Research Article

Biosynthesis, Characterization and Evaluation of Silver Nanoparticles of *Alternanthera sessilis* (linn.) and its Ethanolic Extract in High Fat Diet Induced Dementia Model

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ABSTRACT

Nanoparticles biosynthesis employing plants can potentially reduce the toxicity problems. These nanoparticles can be prepared by different physical, chemical and biological approaches. The biological approach is an emerging approach for preparation as this is eco friendly and easier than the other methods. The synthesis of silver nanoparticles was done by *Alternanthera sessilis* (Linn.) ethanolic extract and silver nitrate. The nootropic action of ethanolic extract of *Alternanthera sessilis* (Linn.) (EAS) and *Alternanthera sessilis* (Linn.) silver nanoparticles (ASNPs) were evaluated using High Fat diet (DHF) Model. By administration of DHF, dementia was induced. The effect of EAS (200 mg/kg) and ASNPs (20mg/kg) were assessed by determining plasma corticosterone level. Behavioural parameters like inflexion ratio, reference memory and working memory, were analysed using elevated plus maze and radial arm maze. The administration of DHF created cognitive dysfunctions like RME and WME. EAS and ASNPs have shown protective effect against model of DHF induced dementia. Significant nootropic activity was created by EAS. It resulted in a significant ($p < 0.001$) decrease in RME and WME with significant increase in inflexion ratio as compared to the DHF group. An increase in the learning memory with a reduction in the corticosterone level indicated nootropic activity. It was concluded that *Alternanthera sessilis* (Linn.) may be used as a phytomedicine for dementia.

Keywords: Ethanolic extract of *Alternanthera sessilis* (Linn.) (EAS), *Alternanthera sessilis* (Linn.) silver nanoparticles (ASNPs), high fat diet (DHF), Reference memory error (RME), Working memory error (WME).

INTRODUCTION

Hippocampal neurogenesis is inhibited by increased levels of corticosterone[1] and adrenalectomy increases the number of surviving and newly growing neurons supporting the role of corticosterone in hippocampal neurogenesis regulation.[2]

Fat rich diets have formerly known to elevate serum corticosterone levels in male rats as early as in seventh day after commencement of diet with high fat, and correspondingly the level of corticosterone in serum have stayed elevate up to even five months on feeding diet with high-fat.[3]

The phenomenon that the reduction in hippocampal neurogenesis in high fat fed females rats can be related to estrogen neuroprotective effect. In female rats the estrogen promote generation of neurons in the *dentate gyrus* through stimulation of IGF-1.[4]

High-fat feeding over short time period has showed detrimental cognitive function effects in rats. Studies suggest that fat rich diet can influences normal development of CNS and can hinder cognitive performance.[5-7] Diets containing high amount of fat have shown impairment in spatial learning in normal rats and rodents subjected to traumatic brain damage and this behavioral impairment may due to the decrease in hippocampal neurogenesis.[8]

Several study reports suggest that newly developing hippocampal neurons contribute to memory and learning. Several clinical studies has shown that diets having high saturated fats may cause decline in cognitive functioning even in humans afflicted by dementia and with aged individuals.[5-6]

A decrease in hippocampal neurogenesis was seen in male rats fed with a DHF for 28 days. The reduction of neurogenesis in hippocampal was very much independent of adipose tissue fat accumulation and these animals not exhibited more fat accumulation compared those fed with low fat diet. Rather this effect was connected with the real ingestion of dietary fat. Regardless of diet, a lower neurogenesis was found in female rats compared to male.

