Synthesis of silver and gold nanoparticles from leaf of *Litchi chinensis* and its biological activities

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Objective: To synthesize and isolate silver and gold nanoparticles from *Litchi chinensis* leaf methanolic extract, and to evaluate its comparative biological activities including muscles relaxant, analgesic, anti-inflammatory and antidiarrheal. Methods: The gold and silver nanoparticles were synthesized by dissolving methanolic extract in gold chloride and silver nitrate solution separately which were confirmed by colour change and UV-Vis spectroscopy, and pellets were collected through centrifugation. Biological activities of the extract were conducted on BALB/c mice through various standard methods and the data were subjected to One-way ANOVA. Results: The colorless gold chloride solution changed to purple soon after the addition of plant extract, demonstrating that the reaction took place and gold ions were reduced to gold nanoparticles, while colorless silver nitrate solution changed to light and dark brown that was indicative of silver nanoparticles. The muscles relaxant activity showed that silver nanoparticles were more effective than gold nanoparticles and methanolic extract in traction test. The analgesic activity showed that silver and gold nanoparticles showed highest percentage decrease in acetic acid induced writhing at the doses of 50, 100 and 150 mg/kg b.w. The highest anti-inflammatory activity was produced by gold nanoparticles followed by silver nanoparticles, while low activity was observed in methanolic leaf extract. Only the crude methanolic extract showed significant anti-diarrheal activity as compared to the standard drug atropine sulphate, while anti-diarrheal activities of gold and silver nanoparticles were non-significant. Conclusions: The present work concludes that isolated silver and gold nanoparticles from leaf methanolic extract shows strong muscles relaxant, analgesic and anti-inflammatory activities while crude methanolic extract possesses good antidiarrheal activity.

1. Introduction

Plants are among the most common and accessible sources of potentially active drugs for combating various ailments. Therefore, it is imperative to search biological properties of medicinal plants for the development of new drugs. A lot of work has been done on plants but still there is need to work more in this respect. Different bioassays are suggested for screening out various medicinal plants extracts for different purposes. Nanotechnology is a growing field with significant potential for improvement of human welfare. Nanoparticles and nano-materials...
provide a wide range of constantly increasing applications[1]. Green synthesis of noble metals is important because they are environmentally friendly and are beneficial to human health. Therefore, the biological method has a clear advantage over physical and chemical methods[1]. Biological synthesis of nanoparticles is of great interest to scientists due to the rising need to decrease toxicity, increase renewable resources, and provide clean and environmentally friendly solvents. These concerns have captured the attention of major corporations in the last few decades[1].

Skeletal muscle relaxants are agents that treat both muscle spasm and spasticity, acting as antispasmodic and antispasticity agents respectively. Antispasmodic agents like cyclobenzaprine are commonly used to treat musculoskeletal conditions. Antispasticity agents like dantrolene are used to relieve muscle hypertonicity. However, both agents are used with caution due to their side effects on human health[2].

Pain and inflammation are unpleasant feelings caused by diverse factors disturbing people all over the world[3]. Analgesics are drugs that relief pain. Plants have compounds that show significant analgesic effect by lessening pain sensation and have very minute or no side effects[4]. The non-steroidal anti-inflammatory drugs decrease rheumatism and pain sensation and produce harmful effects like gastrointestinal tract (GIT) ulceration, bleedings[5,6]. Diarrhea is the frequent release of loose watery faecal matter from the body 2-4 times a day due to GIT infection. It is hazardous disease which causes millions of deaths per year worldwide and affects every type of sex and climatic area[7]. Each year more than 4-9 million deaths occur in newborns and small children mostly at the age below seven, it occurs due to unhygienic conditions, contaminated water and starvation[8].

*Litchi chinensis* (L. chinensis) Sonn. Locally known as lychee nut, litchi, lychee is an evergreen tree originated in South China, North Vietnam, and the Malay Peninsula. Now it is currently cultivated in over 20 countries in the tropical and subtropical regions of the world[9]. Litchi flower contain phenols, flavonoids, and condensed tannins, showing a strong antioxidative capacities and anti-inflammatory effect[10]. *L. chinensis* was used as hypoglycemic, anticancer, antibacterial, antihyperlipidemic, antiplatelet, antitussive, antipyretic, diuretic and antiviral activities[11]. The present research was conducted to evaluate *L. chinensis* isolation of silver and gold nanoparticles and also determine it or its muscle relaxant, analgesic, anti-inflammatory and anti-diarrheal potentials.

