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

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ABSTRACT

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 antidiarrheal activity as compared to the standard drug atropine sulphate, while antidiarrheal 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

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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, bleeding[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 antidiarrheal 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 ℃. 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 ℃[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 ℃[13,14].

2.3.2. Preparation of solution

For gold nanoparticles of 1 M stock solution of chloroauric acid, 1 g of $HAuCl_4$ took in 3.3 mL of distilled water and was dissolved well, while for silver nanoparticles $AgNO_3$ solution was prepared by dissolving 1 g of $AgNO_3$ 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 this 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 ($HAuCl_4$) 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 ℃, 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 Ⅲ-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 Ⅵ-Ⅺ 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 droped on ground and were unable to grasp their hind feet after five second, it was considered as a failure.

2.4.2. Analgesic activity

The antispasmodic activity was performed following 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-Ⅺ. 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 Misar[20].

Percent inhibition of writhing = (A-B)/A×100

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).

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 (Ⅰ)	10 mg/kg	15.200±1.330	
Diazepam (Ⅱ)	1 mg/kg	13.000±0.577	85.85
Methanolic extracts	100 mg/kg (Ⅲ)	8.000±1.732	52.60
	200 mg/kg (Ⅳ)	8.667±0.882*	56.10
	400 mg/kg (Ⅴ)	12.000±0.577**	78.90
Silver nanoparticles	50 mg/kg b.w (Ⅵ)	9.000±1.155**	59.20
	100 mg/kg b.w (Ⅶ)	10.000±0.577**	65.70
	150 mg/kg b.w (Ⅷ)	12.667±0.667**	82.84
Gold nanoparticles	50 mg/kg b.w (Ⅸ)	7.667±0.882	50.00
	100 mg/kg b.w (Ⅹ)	11.000±0.577**	72.30
	150 mg/kg b.w (Ⅺ)	12.333±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.

3.2.2. Analgesic activity

The analgesic activity showed that among all the groups the gold nanoparticles were most significant. The gold nanoparticles showed significant result (P<0.05) at low dose 50 mg/kg b.w, while highly significant (P<0.01) at high doses 100 and 150 mg/kg b.w. The silver nanoparticles and methanolic extracts demonstrated significant effect at high doses, while at low dose produced non-significant (P>0.05) effect (Table 2).

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

Analgesic activity of leaf methanolic extract and nanoparticle of *L. chinensis*.

Treatment	Dose	Number of writhing
Normal saline (Ⅰ)	-	72.440±2.345
Diclofenac sodium (Ⅱ)	10 mg/kg b.w	17.667±3.844
Methanolic extracts	100 mg/kg (Ⅲ)	42.333±5.487
	200 mg/kg (Ⅳ)	30.333±3.480*
	400 mg/kg (Ⅴ)	22.000±7.506**
Silver nanoparticles	50 mg/kg b.w (Ⅵ)	39.667±8.090
	100 mg/kg b.w (Ⅶ)	28.333±2.906*
	150 mg/kg b.w (Ⅷ)	21.333±5.667**
Gold nanoparticles	50 mg/kg b.w (Ⅸ)	37.667±7.265*
	100 mg/kg b.w (Ⅹ)	26.000±4.726**
	150 mg/kg b.w (Ⅺ)	19.333±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 non-significant 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 3

Anti-inflammatory activity of leaf methanolic extract and nanoparticle of *L. chinensis*.

Groups	Dose (mg/kg)	Normal paw volume	Carrageenan injected paw volume	Paw volume after drug administration			% decrease in paw volume after 3 h
				After 1 h	After 2 h	After 3 h	
Normal saline (Ⅰ)	-	0.163±0.015	0.197±0.016	0.207±0.011	0.317±0.016	0.330±0.002	-
Indomethacin (Ⅱ)	10 mg/kg	0.165±0.008	0.197±0.008	0.215±0.008	0.225±0.008	0.095±0.008	72.72
Methanolic extracts	100 mg/kg (Ⅲ)	0.138±0.006	0.212±0.006	0.223±0.007	0.230±0.004	0.230±0.004	33.33
	200 mg/kg (Ⅳ)	0.128±0.007	0.182±0.011	0.208±0.009	0.222±0.007*	0.180±0.007*	42.30
	400 mg/kg (Ⅴ)	0.145±0.008	0.205±0.008	0.188±0.007*	0.197±0.006**	0.150±0.056**	57.77
Silver nanoparticles	50 mg/kg b.w (Ⅵ)	0.145±0.008	0.215±0.008	0.213±0.007**	0.207±0.006*	0.127±0.056*	62.44
	100 mg/kg b.w (Ⅶ)	0.153±0.007	0.182±0.006	0.203±0.007	0.223±0.007**	0.117±0.007**	65.60
	150 mg/kg b.w (Ⅷ)	0.142±0.009	0.215±0.008	0.210±0.004	0.218±0.006**	0.093±0.006**	71.22
Gold nanoparticles	50 mg/kg b.w (Ⅸ)	0.150±0.009	0.218±0.006	0.212±0.003**	0.222±0.009**	0.103±0.009**	63.55
	100 mg/kg b.w (Ⅹ)	0.142±0.009	0.195±0.004	0.210±0.007*	0.218±0.011**	0.092±0.008**	70.70
	150 mg/kg b.w (Ⅺ)	0.137±0.009	0.203±0.009	0.217±0.007**	0.227±0.002**	0.088±0.011**	73.50

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 $P<0.01$.