Another possible reason could be differences in levels of serum corticosterone between male and female rats. A low-fat diet fed female rats possessed superior corticosterone levels than males. Likewise in females, with a diet containing high-fat, corticosterone levels remained unchanged, whereas in males a significant increase was observed. This elevation in male rats fed with diet containing high-fat may be due to stress effects occurring independent of diet.[9]

Alternanthera sessilis (Linn.) commonly named sessile joy weed, is found mainly in warm, humid regions and is mainly used to relieve headache, dizziness etc. In the present investigation the preliminary phytochemical tests

on ASNPs and ASE gave positive results for steroids, flavonoids, carbohydrates glycosides, and sterols and these may be responsible for its biological activity. Silver nanoparticles can be used as alternative strategies for drug delivery to brain since these can cross the Blood brain barrier and penetrate into cytoplasm of cell.

MATERIALS AND METHODS

Experimental Animal

Healthy Wistar albino rats (Male weighing 150 - 200 gram) were obtained Department of pharmaceutical sciences, M.G University, Kottayam, Kerala. The animal housing had large spacious hygienic cages with sufficient ventilation, with proper animal husbandry. An alternate 12 hour light-dark cycle with 22-28°C temperature and with relative humidity of 55±5 % conditions were maintained. These animals were fed with water ad libitum and standard food. Prior to study animals were acclimatized to the experimental condition.

Plant

Whole plant was collected from village Kanjiramattom, Ernakulum (Dist), Kerala. Plant was authenticated from CMS College, Kottayam, Kerala. Specimen voucher is preserved with collection number 782 at the Herbarium.

Preparation of *Alternanthera sessilis* (Linn.) Silver Nanoparticles

Sillver nitrate (3 mM) solution was prepared. The plant extract (20ml)was mixed with silver nitrate solution (80 ml). Colour change from yellow to reddish brown indicated silver nanoparticles formation. These were then purified by repetitive centrifugation for 10 min at 7000 rpm. Finally the pellets were collected and dried. The chemical tests were carried out in ASNPs for vitamin C and proteins.

Characterization of the biosynthesized silver nanoparticles of *Alternanthera sessilis* (Linn.)

UV spectrum analysis: Silver ions reduction was confirmed by measuring UV spectrum of reaction mixture. Distilled water was used as blank. The UV analysis was done by double beam spectrophotometer, Shimadzu 1800, at resolution 1 nm from 250 to 450 nm.

FT-IR analysis: Spectrometer (Perkin Elmer Spectrum. 400) in the range of 4000 – 450 cm−1 at a resolution of 4 cm^{-1} was used.

SEM analysis: FE-SEM (JEOL JSM 3600) was used to do the Morphological characterization of the samples. A small amount of dried sample was coated on the carbon tape. It was again coated with platinum then the material was subjected to analysis.

Particle size measurement: Malvern Instrument (Zetasizer Ver. 7.03) Nano- ZS laser diffractometry, serial No.MAL1008884 was used. The particle size measurements between range 0.1 and 10,000 nm were taken.

Acute Oral Toxicity Study

The adult female albino rats were used in oral acute toxicity study with “fixed dose” method. This was done as per the OECD, 2000 (Guideline No.423, Annex 2d).

DHF Model

Male wistar rats were selected and grouped into five containing six rats each. Initially all rats fed on normal diet. There after 3 groups were allowed to feed custom-made high-fat diet (DHF), out of which 2 groups was administration with ASNPs and EAS respectively. The composition of the HF diet /100g: Fibers 9.5g, Coconut butter 18g, Protein 26g, Starch 38g, Corn oil 3g, Vitamins 1g, Minerals 0.5g, Salt 4g.

Energy content: Fat 42.1 kcal%, Protein: 23.9 kcal%, Carbohydrate 34 kcal%.[9]

Initially the rats were given four days to familiarize with the changed diet, following which they underwent 4 weeks of DHF, the performance testing was done using elevated plus maze and radial arm maze. For corticosterone estimation: Blood samples were collected by (non-surgical/terminal) retro-orbital method into separate tubes.

