



GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *GLYCINE MAX* SEEDS AQUEOUS EXTRACT AND STUDY OF ITS ANTI-DEPRESSANT ACTIVITY AGAINST FORCED SWIM TEST

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ABSTRACT

Present study investigates the synthesis of silver nanoparticles using aqueous extract *Glycine max* (AEGM) and evaluating its antidepressant activity in forced swim test (FST) by using amitriptyline as a standard anti-depressant drug. Synthesized Silver Nanoparticle from Aqueous Extract of *Glycine max* (SNAEGM) were characterized by advanced techniques like UV, XRD, SEM, FTIR and Zeta potential, and its anti-depressant potential by FST. Successful synthesis of SNAEGM by ecofriendly and low cost method was justified by change of color of solution to dark brown, appearance of peak at 440nm in UV spectroscopy, average particle size with in nano range by XRD and SEM, zeta potential of -12.9mV and appearance of functional group bands of different plant phytoconstituents in FTIR. Control group animals showed increased immobility time which was significantly ($p < 0.0001$) decreased in amitriptyline (20mg/kg), AEGM (200mg/kg) and SNAEGM (160mg/kg). Moreover it was observed that SNAEGM was more effective in decreasing immobility time as compared to AEGM and amitriptyline. Thus we conclude *Glycine max* is effective tool for synthesis of nanoparticles and have potential anti-depressant activity. Further research is required to explore the in-depth molecular mechanism involved in treatment of depression.

KEYWORDS: Anti-depressant, *Glycine max*, Force swim test, Silver nanoparticles.

INTRODUCTION

Nanoparticles derived from different metals like silver, platinum and gold is gaining importance in modern research due to their application in different fields of biotechnology and bioengineering.^[1] Moreover silver is known to possess many biomedical applications like anti-bacterial^[2], anti-angiogenesis^[3], anti-viral^[4] and anti-fungal.^[5] Silver derived nanoparticles of size ranging from 1-100nm also gained importance due to their optical, electrical and biological properties.^[6] Physical and chemical methods applied for the synthesis of silver nanoparticles (AgNPs) are toxic and expensive.^[7] Therefore plant extract mediated synthesis of AgNPs emerged as an economical, safe, easy and eminent tool. Reduction of Ag⁺ to Ag⁰ and stabilization of AgNPs is possible due to the presence of different phytoconstituents in plant extracts.^[8] *Glycine max* .L is an annual, subtropical plant belonging to family Leguminosae. It contains different phytoconstituents like Proteins^[9], oils majorly linoleic acid^[10], phospholipids, phytosterols^[11], Carbohydrates^[12], Isoflavones.^[13] *Glycine max* was reported to possess efficacy in the treatment of Hypertension^[14], Cardio protective

activity^[15], Anti-arthritis activity^[16], Antioxidant Activity^[17], Ant diabetic and Antihyperlipidemic Activity^[18], Anti-obesity effect^[19], Antimicrobial Activity.^[20]

Depression is majorly characterized by anhedonia, insomnia, depressed mood, and difficulty in concentrating, abnormal appetite and irritability.^[21] According to World Health Organization, it is considered as one of the leading cause of mortality, affecting about more than 20% of population and may become 2nd largest dreadful disease for mankind by 2020.^{[22],[23]} Different anti-depressant drugs which are available for the treatment are reported with unwanted side effects, long duration of treatment.^[24] Even though anti-depressant drugs are used since many years, challenge for the treatment of depression remained uncertain. Present days, research is majorly focused on use of medicinal plants for the treatment of which are devoid of severe adverse effects. Based on these above discussed benefits of *Glycine max* we conducted the synthesis of silver nanoparticles of AEGM and evaluated its anti-depressant activity by FST in wistar albino rats.

MATERIALS AND METHODS

Plant material

Soya bean seeds were obtained from local grocery shop located at Annaram, Hyd during the months of November. Plant was subjected to authentication at Botanical Survey of India, Hyd unit, Telangana, India. After identification 1000gm of seeds were grinded to form a uniform powder. 200gms of dried powder of *Glycine max* seeds was extracted in soxhlet apparatus by using water as a solvent at 100°C. Finally semi solid form of extract was obtained and refrigerated in an air tight container. Percentage yield of extract was calculated by using the formula.

$$\% \text{ yield} = \frac{\text{Weight of extract in grams}}{\text{Weight of sample in gms}} \times 100$$

Animals

Care and use of animals was carried out in accordance with CPCSEA guidelines. 150-200gm weight of female wistar rats was used. Work was approved by Institutional Animal Ethics Committee IAEC, Pulla reddy institute of pharmacy, Hyd, Telangana, India.

Synthesis of silver nano particles from aqueous extract of *Glycine max* (SNAEGM)

1mM of AgNO₃ solution and AEGM solution (1000mg in 100ml) in Double Distilled Water (DDW) was prepared separately. By stirring the AgNO₃ solution on a magnetic stirrer at a temperature of 60°C AEGM solution was added drop wise and kept on incubation for 15hrs.