Table 4

Antispasmodic of leaf methanolic extract and nanoparticle of *L. chinensis*.

Treatment	Dose	Total length of intestine (cm)	% inhibition of charcoal	% charcoal motility inhibition
Normal saline (Ⅰ)	10 mL/kg	57.440±1.440	11.330±1.440	-
Atropine sulphate (Ⅱ)	10 mg/kg	52.667±2.333	1.667±0.333	85.33
Methanolic extracts	100 mg/kg (Ⅲ)	51.667±4.842	7.000±1.155*	38.22
	200 mg/kg (Ⅳ)	48.667±2.028	5.000±0.577**	56.66
	400 mg/kg (Ⅴ)	48.667±2.728	3.000±0.577**	74.55
Silver nanoparticles	50 mg/kg b.w (Ⅵ)	53.333±1.856	8.667±0.882**	24.55
	100 mg/kg b.w (Ⅶ)	51.000±2.082	7.500±0.764	37.40
	150 mg/kg b.w (Ⅷ)	51.000±2.887	5.333±0.882	56.77
Gold nanoparticles	50 mg/kg b.w (Ⅸ)	50.667±2.603	10.000±1.202	17.00
	100 mg/kg b.w (Ⅹ)	49.000±1.732	9.467±0.555	21.33
	150 mg/kg b.w (Ⅺ)	53.667±3.180	8.685±0.456	24.44

Values are reported 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 $P<0.01$.

($P<0.05$) at low dose 100 mg/kg while highly significant ($P<0.01$) at higher doses 150 and 200 mg/kg as compared to the Atrophine sulpahte. However, silver and gold nanoparticles produced non-significant activity (Table 4).

4. Discussion

The nanotechnology is an advance field and the nanoparticles have significant uses in the field of high sensitivity bio molecular detection, therapeutics, diagnostics, catalysis, micro-electronics and have possible uses as an antimicrobial agent[24].

In the present study, color change indicated presence of silver and gold 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. Srikanth and Muralidharan[23] investigated the muscle relaxant activity of methanolic extract of pericarp of *Sapinduse marginatus* (Sapindaceae) in Swiss albino mice and revealed that the methanol

extract caused reduction in muscle relaxant activity in traction tests. Prakash and Kuppast[24] studied the alcoholic and aqueous extracts of *Cardiospermum halicacabum* and *Dodonea 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. Kalriya *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 S*apindus 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 as 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. Other several plants have been reported to have analgesic effect like Nisar *et al*[27] who documented that *Taxus wallichiana* extracts significantly (P<0.05) showed analgesic effect. Roslida *et al*[28] reported that the *Pluchea indica* extracts have the potential to treat analgesia. Various other researchers like Amresh *et al*[29] carried out similar work on several medicinal plants like *Portulaca oleracea*, reported similar observation and suggested that these plants have analgesic properties due to presence of phytoconstituents like alkaloids, flavonoids phenols *etc*, which are actually pain-relieving agents and are responsible for such effect. The results of these workers strongly support this present work. The non-steroidal anti-inflammatory drugs decrease rheumatism and pain sensation producing harmful effects like GIT ulceration and bleeding. Phytochemicals derived from any parts of the plant are considered to be a new precious source of analgesic, anti-depressant and anti-inflammatory agents[30].

Inflammation is a complicated biotic reaction of vascular tissues beside hostile broker such as pathogens, damaged cells, irritants or pathogens. The typical signs of redness are showed through enhanced blood stream, vasodilatation, raised cellular metabolism, ease dissolvable intermediators, cellular inflow and extravasation of fluids[31].

In the present observation gold nanoparticles showed good anti-inflammatory effect followed by the silver nanoparticles and then methanolic extract. Several other researchers such as Reddy *et al*[31]; Kumar *et al*[5] and Ali *et al*[32] reported good anti-inflammatory activities of plant like *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 vernalis* (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 antidiarrheal activity of *Mangifera indica* that was comparable with standard drug loperamide. Abubakar *et al*[12] reported a significant antidiarrheal 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 antidiarrheal effects. Suleiman *et al*[35] studied antidiarrheal bioassay of *Annona senegalensis*. Semwal *et al*[36] reported *Cissampelo spareira* possesses significant antidiarrheal potential. Al-Snafi[37]; Paul *et al*[38]; Shrinivas *et al*[39]; Rahman *et al*[40] reported that various extracts of plants *Beninca sahispida*, *Alpinia conchigera*, *Dillenia indica*, *Cyperus tegetm* 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 *Symploco spaniculata*, *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 antidiarrheal potential.

In conclusion, gold and silver nanoparticles from methanolic extract of *L. chinensis l*eaf 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 antidiarrheal 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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