International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 9 (5): 220-234, 2015
ISSN 1819-155X / DOI: 10.3923/ijbc.2015.220.234

Green Synthesis of Gold Nanoparticles Using *Hypericum hookerianum* and its Antiparkinson like Effect in Haloperidol Induced Swiss Albino Mice

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ABSTRACT

Gold has been used as antidepressants in Indian traditional medicine. *Hypericum* species are used as alternative to conventional medicines for the treatment of neurodegenerative diseases. The present study focuses on synthesis and characterization of gold nanoparticles (AuNPs) using *Hypericum hookerianum* and its antiparkinson like effect in haloperidol (1 mg kg^{-1}; ip) induced swiss albino mice. The synthesized AuNPs were characterized by ultraviolet-visible spectroscopy, scanning electron microscopy; energy dispersive X-ray spectrum and fourier transform infrared spectroscopy. The parkinson induced mice were treated with ethanolic extract of *H. hookerianum* (EEHH) (400 mg kg^{-1}) and *H. hookerianum* synthesized gold nanoparticles (HHGNps) (40 mg kg^{-1}). Behavioral analyses of mice were examined by rota rod test, gait analysis, wire hang test and their neurobiochemical analysis (dopamine and glutamate) by spectrofluorimetry. In this study, the haloperidol induced group showed depleted dopamine and increased glutamate levels, whereas treated groups exhibited significantly restored values. Among the extracts, EEHH showed well pronounced antiparkinson like effect due to its neuroprotective flavonoids and surprisingly HHGNps treated groups showed greater antiparkinson like effect. The green synthesis of AuNPs showed promising effects for the modern therapeutic era especially with regard to parkinson's disease.

Key words: Rota rod test, gait analysis, dopamine, glutamate

INTRODUCTION

Parkinson's Disease (PD) is a persistent neurological disorder. Degeneration of dopaminergic neurons in ventral midbrain, particularly in the substantia nigra portion, which causes a subsequent reduction of dopamine (DA) levels in the striatum. The imbalance in the functions of acetylcholine and dopaminergic neurons in the striatum leads to PD. The PD patients show symptoms like tremor, myotonia, dyskinesia and motor dysfunction (Liu *et al*., 2012). Recent developments that have led to evolution in the medical management of PD are to: (1) Improve the dopaminergic therapies, (2) Identification of non-dopaminergic drugs for symptomatic improvement and (3) Discovery of novel compounds to positively alter the course of PD. The pre-clinical study models of PD (biochemical, cellular and animal) have contributed much to the understanding of PD in human (Schapira *et al*., 2006).

Nanotechnology is an upcoming area of research associated with assembly of nanoparticles of variable size (1-100 nm), shape and chemical composition, with controlled dispersity, as well as their promised benefits in clinical findings (Andeani *et al*., 2011). Across the globe, nanoparticles have been extensively used in various applications, including drug discovery (Liversidge and Cundy, 1995) and tissue engineering (Langer, 2000). Gold nanoparticles have an emergent role in medical biotechnology (Song *et al*., 2009) and production of nanoparticles can be achieved mainly through chemical, physical and biological methods. Natural methods for nanoparticle synthesis using plants or plant extracts have been suggested as possible alternatives for disease treatments when compared to chemical and physical methods (Willner *et al*., 2006). Green synthesis of nanoparticles is a representative juncture of nanotechnology and biotechnology and received much attention due to the growing need to develop ecofriendly technologies for nanoparticle synthesis (Sathishkumar *et al*., 2009).

Herbal medicines play pivotal role in the treatment of neurodegenerative disorders for their minimal side effects with more beneficiary profiles. Advanced tool of green synthesis of gold nanoparticles with medicinal plants will fulfill the emerging needs of PD treatment with most successful events. According to ancient era pharmacopeias, gold is mainly used in anti aging and brain cell (neurons) nourishing.

Hypericum hookerianum is a small woody yellow flowered shrub in India, it is mainly available in the high altitudes of hills and commonly known as the Golden Lotus of *H. perforatum*. These *Hypericum* species are mainly used for the treatment of neurodegenerative diseases. Among 20 different species found in India, *H. hookerianum* wight and Arnott is identified as a well known ornamental plant. Different extracts of *H. hookerianum* have already been reported to have antitumor (Dongre *et al*., 2007), antibacterial (Mukherjee *et al*., 2001) wound healing (Mukherjee and Suresh, 2000; Mukherjee *et al*., 2000) and anxiolytic (Subakanmani and Umadevi, 2012) activities. Due to the neuroprotective potential of *Hypericum* species and gold in the treatment of neurological disorders and anti- aging mechanisms, the present study investigated and compared the antiparkinson like effect of EEHH and HHGNps in haloperidol induced Swiss albino mice, based on behavioral and neuro biochemical parameters.

MATERIALS AND METHODS

Chemicals: Haloperidol 4-[4-(4-chlorophenyl)-4-hydroxy piperidinol]-4'-fluorobutyrophenone, chloro aurate ($HAuCl_4$), Thio Barbituric Acid (TBA), reduced glutathione and 3, 5-dithio-bisnitrobenzoic acid were purchased from Sigma Aldrich, Bangalore, India. All other chemicals were of analytical grade.

