds, inflammations, skin rashes, and hemorrhoids. The objective is to synthesize silver nanoparticles (AgNPs) conjugated Leaf methanol extract of B. suaveolens analyzed and characterized based on multiple (X-ray diffraction (XRD); Scanning electron microscopy (SEM); Fourier transform infrared (FTIR) spectroscopy and ultraviolet–visible (UV–Vis) spectroscopy) analytical methods. After characterization, the analgesic activity of extract bio-synthesized with silver nanoparticles was evaluated and compared with different concentrations of leaf methanol extract alone. Analgesic activity was examined by writhing and tail-flick testing. Reduction in the writhing and tail withdrawal time after administration of 250 mg/kg b.w./d of leaf methanol extract bio-synthesized with silver nanoparticle is more significant than 250, 500, and 750 mg/kg b.w./d extracts alone and is slightly less significant when compared to the standard drug. This has proved that the bio-synthesized silver nanoparticles may have played a fundamental role as drug delivery vehicles to target tissues more conveniently and efficiently compared to nascent extract.

Keywords: Brugmansia suaveolens, Analgesic Activity, Silver Nanoparticles, Tail-Flick Testing, Writhing Test.

INTRODUCTION

Traditional plant-based medicines consist of secondary metabolites, which serve as rich sources of biologically active molecules with multiple activities comprising more than 40% of recommended drugs.¹,² It has proven helpful in medicine for its narcotic, analgesic, anti-oxidant, anti-asthmatic, spasmyloytic, and anesthetic qualities.³,⁴ The plant B. suaveolens Bercht. & Presl belongs to Solanaceae, Muccillo-

Baisch et al. (2010) tested the analgesic effectiveness of a B. suaveolens flower aqueous extract in mice.⁶ Steroids, alkaloids, anthraquinone tannins, glycodies, flavonoids, triterpenes, saponins, and phenolic substances were detected in the initial phytochemical screening.⁷ Numerous researchers have documented the secondary metabolite's analgesic properties.⁸ Tannins and terpenoids are essential for analgesic action in addition to saponins and flavonoid compounds.⁹ Flavonoids mainly target prostaglandin synthesis, which is implicated in pain perception, whereas alkaloids are well known for decreasing pain perception.¹⁰ Nanoparticles have a 1–100 nm size with distinctive stability and superior physico-chemical properties and exhibit various biomedical applications.¹¹ AgNPs are nontoxic, with antibacterial properties used for centuries, and can inhibit more than 500 disease-causing micro-organisms.¹² It has significant leverage for many biological ramifications, such as preventing infections, healing wounds, and anti-inflammatory, and is used to treat antibiotic-resistant bacteria.¹³ For silver nanoparticle preparation, while several methods are available, we used biological methods for synthesis.¹⁴ A literature survey led to the unavailability of analgesic activity reports on AgNP synthesis using B. suaveolens leaf methanol extract (LME). Hence, the

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current research work was executed to understand the analgesic activity of synthesized silver (Ag) metal ions using LME.

**EXPERIMENTAL**

**Plant Material Gathering and Leaf Methanol Extract Preparation (LME) and Animal Model**

During the flowering stage, the plant material was collected in October 2018 at Hosanagar Taluk, Shivamogga Dist., Karnataka, India. Prof. Y. L. Krishnamurthy verified the specimen, which was then housed at Kuvempu University's Department of Applied Botany in Shankaraghatta, Shimoga, Karnataka, India. KU/AB/HS/1010 is the voucher number. As shown in Figure 1a & 1b, the shadow-dried leaf plant sample crushed, soxhlet extracted and directed to sequential based on multiple solvents including methanol, petroleum ether and chloroform. It was concentrated using a rotary evaporator and stored in desiccators containing calcium carbonate. LME formulations of *B. suaveolens* (250, 500, and 750 mg/kg bw/d) were prepared for oral administration in 1% DMSO and assessed for analgesic effectiveness alongside the standard drug diclofenac sodium. Swiss albino male mice weighing 30–35 grams were procured and used in the experiment, with Institutional Animal Ethics Committee approval.