2. Materials and methods

2.1. Collection of plant parts

*L. chinensis* fresh leaves were collected in April 2016, from botanical garden Islamia College Peshawar. The leaves were detached from branches. The fresh leaves were utilized for macroscopic and microscopic studies. The leaves were dried under the shade for 15 d, then ground into powder. The powder was preserved for the further research work.

2.2. Extraction process

About 400 g of powder was dissolved in 2 L of 95% methanol leaves and placed at room temperature for 7 d. After 7 d, the extract was filtered off through Whatman No. 1 filter paper. The filtrate was evaporated through a rotary vacuum evaporator (R-300 manufactured by Abbas Scientific Pakistan) under reduced pressure below 50 °C. The saturated or thick filtrates were collected in a china dish and let to air dry for entire dissipation of methanol. The extract was stored in refrigerator at 4 °C[12].

2.3. Green synthesis of gold and silver nanoparticles

2.3.1. Preparation of broth

Aqueous extract-broth was prepared by admixing 2 g of leaf extract in 80 mL of distilled water separately in two beakers and well dissolved followed by filtering. The broth was then kept in refrigerator at 4 °C[13,14].

2.3.2. Preparation of solution

For gold nanoparticles of 1 M stock solution of chloroauric acid, 1 g of HAuCl₄ took in 3.3 mL of distilled water and was dissolved well, while for silver nanoparticles AgNO₃ solution was prepared by dissolving 1 g of AgNO₃ in 5.91 mL of distilled water. From this 1 M stock solution was prepared 2 mM solution of gold chloride and silver nitrate in 250 mL Erlenmeyer flask by dissolving 31 µL from stock solution in 99.969 µL of distilled water[15,16].

2.3.3. Synthesis of nanoparticles

In descriptive experiment, 2 mM aqueous chloroauric acid (HAuCl₄) and silver nitrate solution were added to the methanolic extract of leaf in different ratios of 1:1, 1:10, 10:1, 1:2, 1:3, 1:4, 1:5, 5:2, 5:3 and stirred/stimulated on magnetic stirrer continuously for 15-30 min. In leaf extract the reduction of gold ions to gold nanoparticles was centrifuged by Advanced Equipment & Technologies (Pvt) Ltd. Karachi, Pakistan and was completed within 2 h while that of silver nanoparticles in 2, 24 and 48 h. The nanoparticles formation was confirmed by the modification in color visually and by measuring with UV–visible spectrophotometer in the wavelength range 450–800 nm for gold nanoparticles (AuNPs) and wavelength range of 300-500 nm for silver nanoparticles[17].

2.3.4. Collection of nanoparticles

After 24 h, the mixture was subjected to centrifugation at 15 000 rpm for 15 min. The supernatant was throwaway and the pellet was
maintained in centrifuge tubes. The centrifuge tubes were kept in an oven, all night, at 50 °C, to heat and dry the pellet. Using a small spatula, the desiccated pellets were scratched out and the gold and silver nanoparticles were collected and used for various biological activities[17].

2.4. Biological activities

The following biological activities were performed on nanoparticles and crude methanolic leaf extract of L. chinensis using standard methods from the literature.

2.4.1. Muscle relaxant bioassay

The muscles relaxant activity of L. chinensis was carried out using traction standard method of Hosseinzadeh et al[18]. Previous to the experiment the BALB/c mice were kept on fast for 24 h by keeping away from food. After that the mice were divided into 11 groups. Group I was treated with normal saline as negative control, group II with standard drug diazepam as positive control, groups [I] - [V] were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the methanolic extract of L. chinensis and the remaining groups [I] - [V] were treated with silver and gold nanoparticles at the doses of 50, 100 and 150 mg/kg body weight (mg/kg b.w) respectively. Experiment was performed in triplicate for each group. All the doses were applied intra-peritoneally using 1 cc syringes.