Collection and validation of plant samples: *Hypericum hookerianum* is a yellow flowered woody shrub which was collected from Western Ghats of Nilgiris, South India and botanically authenticated by Dr. S. Rajan, Field Botanist, Department of Ayush, Government of India, Ooty, South India. The plant parts were air-dried at room temperature, finely powdered, pulverized, sieved and stored in airtight container for further studies.

Preparation of plant extract: The shade dried aerial parts of *H. hookerianum* was subjected to extraction with petroleum ether, chloroform and ethanol successively by soxhlation method at room temperature and concentrated over water bath. The ethanolic extract obtained was filtered and the solvent was evaporated at 50°C under reduced pressure and then lyophilized.

Green synthesis of gold nanoparticles from the ethanolic extract of *H. hookerianum* (HHGNps) Exactly 20 mL of EEHH (100 mg) supernatant was added to 1 mM (80 mL) chloro auric acid solution in a 250 mL Erlenmeyer flask. About 95% bioreduction of $AuCl_{4^-}$ ions occurred within 30 min and the yellow colored solution which turned to ruby red slowly indicated the formation of gold nanoparticles (Prakash *et al*., 2010). The bioreduction of gold ions was monitored periodically using UV- Vis spectroscopy (200-700 nm). The gold nanoparticles obtained from EEHH were purified by repeated centrifugation (12000 rpm) and the pellet was collected. The structure, size and reduction of functional groups were studied by Scanning Electron Microscopy (SEM), Energy Dispersive X-ray spectroscopy (EDAX) and Fourier transform Infra Red spectroscopy (FTIR) respectively.

Characterization of HHGNps

UV-Vis spectra analysis: The reduction of $AuCl_{4^-}$ was confirmed by using UV-V is spectroscopy (Hitachi u-2910 spectrophotometer, Japan) in the range from 200-700 nm with periodical time intervals by diluting a small aliquot (100 µL) of the sample (10-fold) in double distilled water.

SEM analysis and EDAX analysis: The purified HHGNps powder was characterized for nanoparticles size and shape by scanning electron microscope (JEOL-JSM 6390, Japan). The EDAX analysis was carried out to confirm the presence of elemental gold in the HHGNps.

FTIR analysis of EEHH and HHGNps: To identify the functional groups of bioactive constituents in EEHH and to recognize the specific functional group responsible for the formation of gold nanoparticles in HHGNps were confirmed by FTIR analysis (FTIR Shimadzu 8400s, Japan) within the range of 400-4000 cm^{-1}. The FTIR analysis of EEHH and HHGNps was performed with KBr pellets at PSG College of Arts and Science, Coimbatore, South India. The various modes of vibrations were identified and assigned to determine the different functional groups present in the EEHH and HHGNps.

Antiparkinson like effect of EEHH and HHGNps

Experimental animals: Swiss albino mice of either sex, weighing 20-30 g were used in the present study and they were obtained from the Small Animal Breeding Station, Agricultural University (Mannuthy, Thrissur, Kerala, South India). They were housed individually with *ad libitum* access to food and water under controlled laboratory conditions and exposed to a 12 h cycle of light and dark. All the observations were made at room temperature in a noiseless diffusely illuminated experimental room between 9.00-17.00 h. All the experimental protocols were approved by the Institutional Animals Ethics Committee (IAEC) as per provisions of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India (KMCRET/PhD/11/2011).

Gait analysis: To measure gait, mice were trained to walk through a narrow alley directing into their home cage. Once trained, paper was placed along the alley floor and each mice forelimb and hind limbs were brushed with non toxic blue ink. The animals subsequently placed at the beginning of the alley. As they walked into their home cage, they left their paw prints on the paper underneath. Stride length was calculated by measuring the space between paw prints. Only strides made continuously while walking (no stopping) were included in the analysis. Stride lengths at the

beginning and the end of the alley was not counted because animals usually make irregular steps at the beginning and typically stop and make smaller steps just before entering the cage. Stride width was calculated by measuring the distance between the hind limbs (calculated by subtracting the shortest stride length from the longest stride length). Distance between fore paw and hind paw length and stance width was also calculated (D'Hooge *et al*., 1999).

Wire hang test (Muscular traction test): Muscular traction of experimental mice was performed by placing the forepaws of the animals in a small twisted wire rigidly supported above a bench top. Normally, an experimental mouse grasps the wire with the forepaws and place at least one hind foot on the wire within 5 sec when permitted to hang freely. Inability to put up at least one hind foot is considered as a failure in the traction test (Turner, 1965).

Rota rod test: A custom build rota rod (Medicraft Rota Rod, Model No.519/E-2C, S.NO: MRA-036, Medicraft electro medicals (P) Ltd., Lucknow) consists of a rotating spindle (diameter 7.3 cm) and individual compartments for each mouse with varying rotational speeds. Initially, animals were trained for four training sessions on succeeding days (each constituted by a maximum of 10 trials) to achieve the maximal performance. The animals were exposed individually on a rotating rod at 5-25 rpm (speed of rotation) at 5 min intervals with a cut off time of 180 sec. The average retention time and grip strength of muscle coordination on the rod was calculated (Rozas *et al*., 1997).