**Acute Toxicity Study**

The acute toxicity (LD50 p. o.) of oral *B. suaveolens* LME and LME biosynthesised AgNPs was evaluated in mice. It is considered that there were no observable behavioural alterations or mortality differences between 1000 and 5000 mg/kg bw/d LME and 5000 mg/kg bw/d bio-synthesized AgNPs. As a consequence, the LD50 of manufactured AgNPs was determined to be one-tenth of the 5000 mg/kg bw/d concentration, and 2500 mg/kg bw/d is regarded to be the safe and effective dose for animal survival.

**Biosynthesis of AgNPs**

10 ml of LME was dissolved in aqueous solution containing 1mM silver nitrate. As the LME was added to the aqueous AgNO₃ solution, the alteration in color was observed, resulting the formation of AgNPs. After a 24-hour incubation period, the sample was measured using UV-visible spectrophotometry. The LME solution with AgNPs was centrifuged (4,000 rpm for 15 min) and (at 25,000 rpm for 30 min) consequently to eliminate any insoluble material existing in the solution. The resultant pellet was collected by removing the supernatant, the process was repeated. Finally, in aqueous medium bio-synthesized AgNPs, a reddish-brown color strongly substantiates its synthesis due to excitation surface plasmon vibrations.

**Characterization of Bio-Synthesized AgNPs**

Characterization of bio-synthesized AgNPs was carried out by UV-vis absorption spectroscopy, FTIR, SEM, and XRD. The chemical composition of the bio-synthesized silver nanoparticle was studied using an FTIR spectrometer. The solution was dried at 75 °C, and the dried powder was suspended in KBr for characterization at 400 – 4000 cm⁻¹ wave number. The synthesized silver nanoparticle's morphology and size were investigated using a Zeiss-Ultra 55 model microscope equipped with an energy dispersive X-ray spectroscopy (EDS) with a scan range of 200 nm was used to measure synthesized nanoparticle (IISc, Bangalore). The phase composition of bio-synthesized nanoparticles was recorded by XRD with CuKα radiation at 50 kV and 20 mA at a scan rate of 2 theta (range 10°-80° at a scan rate of 4°/min in the increments of 0.1°, 20).
The Analgesic Property of Bio-Synthesized AgNPs in Mice Model

Abdominal Writhing Test

The test for writhing study was carried out, as stated by Koster et al. (1959). Adult Swiss albino mice (SBM) with 20-25g weight were used to evaluate the LME's analgesic effect; seven groups with six animals. Group I mice got no therapy; Group II mice were given 1% DMSO (10 ml/kg bw/d); Group III mice were given the usual medicine acetylsalicylic acid (10 mg/kg b. w.); and Group IV, V, VI animals were given LME suspended in 1% DMSO at dosages of 250, 500, and 750 mg/kg bw/d, respectively. Group VII was given 250 mg/kg b.w. of bio-synthesized AgNPs orally. Finally, except for Group I, all groups received 0.6% CH₃COOH (10 ml/kg bw. i. p.). After 5 minutes of CH₃COOH delivery, the number of writhing motions was measured consisting of back bending, hind limb extension, and body elongation for 20 minutes. Each group's mean value was computed. A decrease in the number of writhing in the treatment groups (Standard drug/LME/Bio-synthesized nanoparticle) compared to the positive control Group II was regarded evidence of analgesia.