Elevated Plus Maze[10]

Elevated plus maze was made of wood with two enclosed (length 35 cm × breath 6 cm x height 15 cm) and two open arms (length 35 cm × breath 6 cm). It was elevated from ground to a height 40 cm. Rats were kept one at a time, at one arm end facing away from center of the plus maze. The time that (Transfer latency, TL) the rat move with all four legs from the open arm to any one of the closed arms was recorded.

1st day of study, each rat was put on the end of one open arm, facing away from the centre. The TL was recorded on the first day (27th day of diet administration). If the animal did not enter within 90 sec, TL was assigned 90 sec by giving a gently push into one of the covered arms. Before return to its home cage an extra two minutes were allowed the rats to explore the maze. Retention of this (memory) learned-task was examined 24h (28th day) after the 1st day trial.

Radial Arm Maze[11]

The spatial memory was evaluated by the instrument. Open type radial arm maze was used in the study. It had a circular central arena and 8 equally sized arms. (20 x 60 cm). Small dishes with sucrose pellet or animal food was kept at far end inside each arm was mounted.

Initially animals were habituated to the environment, animals were placed to the central platform and allowed to move freely on each arm they were allowed to explore the maze for a time of 15 minutes. In training periods of first days all the arms were baited and then baiting of alternative arms. Retention latencies RL (time for rat to reach the reward) was recorded on 14th and 28th day. The

spatial memory error was measured in the radial arm maze apparatus. Spatial reference memory error is considered as entries in never baited arm while the spatial working memory error is considered as the double entries into baited arm.

Biochemical Estimations of Corticosterone in Plasma[12]

Fluorimetric method was used to estimate the plasma corticosterone. This is considered as an index for the hypothalamic pituitary adrenocortical function. For preparation of reaction mixture 1 ml of plasma added to 7.5 ml of dichloromethane. Shaken for about 2 minutes, centrifuged (which allow phase separation), and plasma layer removed. At 0 time, 2.5 ml of reagent fluorescent (7:3 v/v conc. sulfuric acid: ethanol) were added. Supernatant liquid was discarded, after about 10 -12 minutes, the acid extracts were read with 470 nm excitation at 530 nm emission.

Preparation of Corticosterone standard (20 µg./ml): 20 mg of corticosterone was dissolved in 5 ml absolute ethanol after that diluted quantitatively to 1 litre with distilled water. Before use, diluted to 0.1 or 0.2 µg/ml with distilled water.[13]

Statistical Analysis

The difference between treated and control were analyzed using ANOVA (one way analysis of variance). P-values < 0.05 were taken to be statistically significant. Tukey's test was used for multiple comparisons. The results of studies were expressed as standard error of mean (SEM). Statistical analysis were done using graph pad prism version 6.05.

RESULTS

UV–vis Spectra Analysis

Extracts from whole plants under study showed rapid conversion of silver nitrate into silver nanoparticles indicated by colour change within few minutes of addition of extract in 3mM $AgNO_3$ solution, from pale yellow to red-brown. The UV spectrum is shown in Figure 1. The spectrum showed a maximum absorption in range between 420 - 450 nm This may be due to the conduction of electrons on surface of silver nanoparticles This unique possession shown by metal nanoparticles is called surface plasmon resonance.

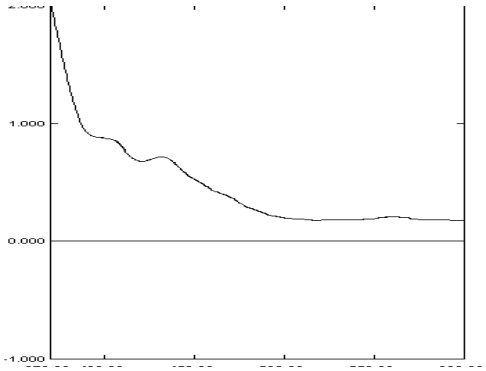

Figure 1: UV absorbance spectra at 444nm

Scanning Electron Microscopy

Under the Scanning electron microscopy study the characterization of ASNPs, revealed the nanoparticles formed had spherical shape. (Figure 2)