Estimation of lipid per oxidation levels: At the end of the experimental period, whole brain of the experimental mice was dissected out and weighed and 10% tissue homogenate was prepared with 0.025 M Tris-HCl buffer (pH-7.5). After centrifugation at 10,000 rpm for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances (TBARS). The quantitative measurement of lipid per oxidation in the whole brain was assessed as per the method previously described by Ohkawa *et al.* (1979).

Estimation of dopamine brain tissue extraction: At the end of the experimental period, whole brain of the experimental mice was dissected out and separated the sub cortical region (including the striatum). A specific amount of tissue was weighed and homogenized (Remi Homogenizer, Mumbai, India) in 3 mL of HCl and butanol (1:1) in a cold condition. The samples were then centrifuged at 2000 rpm for 10 min. Exactly 0.8 mL of supernatant was removed and added to a micro centrifuge tube containing 2 mL of heptane and 0.25 mL of 0.1 M HCl. After 10 min it was mixed vigorously and centrifuged under the same conditions to separate the organic and aqueous phase. Upper organic phase was discarded and the aqueous phase was used for dopamine assay.

Dopamine assay: To 0.02 mL of HCl phase, 0.005 mL of HCl and 0.01 mL EDTA/sodium acetate buffer (pH 6.9) were added which is accompanied by the addition of 0.01 mL I_2 solution for oxidation. The reaction was stopped after 2 min by the addition of 0.1 mL sodium thiosulphate in 5 M NaOH and after 1.5 min, 10 M acetic acid was added to this reaction mixture. The solution was then heated at 100°C for 6 min. At room temperature, excitation and emission spectra of samples were read (330-375 nm) in a spectrofluorimeter. Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium thiosulphate before iodine).

Unknown concentration of dopamine was calculated from known concentration of dopamine (internal reagent standards were obtained by adding 0.005 mL double distilled water and 0.1 mL HCl butanol mixture to 20 ng of dopamine standard) (Schlumpf *et al*., 1974).

Estimation of glutamate: Brain tissue was homogenized with 2 parts by weight of perchloric acid and centrifuged at 3000 rpm for 10 min. The supernatant was made up to 3 mL and pH was adjusted to 9 by the addition of 1 mL phosphate buffer. Mixtures of above solutions were kept for 10 min, in an ice bath and then filtered through a small, fluted filter paper. The filtered portion was kept at 30°C, warmed up and then diluted to 1:10 ratio with distilled water and from that 1.0 mL of the sample was taken for the assay. Absorbance of the samples and blank (reagents only) were measured at 340 nm. The level of glutamate was expressed as micromole per gram tissue (Bernt and Bergmeyer, 1965).

Statistical analysis: The values are expressed as Mean±SD, (n = 6) mice in all experimental groups. A statistical significance difference was assessed by Graph pad prism-V version software with Post-ANOVA (Bonferroni test for multiple comparisons). The p values, $^{a}p<0.001$(***), $^{b}p<0.01$(**), $^{c}p<0.05$(*) when compared with all the groups.

RESULTS

UV-Vis spectroscopic study of HHGNps: The change in color of the plant extract was noticed visually when the *Hypericum hookerianum* extract was incubated with $AuCl_4$ solution. The absorption spectrum of HHGNps revealed between 200-700 nm and the maximum peak was observed at 520 nm (Fig. 1b). The plant extract (EEHH) showed visually yellowish green in color and the color of EEHH changed to intense ruby red color when it was incubated with an auric chloride solution. The strong resonance at 540 nm is clearly seen in all curves due to the excitation of surface phenomenon vibrations of gold nanoparticles. Curves clearly indicating that the gold nanoparticles are extremely stable with no precipitation observed even after four months storage (data not shown). The stability for such a long period was possible due to flavonoid enriched phytoconstituents present in the *H. hookerianum* extract.

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Characterization of gold nanoparticles: The SEM analysis was carried out to analyze the size and morphology of gold nanoparticles (Fig. 2). The SEM image showed that the nanoparticles formed as a result of reduction of gold ions with EEHH (magnification-16000X) and this indicated that nanoparticles formed with diameter range of 33.86-60.47 nm.