Tail-Flick Test

D'Amour et al. (1941) used the tail flick technique to evaluate the analgesic effect of LME. Seven groups of six SBM weighing 20 -25 g were formed. Group I received no treatment. As a control, Group II mice were administered 1% DMSO (10ml/kg b.w.). Diclofenac sodium (10 mg/kg b. w.) was given orally to Group III. LME dissolved in 1% DMSO was given orally to groups IV, V, and VI at varied doses (250, 500, and 750 mg/kg b.w.), correspondingly. Animals in Group VII were given 250 mg/kg b.w. of biosynthesized AgNPs dissolved in 1% DMSO orally. Later, a two-centimeter-long tagged experimental mouse tail was submerged in Luke warm water (50° C). The pain reaction time was assessed when mice deflected their tails after being immersed in a warm water bath. The initial reading was redundant, later by taking the average of the next two readings, the reaction time was computed. The latent time of the tail-flick response was assessed in intervals (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h) before and after administration of drug as an indication of analgesic activity. The reaction time was limited to 30 minutes and the maximum allowable analgesia (MPA) level were established. The mice swiftly remove their tails in response. A stopwatch is used to time the reaction time (30 minutes). The analgesic activity data was statistically analysed as the mean ± S.E.M of all the six animals in individual group based on one-way ANOVA continued by Turkey's t-test. The variation in values at P≤ 0.01 was found to be statistically important.

RESULTS AND DISCUSSION

Acute Toxicity Study

The acute toxicity (LD₅₀, p.o.) of orally given B. suaveolens LME and AgNPs was studied in mice. From 1000 to 5000 mg/kg b.w. LME and 500 to 2500 mg/kg b.w/d. AgNPs, no obvious behavioural abnormalities or mortality were observed. Hence, this array of LME and AgNPs is a safer dose for further studies. Thereby, LD₅₀ was recorded at 1/10th of the concentration of 5000 to 2500 mg/kg b/w of AgNPs are considered the safe and effective dose for animal survivability.

Biosynthesis of AgNPs

Surface plasmon vibrations cause AgNPs to look reddish brown in aqueous media. An aqueous silver nitrate solution's color changed from light yellowish to brown and finally to colloidal brown upon the addition of LME (Fig.-1c), signifying the formation of bio-synthesized AgNPs. Mechanism of Bio-Synthesized AgNPs

Secondary metabolites found in B. suaveolens LME extract include alkaloids, flavonoids, saponins, terpenoids, polysaccharides, tannins, amino acids, and vitamins. Silver ions become stuck on the surface of alkaloids and are bio-reduced by proteins, resulting in the production of silver nuclei. These silver nuclei aggregate and expand in size, finally becoming AgNPs. These AgNPs were further encapsulated with alkaloids to avoid aggregation and to aid in their stability.

Characterization of Bio-Synthesized AgNPs

By silver nanoparticle nucleation, growth, and particle combination, the linked vibrations of electrons of silver ions caused by resonance with the light wave can give rise to an absorption band. AgNPs have the
maximum UV-visible absorption wavelength of 338-450 nm. The current study discovered an absorption peak about 420 nm, which is characteristic of AgNPs. The peak was discovered after 24 hrs of reaction time, and the absorption band confirmed the poly-dispersity of the nanoparticles. The UV-visible spectrum of bio-synthesized AgNPs is depicted in Fig.-2a and 2b. FTIR was used to examine the bio-synthesized AgNPs. The presence of biomolecule functional groups in LME was detected by FTIR following conjugation with AgNPs, as seen in the spectrum (Fig.-2c). For the functional groups of phenols and alcohols, the absorption bands at 3419 cm⁻¹ are caused by free -OH group stretching. The band at 2937 cm⁻¹ reflects the aldehyde's C-H stretching area. The existence of symmetric stretching of CC in biosynthesized nanoparticle solution is shown by the area of 2100 cm⁻¹. At 1638 cm⁻¹, the C=C stretching (aromatic compound) was seen. The presence of amide I and amide II may be seen in the spectrum between 1600 and 1500 cm⁻¹. The C-O stretching peak is overlaid on the band at 1100 cm⁻¹.