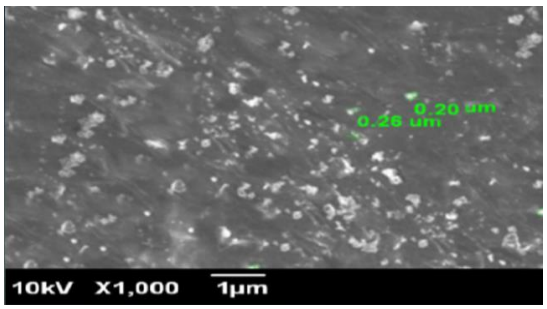

Figure 2: SEM

IR Spectra Analysis

Typical IR spectrum of EAS showed presence of peak at 876 cm^{-1}, 1044 cm^{-1}, 1382 cm^{-1}, 1640 cm^{-1}, 2982 cm^{-1}, 3006 cm^{-1}, 3370 cm^{-1}. These corresponds to aromatic CH bending, CN stretching of aliphatic amines, CO stretching (alcohols), NH bending, C H stretching, CH stretching, polyphenols, aromatic. (Figure 3)

IR spectrum of ASNPs showed following observations. The peak at 3370 cm^{-1}, 3006 cm^{-1}, 2851 cm^{-1} is nearly disappeared in spectrum of ASNPs suggesting role of protein in capping around formed nanoparticles. The ascorbic acid present may act as the reducing agent while protein as the capping agent. (Figure 4)

The intense peak at 1382 cm-1 is due to NO_3. Similarly after ASNPs synthesis, several other peaks are disappeared, showing change in transmission value and decreased in intensity. Findings suggest the association of proteins and other bioorganic compounds of extract in the formation and stabilization of ASNPs.

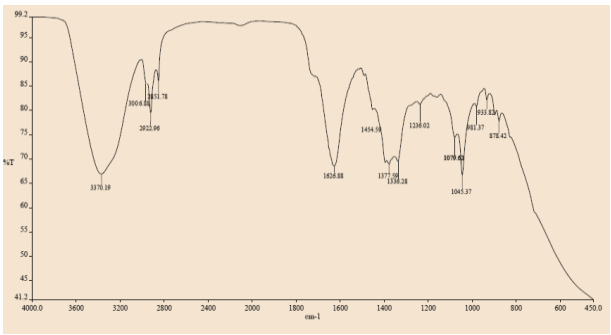

Figure 3: IR spectrum of EAS

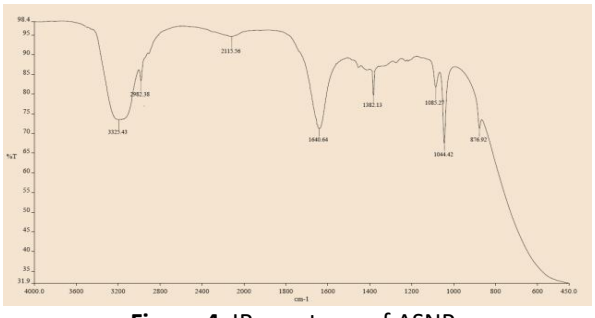

Figure 4: IR spectrum of ASNPs

Particle Size Analysis

Particle size analysis revealed that the nanoparticles formed had average size of 122- 396 nm. Well dispersed particles were found with respect to intensity. (Figure 5)