For traction method twisted wire was used, which was tightly and straight supported by tops of benches. After that forepaws of the mouse were grasped to wire and allow to hang free, if the mice placed at least grasped their one hind foot within five second, the drug was showed to be muscle relaxant and if the mice dropped on ground and were unable to grasp their hind feet after five second, it was considered as a failure.

2.4.2. Anti-inflammatory activity

The anti-inflammatory activity was carried out using standard method of Ior et al[19]. Previous to the experiment the mice were kept on fast for 24 h. After that the mice were injected by 1% acetic acids intraperitoneally and divided into respective groups Group I - [II]. Diclofenac sodium (+ve control) 10 mg/kg b.w of mice was used as standard drug. Experiment was performed in triplicate for each group. The writhing’s (contraction of abdomen, turning of trunk and extension of hind limbs) that occurred within the next 10 min following acetic acid administration were counted and recorded for 10 min and the result was expressed as percentage inhibition. The percent decrease in writhes was calculated using the following formula of Mujumdar and Misra[20].

\[
\text{Percent inhibition of writhing} = \frac{(A-B)}{A} \times 100 \\
\text{Where A= Mean of writhes in +ve control group, B= Mean number of writhes in tested group.}
\]

2.4.3. Anti-inflammatory activity

Anti-inflammatory activity of methanolic extract of L. chinensis was carried out using standard method of Elaya et al[21]. Before the experiment the mice were kept on fast for 24 h. After that the mice were divided to 11 groups. All the groups were injected with carrageenan 1% suspension in right hind paw of rats to cause oedema. Initial paw volume after swelling was noted using Plythesmometer. After drug administration paw volume was measured for next 1, 2 and 3 h and the decrease or increase in paw volume was examined. Indomethacin 10 mg/kg was used as standard drug.

2.4.4. Antidiarrheal bioassay

The antidiarrheal activity was performed following standard method of Kalriya et al[22]. Previous to the experiment the mice were kept on fast for 24 h. After that the concentrated charcoal solution was administered by oral route to all the animals in each group. After 50 minutes of administration the mice were killed by cervical dislocation, which was ethically approved. After killing mice were dissected and small intestine was removed. The percent charcoal meal inhibition was calculated by dividing charcoal movement to the length of total intestine. Atropine sulphate 10 mg/kg was used as standard drug and positive control.

2.5. Statistical analysis

The data was subjected to statistical analysis, mean±SEM was determined through Microsoft excel version 2016, while One-way ANOVA through IBM SPSS Version22 statistical computer software Manufactured by Microsoft company and for multiple comparison between control and tested treatments, Dunnet test was used. The probabilities \( P<0.05 \) were considered as significant difference and \( P<0.01 \) as highly significant difference[23].

3. Results

3.1. Synthesis result of nanoparticles from L. chinensis leaf methanolic extract

The colorless gold chloride solution changed to purple soon after the addition of plant extract which indicated that the reaction took place and gold ions were reduced to gold nanoparticles while colorless silver nitrate solution changed to light and dark brown which indicated that silver ions were reduced to silver nanoparticles. This was only the visual indication about nanoparticles synthesis, which was further confirmed by UV-Vis spectrophotometry. The maximum absorbance peak was seen at 535 nm for gold and at 410 nm for silver nanoparticles. The gold and silver nanoparticles were collected and used in comparison
with crude methanolic extract of leaf of *L. chinensis* for the following biological activities.

### 3.2. Results of biological activities

Following biological activities were performed on nanoparticles and crude methanolic leaf extract of *L. chinensis* to check their pharmacological potentials.

#### 3.2.1. Muscle relaxant activity

The present result showed that silver nanoparticles had more significant activity as compared to gold nanoparticles and methanolic extract. The silver nanoparticles showed relatively significant (*P*<0.01) activity even at low dose 50 mg/kg b.w, while the gold nanoparticles and the methanolic extract showed highly significant activities at high doses 100 and 150 mg/kg b.w as well as 400 mg/kg b.w, respectively (Table 1).