It is observed that the bio-synthesized AgNPs exhibited spherical and cuboidal shape and were well dispersed without any aggregation, as shown in Fig.-3a. The size of the NPs, as assessed using SEM, was 50 - 110 nm. This kind of typical variation (size and shape) in NPs is common in the presence of biological samples used during the synthesis. The particle morphology was determined by scouring the SEM images with a Zeiss-Ultra 55 model microscope fitted out with an energy-dispersive X-ray spectroscopy (EDS) at a scan range of up to 200 nm. As the SEM images revealing the adsorption of LME of *B. suaveolens* on the surface of silver particles being agglomerated with low tap density, the samples were scanned with a magnification range of 1500 X to 5000 X (Fig.-3b, 3c & 3d) with a scale of 50 m to 4 m, and the agglomerated particle size was determined ranging 50 nm to 2.0 m displaying amorphous condition. Nonetheless, the XRD data showed the particle size were in 50 to 111 nm range. as shown in Fig.-3e.

XRD image of biosynthesized AgNPs with LME of *B. suaveolens* is shown in Fig.-3e. The peak positions of 38°, 44°, 65°, 74° and 78° at relative intensities of 150, 115, 80, 70, and 60 from the powder X-ray diffraction pattern attained were paralleled with Joint Committee for Powder Diffraction Standards (JCPDS) library (ICDD 26-0339) to reason for data as reported by Liu *et al.*, (2007). Particle size was
calculated based on Debye-Scherrer’s equation and was estimated, as shown in Table-1. The results uncovered that the average size is found to be 111 nm. Comparative analysis of SEM and XRD results holds good regarding the size of the bio-synthesized silver nanoparticle and found to be in the range of 50 nm to 2.0 µm size.

Table-1: Calculation of AgNPs size of LME of *B. suaveolens* using Scherrer’s Relation

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Peak (2θ)</th>
<th>Wavelength (Angstroms)</th>
<th>Cos θ</th>
<th>Width of baseline β (degree)</th>
<th>Width of baseline β (radians)</th>
<th>Particle Size Dp (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>1.54</td>
<td>0.88</td>
<td>1.5</td>
<td>0.02618</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>0.71</td>
<td>0.42</td>
<td>2.0</td>
<td>0.0349</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>0.27</td>
<td>0.27</td>
<td>2.0</td>
<td>0.0349</td>
<td>94.55</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>0.20</td>
<td>2.0</td>
<td></td>
<td></td>
<td>147</td>
</tr>
<tr>
<td>5</td>
<td>78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>198</td>
</tr>
</tbody>
</table>

Average particle size 111

From the above table, the size of the AgNPs increases with the rise in the peaks this is due to the decrease in the height of the peak.

**The Analgesic Property of Bio-Synthesized AgNPs in Mice Model Abdominal Writhing Test**

The analgesic effect of *B. suaveolens* LME and bio-synthesized AgNPs in mice was evaluated using an acetic acid-induced abdominal writhing test. The number of abdominal writhes seen in the control group during the 20 minutes following intravenous administration of 0.6% acetic acid was 61.36 ± 4.3. However, at the specific dosage (250, 500, and 750 mg/kg b.w.) treatment of LME, a reduction in the number of writhings to 44.25±2.65 57(56%), 27.0±2.85 (63%) and 43.25±4.07 (57%) respectively. While bio-synthesized AgNPs (at the dose of 250 mg/kg b.w.) significantly shortened the writhing numbers, and it is found to be (14.0±0.40 (86 %)) more significant than LME at the dosage of 250 mg/kg bw/d Nevertheless, the effect of bio-synthesized AgNPs was lesser than standard drug acetylsalicylic acid (ASA) (10 mg/kg b. w.) with 9.04 ± .41 writhes eliciting 91% analgesia, as shown in Table-2. It conforms with earlier reports where *B. suaveolens* flowers' aqueous extract produced antinociceptive effects, as demonstrated in mice using the abdominal writhing test and tail flick test.20-21 Our findings conform with earlier reports of Ricciotti and Fitz Gerald (2011)22 inhibiting the cyclooxygenase and/or lipoxygenase enzyme and intervention with peripheral and peritoneal receptors to bring down the pain. However, the higher activity of bio-synthesized AgNPs is probably due to a resultant increase in the surface area of immobilized phytochemicals present on the surface of AgNPs, which may have propelled their activity.