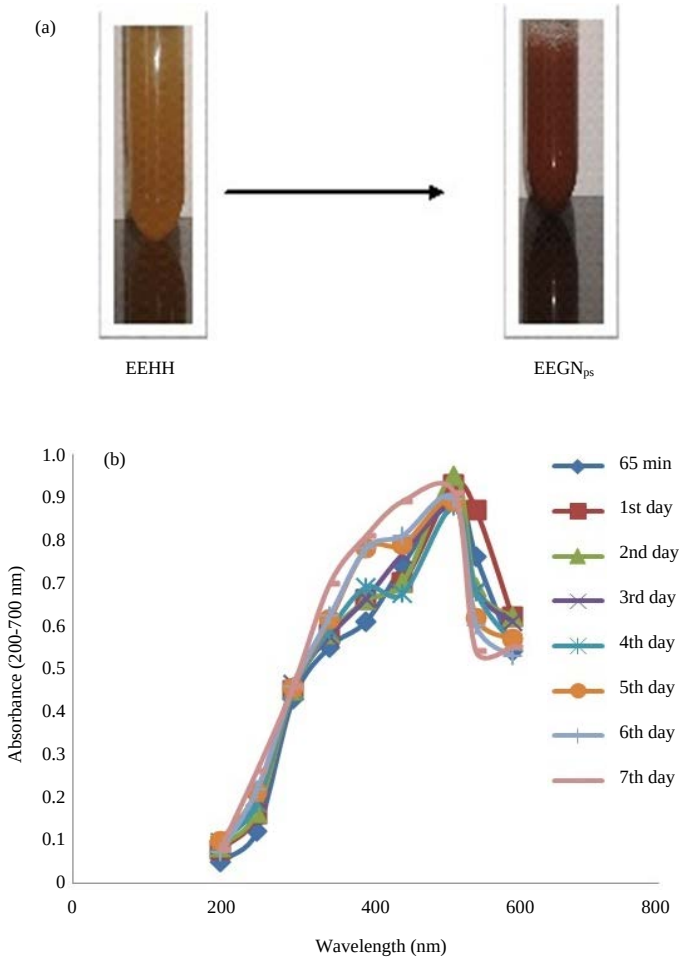

Fig. 1(a-b): (a) Ethanolic extract of *H. hookerianum* (EEHH) and its color change after the addition of chloro aurate in 65 min and (b) UV absorption spectrum of gold aurate and *H. hookerianum* reaction mixture at different intervals

Energy-dispersive X-ray (EDAX) spectrum (Fig. 2b) recorded in the spot-profile mode from one of the densely populated gold nanoparticles area. The strong sharp signals peak of gold strongly confirmed the reduction of auric chloride to auric and weak signals of Cl and O signals were likely due to X-ray emission from carbohydrates/proteins/enzymes present in the cell wall of the biomass.

FTIR analysis: The FTIR measurements were carried out to identify the possible biomolecules present in the *H. hookerianum* extract responsible for the reduction of $AuCl_4^-$ ions and also the capping agents responsible for the stability of the biogenic nanoparticle. The FTIR spectrum of EEHH showed prominent absorption bands at 3525, 1458 and 1080.17 cm^{-1} (Fig. 3a). Similarly, the FTIR spectrum of HHGNps with the absorption bands were observed at 3514, 1454 and 1076.32 cm^{-1}, respectively (Fig. 3b).

Experimental animal studies-behavioral analysis

Effect of EEHH and HHGNps on gait analysis: To prove further whether the EEHH and HHGNps treatment reduced the motor dysfunction in animals, gait analysis was performed.