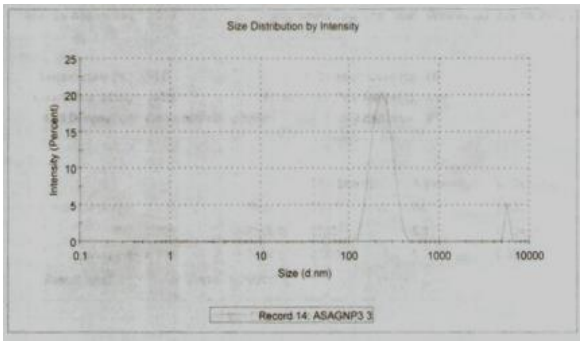

Figure 5: DLS

Radial Arm Maze

Radial Arm Maze test was performed to determine the effect of EAS and ASNPs in reference memory and working memory. DHF group exhibited significant (P<0.001) memory impairment on 28th day compared to normal control. There was a significant decrease in memory impairments in EAS treated groups. EAS treated group produced a significant increase in the reference memory and working memory, time to complete one session on 28th day as compared with DHF group. ASNPs treated group also produced improvement in memory impairment. (Figure 6, 7 & 8)

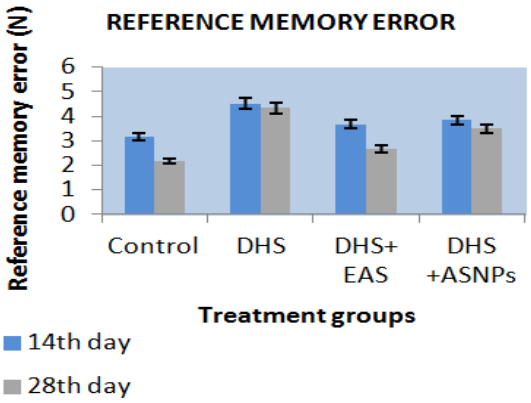

Figure 6: Reference Memory Error

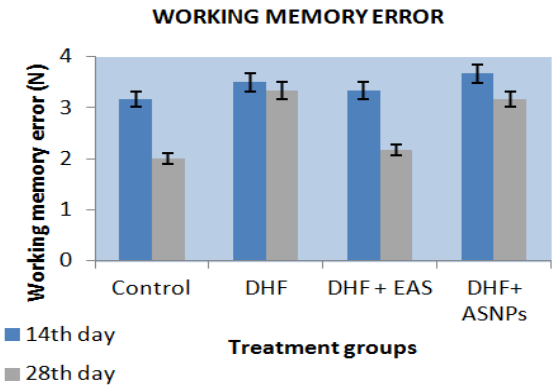

Figure 7: Working Memory Error

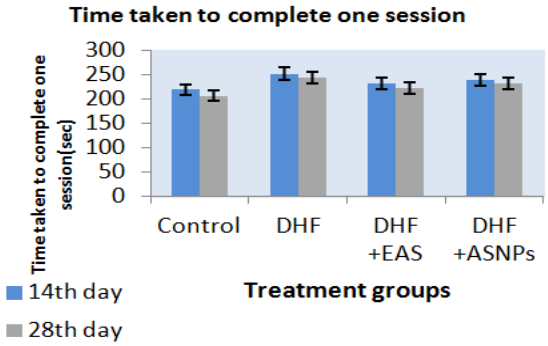

Figure 8: Time taken to complete one session.

Elevated Plus Maze Test

This is a suitable model for measuring special memory in rats. EAS and ASNPs were administered for a period of four weeks. Compared to the normal (control group), the DHF group shown to produce significant memory impairment. The inflexion ratio was significantly increased in EAS (p<0.01) group compared to DHF group, this indicate improvement in spacial memory. ASNPs group non significantly increased the inflexion ratio compared to DHF group. EAS significantly reversed DHF induced memory impairment as compared to respective DHF group. (Figure 9)