Table-2: Comparative Analysis of Analgesic Activity of LME with AgNPs by Writhing Test

<table>
<thead>
<tr>
<th>Treatment (using Drugs)</th>
<th>Dosage</th>
<th>No. of writhes</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mg/kg (i. p.)</td>
<td>61.36 ± 4.3</td>
<td>-----</td>
</tr>
<tr>
<td>Standard</td>
<td>100 mg/kg (p. o.)</td>
<td>9.04 ± .41</td>
<td>91 %</td>
</tr>
<tr>
<td>LME</td>
<td>250 mg/kg (p. o.)</td>
<td>44.25±2.65**</td>
<td>56 %</td>
</tr>
<tr>
<td></td>
<td>500 mg/kg (p. o.)</td>
<td>27.0±2.85***</td>
<td>63 %</td>
</tr>
<tr>
<td>AgNP's</td>
<td>750 mg/kg (p. o.)</td>
<td>43.25±4.07**</td>
<td>57 %</td>
</tr>
<tr>
<td></td>
<td>250 mg/kg (p. o.)</td>
<td>14.0±0.40***</td>
<td>86 %</td>
</tr>
</tbody>
</table>

**= Non-significant control group; ***= Statistical significance.

In measuring analgesia, CH3COOH-induced writhing is an effective approach, its nociceptive effect may have a role through the release of TNF-a, IL-1, and IL-8 secretion by peritoneal macrophages and mast cells,23 prostaglandin pathways and acid-sensing ion channels.24,25 Acetic acid therapy has been demonstrated to increase prostaglandin E2 and F2 levels in mouse peritoneal fluid.26 The significant pain relief provided by LME may be associated with the phytochemicals (flavonoids & alkaloids) activity against the prostaglandin pathways.

**Tail Flick Test**

In the tail-flick experiment, the impact of LME and bio-synthesized AgNPs in mice was evident for 3 hours, while the control group received 1% DMSO and remained non-toxic during the tail-flick response's latent
phase. The original reading was documented before the administration of LME. After a 3-hour observation period, the impact of LME of *B. suaveolens* at three varied dosages (250, 500, and 750 mg/kg b.w./d) was less significant (P 0.01) than bio-synthesized AgNPs at the dose of 250 mg/kg b.w. The analgesic effect of LME and bio-synthesized AgNPs was recorded every 30 minutes. intervals of time up to 3 hours were found to be evident within 30 min. of the experiment.

The analgesic effect of bio-synthesized AgNPs at 250 mg/kg b.w./d has been observed to be increased from 7.83± 0.72 to 9.5 ± 0.57% within 1.30 hours. Similarly, the analgesic activity of LME at a specific dose of 500 mg/kg b.w./d has increased from 7.66 ± 0.44 to 9.0 ± 0.76 %. While analgesic activity at 250 and 750 mg/kg b.w. doses was (P<0.01) significantly reduced from 5.33 ± 0.44 to 6.5±1.32 and 4.33±0.60 to 6.33±1.92% compared to control group as shown in Table-3.