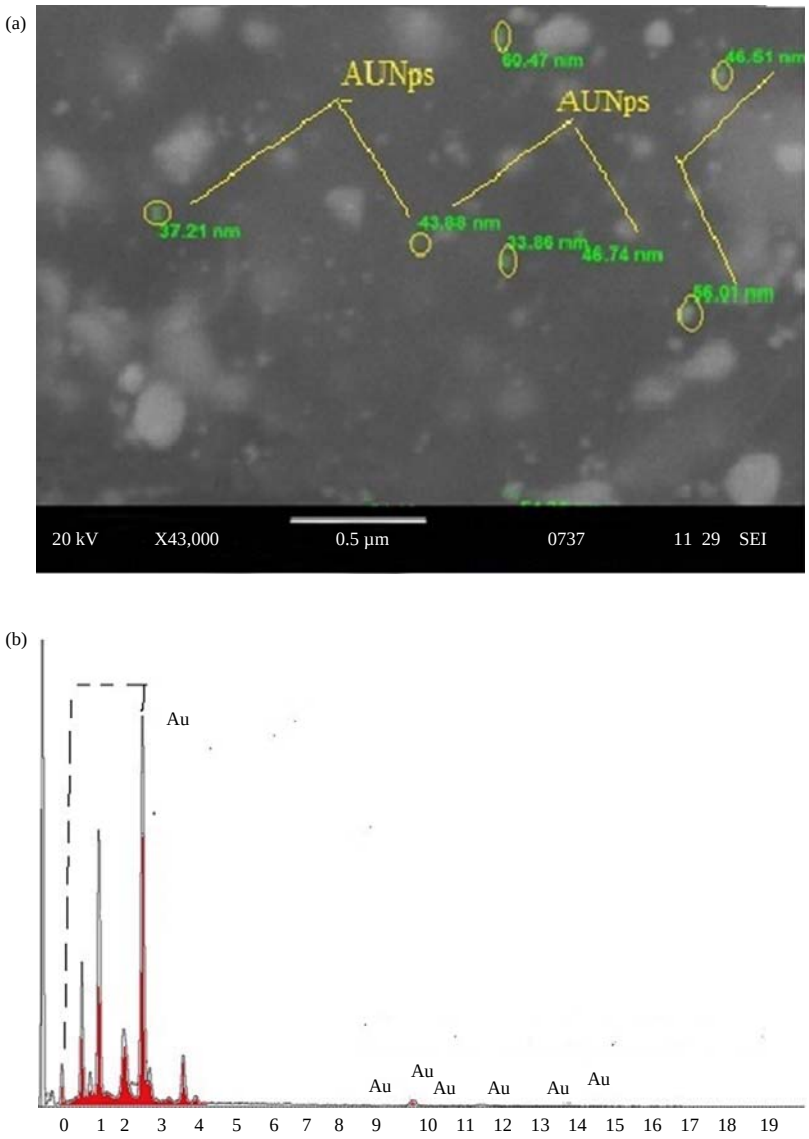

Fig. 2(a-b): (a) SEM and (b) EDAX images of HHGNps

Figure 4a depicts the effects of EEHH and HHGNps on Gait Analysis (GA) on haloperidol induced animals. The haloperidol induced animal showed a significant decrease ($p<0.01$) in forepaw stance width, significant increase ($p<0.01$) in both hind paw stance width and toe spread ($p<0.01$) when compared to the control group. Treatment with EEHH (400 mg kg^{-1}) and HHGNps (40 mg kg^{-1}) significantly ($p<0.01$) increased the fore paw stance width and decreased hind paw stance width with reduced toe spread when compared with haloperidol induced group. Upon comparison of all other extracts, HHGNps (40 mg kg^{-1}) exhibited greater effect when compared to EEHH (400 mg kg^{-1}) treated animals on haloperidol induced animals.

Effect of EEHH and HHGNps on Wire Hang Test (WHT): In WHT, hanging strength was calculated within 5 sec for all groups. The control group showed 100 percentile grip strength,