The percent increase in grasping time showed a dose dependent activity *i.e.* the effect increased with increasing doses in all the test samples. The silver nanoparticles represented highest effect as it increased grasping time followed by the gold nanoparticles at the respective doses, while the leaf methanolic extract exhibited low effect as compared to the standard drug Diazepam which showed 85.85% increase in grasping time (Table 1).

#### 3.2.2. Analgesic activity

The percent inhibition of acetic acid induced writhing showed dose dependent result as the diclofenac sodium inhibited writhing up to 75.00% methanolic extract inhibited writhing up to 42.30%, 58.00% and 69.33% at the respective doses of 100, 200 and 400 mg/kg b.w, while the silver and gold nanoparticles inhibited writhing (45.23%, 60.00% and 71.50%) and (48.02%, 64.30% and 74.44%) at the doses of 50, 100 and 150 mg/kg b.w respectively (Table 2).

#### Table 2

| Treatment                        | Dose       | Number of writhing |
|----------------------------------|------------|--------------------|
| Normal saline                    | -          | 72.44±2.345        |
| Diazepam                         | 10 mg/kg b.w | 17.66±3.844       |
| Methanolic extracts              | 100 mg/kg b.w | 42.33±5.487       |
|                                 | 200 mg/kg b.w | 30.33±3.480       |
|                                 | 400 mg/kg b.w | 22.00±7.506       |
| Silver nanoparticles             | 50 mg/kg b.w | 39.66±8.090       |
|                                 | 100 mg/kg b.w | 28.33±2.906       |
|                                 | 150 mg/kg b.w | 21.33±5.667       |
| Gold nanoparticles               | 50 mg/kg b.w | 37.66±7.265       |
|                                 | 100 mg/kg b.w | 26.00±4.726       |
|                                 | 150 mg/kg b.w | 19.33±9.528       |

Values are presented as mean±SEM for group of six animals. The data was analyzed by one-way ANOVA followed by Dunnett’s test. *Significant at *P*<0.05, **Highly significant at *P*<0.01.

3.2.3. Anti-inflammatory activity

Among all the groups, the gold nanoparticles showed highest effect as compared to the methanolic extract and silver nanoparticles. ANOVA showed that the gold nanoparticles were highly significant (*P*<0.01) at very low dose and after one hour of drug administration. The silver nanoparticles were highly significant at high doses 100 and 150 mg/kg after two and three hours of drug administration and was significant at low dose 50 mg/kg as compared to standard drug Indomethacin, while the methanolic extract was nonsignificant at low dose 100 mg/kg, while highly significant at 400 mg/kg. The percent % decrease in paw volume showed dose dependent results as the most significant effect was observed in gold nanoparticle at the highest dose followed by the silver nanoparticles and methanolic extract (Table 3).

3.2.4. Antispasmodic activity

In the present bioassay, the antidiarrheal of leaf methanolic extracts inhibited (reduced) the percent charcoal motility to 56.66% and 74.55% respectively at higher doses (150 and 200 mg/kg b.w), while silver nanoparticles exhibited 37.4% and 56.77% and gold nanoparticles 21.33% and 24.44% reduction in charcoal meal motility at 100 and 150 mg/kg b.w doses. The one-way ANOVA showed that the effect of leaf methanolic extracts enhanced with gradually increased dose and produced a significant activity.

### Table 1

Percent effect of leaf methanolic extract and nanoparticles of *L. chinensis* on muscle relaxation (traction) in mice.

| Groups                      | Dose       | Traction test (%) | Percent increase in grasp time (%) |
|-----------------------------|------------|-------------------|-----------------------------------|
| Normal saline               | 10 mg/kg   | 15.20±1.330       | 85.85                             |
| Diazepam                    | 1 mg/kg    | 13.00±0.577       | 85.85                             |
| Methanolic extracts         | 100 mg/kg b.w | 8.00±1.732       | 52.60                             |
|                             | 200 mg/kg b.w | 8.66±0.882       | 56.10                             |
|                             | 400 mg/kg b.w | 12.00±0.577      | 78.90                             |
| Silver nanoparticles        | 50 mg/kg b.w | 9.00±1.155       | 59.20                             |
|                             | 100 mg/kg b.w | 10.00±0.577      | 65.70                             |
| Gold nanoparticles          | 50 mg/kg b.w | 12.66±0.667      | 82.84                             |
|                             | 150 mg/kg b.w | 11.00±0.577      | 72.30                             |
|                             | 150 mg/kg b.w | 12.33±0.333      | 80.90                             |