### Table-3: Comparative Analysis of Analgesic Activity of LME with AgNP’s (Tail Flick Analysis)

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Dosage (p. o.)</th>
<th>0 hr.</th>
<th>30 min.</th>
<th>1 hr.</th>
<th>1.30 hr.</th>
<th>2 hr.</th>
<th>2.30 hr.</th>
<th>3 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1% DMSO</td>
<td>10ml/kg</td>
<td>1.0±0.28</td>
<td>1.83±0.166</td>
<td>2.33±0.60</td>
<td>1.0±0.28</td>
<td>1.16±0.16</td>
<td>1.50±0.28</td>
<td>2.0±0.28</td>
</tr>
<tr>
<td>LME</td>
<td>Standard</td>
<td>8.0±1.04</td>
<td>9.0±0.28</td>
<td>11±0.76</td>
<td>9.66±0.44</td>
<td>8.50±0.57</td>
<td>9.83±0.92</td>
<td>8.16±0.72</td>
</tr>
<tr>
<td></td>
<td>250mg/kg</td>
<td>5.33±0.44</td>
<td>6.33±1.45</td>
<td>7.5±0.57</td>
<td>6.5±1.32</td>
<td>7.33±0.44</td>
<td>6.83±0.44</td>
<td>4.66±0.60</td>
</tr>
<tr>
<td></td>
<td>500mg/kg</td>
<td>7.66±0.44</td>
<td>8.5±0.57</td>
<td>7.66±0.44</td>
<td>9.0±0.76</td>
<td>8.16±0.72</td>
<td>9.50±0.86</td>
<td>8.0±0.28</td>
</tr>
<tr>
<td></td>
<td>750mg/kg</td>
<td>4.33±0.60</td>
<td>6.0±0.57</td>
<td>7.5±0.28</td>
<td>6.33±1.92</td>
<td>6.33±1.69</td>
<td>6.16±1.20</td>
<td>4.83±0.44</td>
</tr>
<tr>
<td>AgNP’s</td>
<td>250mg/kg</td>
<td>7.83±0.72</td>
<td>8.66±0.44</td>
<td>10.5±1.32</td>
<td>9.5±0.57</td>
<td>8.33±0.50</td>
<td>9.66±0.44</td>
<td>8.16±0.44</td>
</tr>
</tbody>
</table>

Values represented are the mean ± S.E.M. of four mice. The symbol represents statistical significance. *p < 0.01, ns-not significant, as compare to control group.

The tail flick test is simple and an important test used to examine centrally acting analgesics, as these molecules could increase pain threshold toward heat. Thermal-induced nociceptive tests are more opioid receptor sensitive. Opioids are G-protein and RGS protein composites that are efficient analgesics for the treatment of pain and associated illnesses. Nonsteroidal analgesic drugs suppress only peripheral-mediated pain and interfere with peritoneal receptors, whereas narcotic analgesics are effective in peripheral and central pain mechanisms inhibition. The presence of opioids and/or opioid mimetics, as well as bioactive substances and secondary metabolites such as polyphenols and flavonoid components, may inhibit pain. A possible analgesic response was seen at 250 mg/kg bw/d bio-synthesized AgNPs compared to 500 mg/kg bw/d LME of *B. suaveolens*, but not at an enhanced concentration of 750 mg/kg b.w. LME extract. Among the 250, 500, and 750 mg/kg b.w. dosages, LME of *B. suaveolens* at 500 mg/kg b.w. demonstrated a significant (P0.01) analgesic response in the writhing and tail flick test, although it was shown to be less effective than the impact of 250 mg bio-synthesized AgNPs. It might be attributed to many bioactive constituents in LME reducing silver ions. At the 500 mg/kg bw/d dose, the LME of *B. suaveolens* displayed considerable peripheral analgesic effect by lowering both (writhes and central analgesic) activities, demonstrating a significant (P0.001) influence on the latent latency of tail-flick response. Bio-synthesized AgNPs at 250 mg/kg b.w. had significant analgesic effects when compared to the three LMEs of *B. suaveolens*.

### CONCLUSION

Bio-synthesized AgNPs showed potent analgesic properties compared to the LME of *B. suaveolens*. It may be due to the stability and enhanced activity of conjugated phytochemicals present in the LME of *B. suaveolens* on the surface of AgNPs.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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