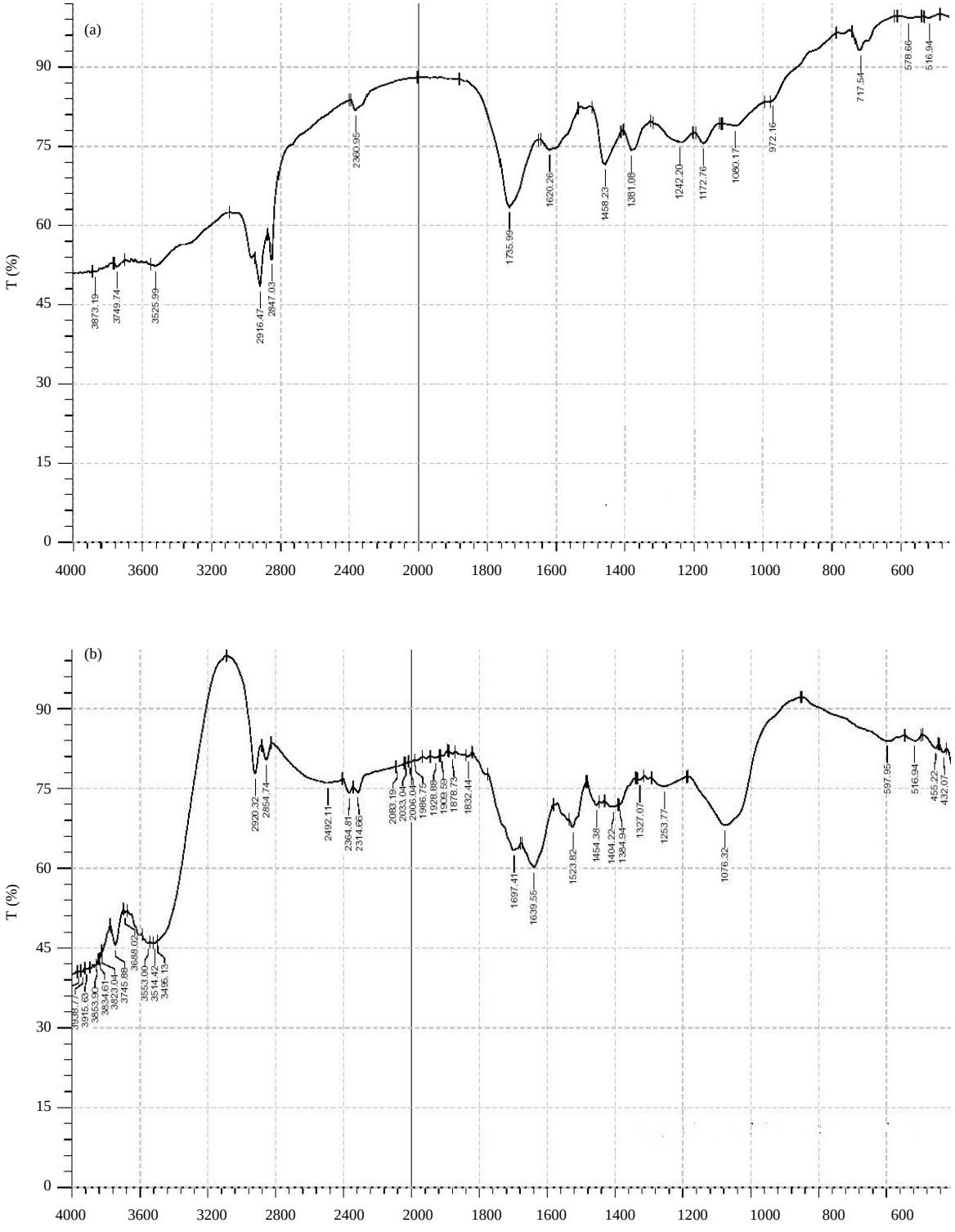

Fig. 3(a-b): (a) FTIR spectrum of EEHH and (b) FTIR spectrum of HHGNps

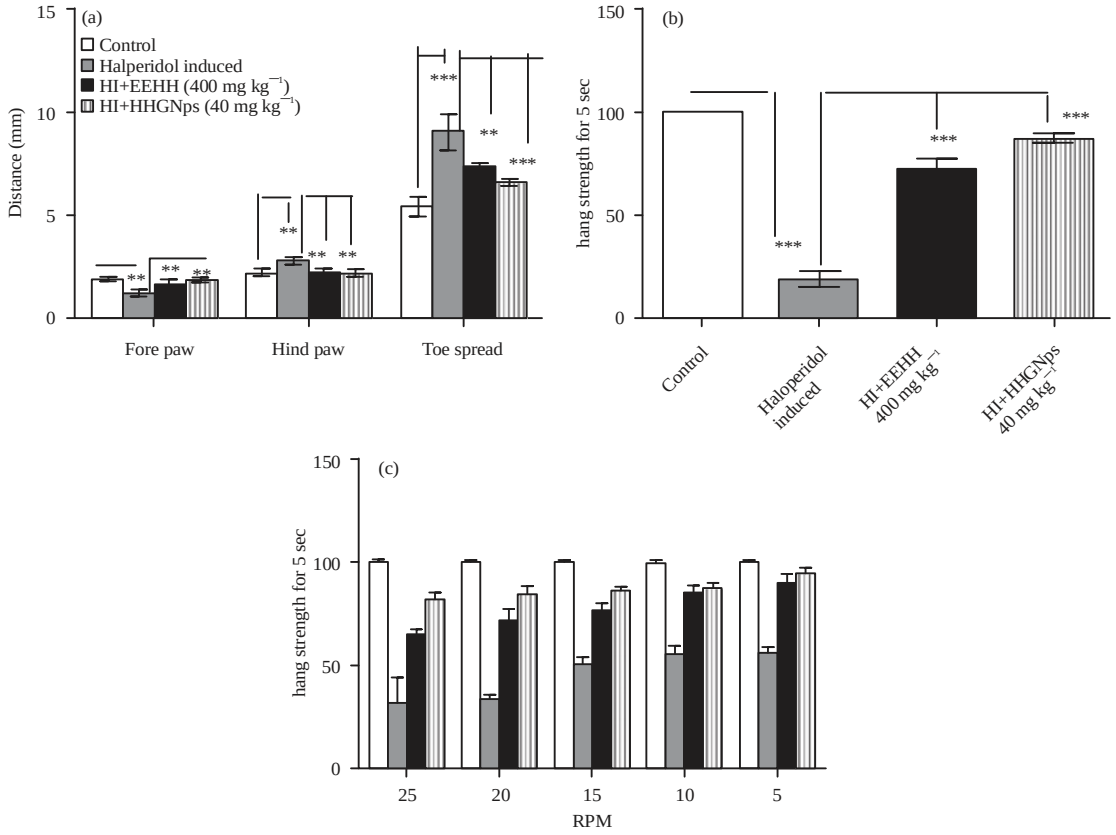

Fig. 4(a-c): Effect of EEHH and HHGNps on (a) Gait analysis, (b) Wire Hang test and (c) Rota rod test

whereas haloperidol induced animals showed very least grip strength ($p<0.001$) when compared to the control group. The EEHH (400 mg kg^{-1}) and HHGNps (40 mg kg^{-1}) treated groups showed significant grip strength when compared with haloperidol induced group ($p<0.001$). Upon comparison, HHGNps showed higher motor co-ordination effect than EEHH treated groups (Fig. 4b).

Effect of EEHH and HHGNps on Roto Rod Test (RRT): Before the treatment period or training sessions, all the groups showed maximum grip strength of the rod in all rpm (5-25 rpm). The results of the rota rod test showed significant ($p<0.001$) decrease in motor co-ordination (i.e., very less grip strength) activity among haloperidol induced group when compared to control group. But the grip strength of motor co-ordination was reversed significantly ($p<0.001$) in EEHH (400 mg kg^{-1}) and HHGNps (40 mg kg^{-1}) treated groups when compared to haloperidol induced animal (Fig. 4c). Moreover, HHGNps (40 mg kg^{-1}) showed more grip strength than EEHH (400 mg kg^{-1}) treated group.

Effect of EEHH and HHGNps on LPO levels in brain: Figure 5 shows the significant reduction of LPO levels in mice treated with EEHH and HHGNps when compared with haloperidol induced group ($p<0.001$). Among them, HHGNps treated group showed a greater reduction in LPO levels when compared to EEHH treated group.

Effect of EEHH and HHGNps on dopamine level in brain: In this study, the dopamine level in brain tissue was significantly ($p<0.001$) decreased in the haloperidol induced group when compared to control group whereas the dopamine level was significantly ($p<0.001$) increased in EEHH (400 mg kg^{-1}) and HHGNps (40 mg kg^{-1}) treated group when compared to haloperidol induced group. Among the treated animals, HHGNps (40 mg kg^{-1}) treated group showed increased dopamine values (Fig. 6a).

Effect of EEHH and HHGNps on L-glutamate levels in brain: In this study, brain L-glutamate level was increased significantly in haloperidol induced group ($p<0.05$) when compared to control group. However, animals treated with EEHH (400 mg kg^{-1}) and HHGNps (40 mg kg^{-1}) showed significantly ($p<0.05$) decreased values of L-glutamate. Among the treated experimental mice, HHGNps (40 mg kg^{-1}) treated group showed a higher reduction in L-glutamate values (Fig. 6b).