Values are presented as mean±SEM for group of six animals. The data was analyzed by one-way ANOVA followed by Dunnett’s test. *Significant at *P*<0.05, **Highly significant at *P*<0.01.
In the present study, color change indicated presence of silver nanoparticles, which was further confirmed by UV-Vis spectrophotometry. The gold and silver nanoparticles were solidified, collected and used in comparison with crude methanolic extract of leaf of *L. chinensis* for the following biological activities.

Gold nanoparticles have many applications in biomedical sciences including drug delivery, tissue/tumor imaging photo thermal therapy and immune-chromatographic identification of pathogens in clinical specimens. Silver nanoparticles are widely utilized for diagnosis and management of diseases such as cancers, genetic and infectious diseases etc. They are utilized for elimination of microorganisms on industrial scale[25]. Lots of investigators such as Ripa et al[4]; Rang et al[6] and Taufikurohmah et al[9] manufactured gold and silver nanoparticles using plant extracts synthesized from *Aloe vera*, *Couroupita guianensis* and *Rosa rugosa* respectively.

In addition, four pharmacological activities of gold and silver nanoparticles have been studied in the present study. The result showed that silver nanoparticles display more significant muscle relaxant activity as compared to gold nanoparticle and methanolic extract. The silver nanoparticle shows highly significant (P<0.01) activity even at low dose 50 mg/kg, while the gold nanoparticle was highly significant only at high doses 100 and 150 mg/kg and the methanolic extract was highly significant only at highest dose 400 mg/kg.

Similar researches were carried out by Elaya et al[21] and Kalriya et al[22] for *Acorus calamus*, *Colocasia esculenta* and recorded that these plants have good skeletal muscle relaxant activity. Srikant and Muralidharan[23] investigated the muscle relaxant activity of methanolic extract of pericarp of *Sapindus marginatus* (Sapindaceae) in Swiss albino mice and revealed that the methanol...
extract caused reduction in muscle relaxant activity in traction tests. Prakash and Kuppat[24] studied the alcoholic and aqueous extracts of *Cardiospermum halycaelum* and *Dodonaea viscosa*, family Sapindaceae for muscles relaxant activity. The result revealed that motor incoordination activity exhibited by the extracts. Same results were obtained by Ripa et al[25] for methanol extracts of leaf of *Nephelium longan* at doses of 250 and 500 mg/kg b.w of rats. The use of modern synthetic drugs as muscle relaxant agents heralds a number of complications. The use of cyclobenzaprine causes confusion, lethargy and anticholinergic. Dantrolene causes severe allergic reactions such as rash, hives, itching and breathing complications. The drug Tizanidine is considered responsible for more serious situations like lowering blood pressure, heart problems and paralysis[26]. It is proved natural plant derived drugs have no side effect and also cost effective. The above mention researchers are in analogy with the present work. Hence it is suggested that *L. chinensis* should be used as muscle relaxing agent. And the specific response substances should be identified and isolated from *L. chinensis*.

Plants have compounds that prove important analgesic effect by lessening pain sensation and have very minute or no side effects[27]. The analgesic activity of *L. chinensis* against BALB/c mice in acetic acid induced writhing test showed that among all the extracts the gold nanoparticles were most significant.

The comparison of the present work with various earlier researchers on different plants strengthens these present findings having similar results. Kaliya et al[22] reported the analgesic activity of various extracts of three major species of *Sapindus* (Sapindaceae) which are one American species, *Sapindus saponaria* and two Asian species, *Sapindus mukorossi* and *Sapindus trifoliatus* and concluded that only the methanolic extract showed analgesic activity. Ripa et al[25] reported methanolic extract of leaf of *Nephelium longan* (Sapindaceae) showed significant (P<0.01) inhibition of acetic acid induced writhing at 37.4% and 54.43%, 36.075% and 52.53%. Ior et al[19] investigated ethanolic extract of the leaves of *Paullinia pinnata* and revealed maximum inhibition by 74.6% and 83.8% acetic acid induced writhing at dose of 200 mg/kg and 400 mg/kg significantly (P<0.05) reduced the induced paw edema in rats. Various researchers such as Reddy et al[31], Kumar et al[5] and Ali et al[32] reported anti-inflammatory activity of *Typhonium trilobatum* L. Schott; *Amorphophallus bulbifer* and *Pistia stratiotes* and suggested these plants as anti-inflammatory agents.