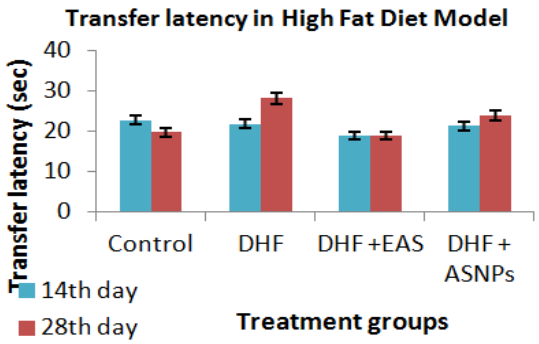

Figure 9: Transfer Latency in High Fat Diet model

Corticosterone Changes in High Fat Diet Model

DHF significantly increased the corticosterone levels in plasma. There was a significant decreased (P<0.05) in plasma corticosterone level of group Treatment with EAS when compared to vehicle group. (Figure 10)

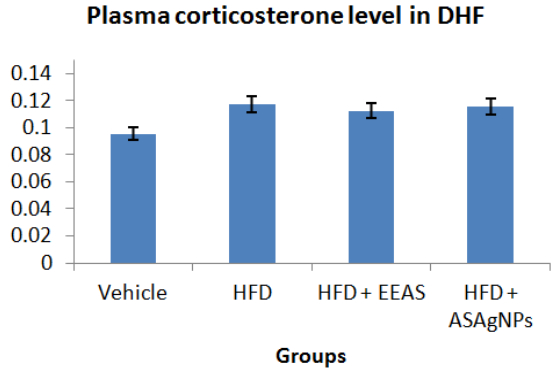

Figure 10: Plasma Corticosterone Level in DHF

DISCUSSION AND CONCLUSION

Literature survey indicates that glycosides, sterols and stanols, flavonoids, carbohydrates, leucin, monoamines, triterpenes and tannins possess wide range of pharmacological activities including neuroprotective activity. In the present study the preliminary phytochemical tests on EAS and ASNPs gave positive results for steroids glycosides, flavonoids, sterols and carbohydrates, and this may be responsible for the biological activity. The use of drug therapy with nanoparticles delivery holds much assurance in targeting remote tissue.

Previously studies with four week feeding with a diet rich in fat *ad libitum* in male rats has indicated in disruption of hippocampal neurogenesis. There was elevated serum corticosterone level but no obesity, compared to those fed standard rat chow. High-fat fed rats over 3 months showed impaired cognitive function.

Similar manifestation has been seen in humans on high fat diet.[14-15] These effects may be due to substrate restriction, since the brain can be considered a glucose-oxidizing organ and fatty acids is unable to cross blood-brain barrier.[16] In this study physical and cognitive performance in rats was investigated with respect to the effect of a high-fat short-term diet model.

In Radial arm maze method, the EAS and ASNPs treated rats show less errors in entering the arms and less time to complete the whole session in the maze. (Table 6.15). As compared with DHF group, EAS treated group produced a significant increase in the time taken to complete one session, working memory and in reference memory, on 28th day. ASNPs treated group also produced improvement in memory impairment but was non significant.

The results has been further strengthened with the positive results given by the EPM (elevated plus maze) test, in which EAS treated rats overcome memory impairment induced by high fat diet model (Table 6.17). The results showed it take EAS treated rats take less transfer latency time.

The negative effects of a DHF on cognitive function were alleviated by EAS and it also showed decrease in plasma corticosterone levels in comparison to the DHF group. (Table: 6.31; Figure: 6.27)

The chronic exposure to higher level of corticosterone in body is considered to play a vital role in synaptic plasticity and learning in non diabetic animals.[17]

Studies has shown that the elevated plasma corticosterone level was seen in 4-week HF-fed rats which shows that plasma corticosterone is an important indicator of cognition.[14-18]

Increased levels of corticosterone can inhibit hippocampal neurogenesis.

With respect to the normal diet group plasma corticosterone levels in all DHF groups were found to be significantly elevated. In the present study, results are in accordance with the previous studies that the decrease in level of plasma corticosterone has been one of the indicators of cognitive ability.

After EAS treatment, significant decrease in plasma corticosterone level was observed. Upon treatment with EAS, the decreased level of corticosterone in the treated groups indicates that the EAS may have the significant Cognitive property.

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