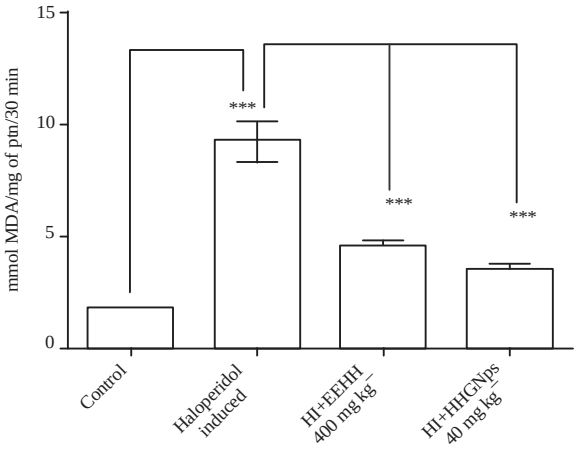

Fig. 5: Effect of EEHH and HHGNps on LPO levels in brain

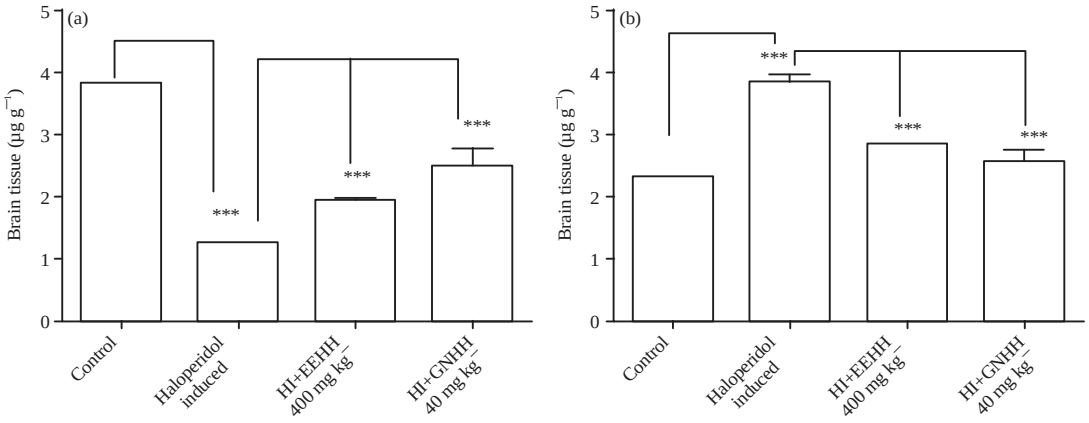

Fig. 6(a-b): Effect of EEHH and HHGNps on brain neurotransmitters (a) Dopamine and (b) Glutamate

DISCUSSION

The present work was carried out to compare the efficacy of phytochemically synthesized gold nanoparticles with that of an ethanolic extract of *H. hookerianum* as neuroprotective agent for haloperidol induced parkinsonian mice and its complications. Behavioral and biochemical parameters of neurotransmitters were assessed to support and explore the mechanism of anti parkinson like effect of EEHH and HHGNps.

Nanotechnology can be defined as research on the design, synthesis and structural manipulation of particles with minute dimensions of 1×10^{-9} m. Biosynthesis of gold nanoparticles with the help of medicinal plants has come into the fame in nanobiotechnology due to the growing need to develop eco-friendly technologies. Plants are a tremendous source of secondary metabolites and have been found to be cost-effective and eco-friendly for the large scale synthesis of nanoparticles (Sastry *et al*., 2003).

In the present study, aerial parts of *H. hookerianum* were used for the preparation of ethanolic extract and also for bioreduction of gold ions into gold nanoparticles. The reduction of chloro aurate ions to gold nanoparticles was rapid upon addition of plant biomass to the aqueous solution of $HAuCl_4$. In the recent past, gold nanoparticles have attracted demanding interest, because of their ease of preparation, low toxicity and greater affinity towards biological molecules. The gold nanoparticles have become more valuable than pretty gold because of their broad use and applications (Giasuddin *et al*., 2012).

The gold nanoparticles synthesized from ethanolic extract of *H. hookerianum* was evaluated using UV spectroscopy at a wavelength range of 200-700 nm; which exposed the characteristic peak for HHGNps at 520 nm (λ_{max}) with the specified ruby red color. Most of the gold nanoparticles synthesized from plant extracts showed UV spectrum in the range of 520-540 nm which is due to Surface Plasmon Resonance (SPR) of gold nanoparticles (Joshi *et al*., 2008).

The SEM image showed the surface architecture of gold nanoparticles as a result of bioreduction of *H. hookerianum* and also high density nanoparticles were observed in the range of 33.86-60.47 nm. Daisy and Saipriya (2012) reported the size range of gold nanoparticles to be 55.2-98.4 nm by *Cassia fistula* leaf biomass by SEM which is consistent with the current study. Energy dispersive spectrometry (EDAX) was performed by measuring the energy and intensity distribution of X-ray signals generated by a focused electron beam on a specimen. The EDAX spectra were recorded from the signaling of gold nanoparticles that clearly indicated that gold nanoparticles presence in the synthesized extract.