Besra et al[33] tested leaf of *Serjania lethalis* and *Cupania verinalis* (Sapindaceae) and reported that these plants contained active compounds at 50 mg/kg of extract and used as anti-inflammatory agents. Ior et al[19] investigated anti-inflammatory activity of ethanolic extract of *Paullinia pinnata* leaves and reported that the extract at doses of 200 mg/kg and 400 mg/kg significantly (P<0.05) reduced the induced paw edema in rats. Various researchers such as Reddy et al[31], Kumar et al[5] and Ali et al[32] reported anti-inflammatory activity of *Typhonium trilobatum* L., *Amorphophallus bulbifer* and *Pistia stratiotes* and suggested that this potential of these plants could be assumed to be related to high levels of phenolic compounds, e.g., flavonoids, present in these plants. Saidu et al[34] worked on leaf methanolic extracts of *Erythrina senegalensis* and reported significant (P<0.05) anti-inflammatory activity at low doses while highly significant results at high doses. These studies strongly support present work. Hence, in the comparison and equivalence of these workers, our results also suggested that the methanolic extract of *L. chinensis* as well as silver and gold nanoparticle possess a good anti-inflammatory activity. Hence it should be further explored and the respective compounds should be isolated and characterized.

Diarrhea is the numerous release of loose watery faecal matter from the body 2-4 times a day due to GIT infection. It is hazardous disease-causing millions of deaths per year worldwide and affects every type of sex and climatic area[35]. In the present study, one-way ANOVA showed that leaf methanolic extract were highly significant (P<0.01) at higher doses as compared to the silver and gold nanoparticles. Various other researchers also reported similar activity like Yakubu and Salimon[10] who studied anti-diarrheal activity of *Mangifera indica* that was comparable with standard drug loperamide. Abubakar et al[12] reported a significant anti-diarrheal activity of crude aqueous and diethyl ether saponin and flavonoid fractions of the leaf of *Anacardium occidentale*. These reports provide a strong support to our present findings.
Other plants have also been reported to have anti-diarrheal effects. Suleiman et al.[35] studied anti-diarrheal bioassay of *Ammorna senegalensis*. Senwal et al.[36] reported *Cissampelo pareira* possesses significant anti-diarrheal potential. Al-Snafi[37]; Paul et al.[38]; Shrinivas et al.[39]; Rahman et al.[40] reported that various extracts of plants *Benincia sahisipida*, *Alpinia conchigera*, *Dillenia indica*, *Cyperus tegetum* and *Holoptelea integrifolia* significantly reduced the charcoal induced gastro intestinal motility in Swiss albino mice.

Several other researchers also agree with us as Schum et al.[41]; Bhogaonkar et al.[42] reported significant antispasmodic activities of various plants like *Symplocos paniculata*, *Myrtus communis*, *Swertia chirata*, *Manilkara zapota*, *Cynanchum viminale* and *Withania somnifera* in custard oil induced diarrhea in mice. Hence in analogy with these workers our current research suggested that the *L. chinensis* possesses a natural significant anti-diarrheal potential.

In conclusion, gold and silver nanoparticles from methanolic extract of *L. chinensis* leaf were synthesized and isolated. The comparative pharmacological test showed that nanoparticles exhibited strong muscles relaxant, analgesic and anti-inflammatory activities while crude methanolic extracts possess good anti-diarrheal activity.

The results of silver and gold nanoparticle showed good pharmacological activities hence it is suggested that the plant should be explored in future for isolation, quantification and identification of active phytoconstituents responsible for specific effect and will be good source for their pharmacological amplification and an inexpensive effective remedy for various diseases and ailments. Conservation measures are adapted for a long term sustainable use of this valuable medicinal plant, which will also be helpful uplifting economic conditions of local inhabitants.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

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