The FTIR spectrum of the HHGNps showed absorption bands at 3514, 1454 and 1076.32 cm^{-1} respectively. The shift in the polyphenolic stretch frequency (3525 cm^{-1}) to lower wave numbers (3514 cm^{-1}) followed by the disappearance at 3525 cm^{-1} may be due to phenolic group binding to the gold nanoparticles. The shift in the alkane deformation frequency (1458 cm^{-1}) to lower wave numbers (1454 cm^{-1}) followed by the disappearance at 1458 cm^{-1} indicated the facilitation of the binding of an O-H group of phenols with the gold nanoparticles. In addition to above supporting evidences, peak shift from 1080.17-1076.32 cm^{-1} is due to the binding of the amines with gold nanoparticles.

According to Vankar and Bajpai (2010), Au^{3+} is a soft metal which binds to a biomass mainly via amino and sulfhydryl groups, which are considered to be soft ligands and carry more positive charge at low pH values, making them available for the binding and reduction of Au^{3+} to gold. In this study, reduction of chloro aurate ions may be due to the presence of secondary metabolites, such as phenols (gallic acid), flavonoids (quercetin, rutin and hyperoside) and ketone derivatives in *Hypericum* species (Butterweck, 2003).

Continuous treatment with haloperidol, a classical neuroleptic drug widely used for the treatment of schizophrenic disease and other affective disorders can lead to parkinson's like symptoms (motor dysfunction). It blocks dopamine receptors and concomitant increase in turnover of this amine may contribute haloperidol neurotoxicity in haloperidol induced animal due to the generation of free radicals in brain mitochondrial redox reactions (Sagara, 1998) and increased lipid per oxidation (Manikandaselvi *et al*., 2012).

Neuropsychiatric disorders are appearing as a major life threatening disease in the future because of extreme mental stress, excessive work load and depression in this fast emerging world. Atmospheric pollutants, toxins can also cause neurodegenerative diseases like parkinson's and Alzheimer's diseases (Fukae *et al*., 2007; Henchcliffe and Beal, 2008). Phytochemicals from plants have diversified effects which are always an alternative option to the synthetic drugs which are well known for their adverse effects. Since the existing antiparkinson's drugs encounter many side effects and the need for prolonged treatment including questionable efficacy in the treatment, may cause parkinson's related movement problems like hallucinations and orthostatic hypotension. These reasons force the researchers to find novel treatment strategies which will overcome the adverse effects and drawbacks of the existing treatments (Chaturvedi *et al*., 2006).

The Nigrostriatal dopaminergic system is more vulnerable to haloperidol toxicity and reported to be the primary pathway playing a significant role in the control of movements and complex motor behavior. Abnormal dopaminergic transmission in these areas may lead to numerous movement disorders, like parkinsonism (Kulkarni and Ninan, 1996). In the current study, chronic haloperidol treatment significantly caused alterations in motor function as observed by behavioral tests such as rota rod, gait analysis and wire hang tests. The rota rod test examined for rodent locomotion skill which can be used for the qualitative examination of posture, forelimb and hind limb stepping in nervous system injured mice. Haloperidol injection showed that the dose of 1 mg kg^{-1} was sufficient to induce catalepsy, motor dysfunction and irregular walk in swiss albino mice. Haloperidol induced oxidative stress and products of lipid per oxidation are implicated in the pathophysiology of various neuropsychiatric disorders. Chronic treatment with haloperidol increases free radical production and oxidative stress (Fleckenstein *et al*., 1997).

The findings of this study showed that the injection of haloperidol associated with enhanced oxidative stress, as evidenced by increased LPO levels is the main pathophysiology of parkinson's due to the formation of free radicals. Further excessive generation of free radicals increased the dopamine turnover which lead to reduced levels of dopamine and increased levels of glutamate. Flavonoids, specifically quercetin and its glycoside derivatives, are a major class of compounds present in the total ethanolic extract of *Hypericum* species. Flavonoids are the major groups of polyphenols present in many plants, known to support a number of physiological benefits, chiefly in cognitive function and memory impairment (Mandel and Youdim, 2004) and also antioxidant activity. Sloley *et al*. (2000) reported that different standardized extracts of *Hypericum* species demonstrated a free radical scavenging activity due to the presence of flavonoidal antioxidants.

Gold nanoparticles have proficient utilities for drug delivery as well as for the diagnosis and treatment of several diseases related to the central nervous system. According to Sperling (1996) conjugation of 12 nm size of gold nanoparticles with the amphipathic peptide increased the *in vivo* penetration in the rat brain which did not alter the integrity of blood brain barrier and also had no effect on cell viability.

The smart features of gold nanoparticles comprised of their surface plasmon resonance, which controlled the interaction with functional groups and their non-toxic nature. These attributes can be exploited to provide an effective and selective platform to obtain a targeted intracellular release

of biological substances. The use of gold nanoparticles can also increase the stability of the payload (Pissuwan *et al*., 2011). In this study, EEHH and HHGNps reduced the lipid per oxidation level in haloperidol induced mice and restored the neurotransmitter levels and HHGNps treated group showed more antiparkinson like effect due to its targeted drug delivery system of gold.

CONCLUSION

In the current investigation, EEHH and HHGNps effectively improved motor function, reduced lipid peroxidation and restored the dopamine and glutamate values. So, it is concluded that both the EEHH and HHGNps can exert a significant motor co-ordination and antiparkinson like effect. However, HHGNps has a significant neuroprotective effect than EEHH.

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