Characterization of SNAEGM

Visual, UV Visible and FTIR mediated assessment of SNAEGM

The formation of AgNPs has been indicated by the color change in the AEGM solution. Color of pure silver nitrate and of pure extract was taken as control. Reduction of Ag⁺ to Ag⁰ was examined by using UV3000, LABINDIA UV visible spectrophotometer. Scanning was carried out in a range of 200-800nm, resolution ±1 nm and DDW was used as blank. SNAEGM obtained was diluted in DDW, centrifuged at 5000rpm for 15 min to remove the unbound moieties, supernatant obtained was discarded, pellet was re-suspended in DDW, centrifuged again at 5000rpm for 15min, procedure repeated for 3 times, final pellet obtained was dried and pure form SNAEGM was screened for the presence of different functional groups by using FTIR.

X-Ray Diffraction mediated assessment of SNAEGM

XRD was carried out by using SHIMADZU, XRD7000, at a current (30mA), scan speed (4.0 deg/min), sampling pitch (0.02 deg), voltage (40kV), monochromator (nickel), preset time (0.30 sec), with Kα1 Cu radiation, 2θ range from 10° to 80°. Sample for XRD analysis prepared as follows, 100μl of SNAEGM applied on a glass slide and dried for 30 min. Average size of SNAEGM was calculated by using the Debye-Scherrer equation^[25]:

$$D = \frac{k\lambda}{\beta \cos \theta}$$

Where, = average size of SNAEGM, k = constant (0.94), λ = wavelength of X-rays (1.546Å), β = full width at half maximum (FWHM), θ = diffraction angle in degrees.

Scanning Electron Microscopy mediated assessment of SNAEGM

SEM was carried out to evaluate morphology, shape and size of AgNPs by using SEM - S-3700, model no-370701-14 operated at Emission current (57000nA), Magnification (30000) accelerating voltage (15000Volt). Sample was prepared by dropping small quantity of AgNPs on copper grid; extra solution was removed by blotting paper and used for SEM analysis.

Zeta Potential analysis of SNAEGM

Stability of SNAEGM was determined by measuring zeta potential by using HORIBA SCIENTIFIC, SZ100, Viscosity (0.894mPa.s), Conductivity (0.604mS/cm) and electrode Voltage (3.3V).

Effect of SNAEGM and AEGM on FST induced depression in wistar albino rats

24 animals were used for the study which are divided into 6 groups with 4 animals in each as follows, Group 1 (negative control) received tween 80,(10% v/v), Group 2 (positive control)received amitriptyline 25 mg/kg, Group3 received SNAEGM 80 mg/kg, Group 4 received SNAEGM 160 mg/kg, Group5 received AEGM 100 mg/kg, Group 6 received AEGM200 mg/kg. All the drugs are given by P.O. route before one hour of commencement of test. After 1 hour, rats were individually forced to swim inside a plastic container (height: 30 cm; diameter: 25 cm) containing 20 cm of water maintained at 25°C. Rat was placed in the cylinder for the first time was initially highly active, however after 2-3 minutes the activity subsided and the phases of immobility increased. After 5-6 minutes, the duration of immobility reached a plateau where the rat remained immobile for about 80% of the time. Each animal was judged to be immobile when it started struggling and remained floating or motionless in the water, making only the movements necessary to keep its head above water. The absence of hind leg movement was recorded as immobility by stop watch accumulation by a single observer during the exposures.^[26]

RESULTS AND DISCUSSION

Characterization of SNAEGM

Visual, UV Visible and FTIR mediated assessment of SNAEGM

In the present study we successfully synthesized AgNPsby using soyabean seed aqueous extract. Maintaining the temperature at 60°C and rotation during the incubation period accelerated the synthesis.First evidence for the formation of nanoparticles is change of color from colorless AgNO₃ solution to dark brown AgNPs solution, which may be due to reduction of Ag⁺ to Ag⁰ and due to excitation of Surface Plasmon

Resonance effect that majorly depends upon chemicals present and particle size in surrounding medium. Reduction of Ag^+ may also be due to various biomolecules present in AEGM. Colorless solution of AgNO_3 was changed to dark brown color with in 8hrs (**Fig. 1**). SNAEGM showed a peak at 440nm in UV Visible spectra which was not shown in spectra of AEGM, silver nitrate solution (**Fig. 2**). 440nm peak showed by SNAEGM in UV-Vis spectra confirmed the formation of AgNPs, our results are in accordance with AgNPs synthesized by Khushboo Singh in 2014^[27], where a strong absorption peak was obtained in the range of 420-430nm which is near to 440nm.

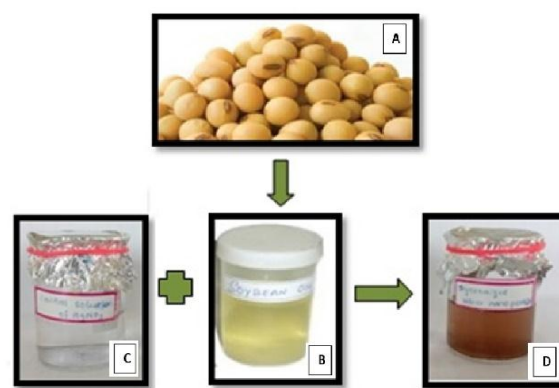


Fig. 1: Synthesis of SNAEGM. A= soya seeds, these are used to produce extract, 1000mg of extract is dissolved in 100ml of DDW to produce AEGM solution (B), C=1mM AgNO_3 solution, D= SNAEGM produced after incubation for 15hrs.

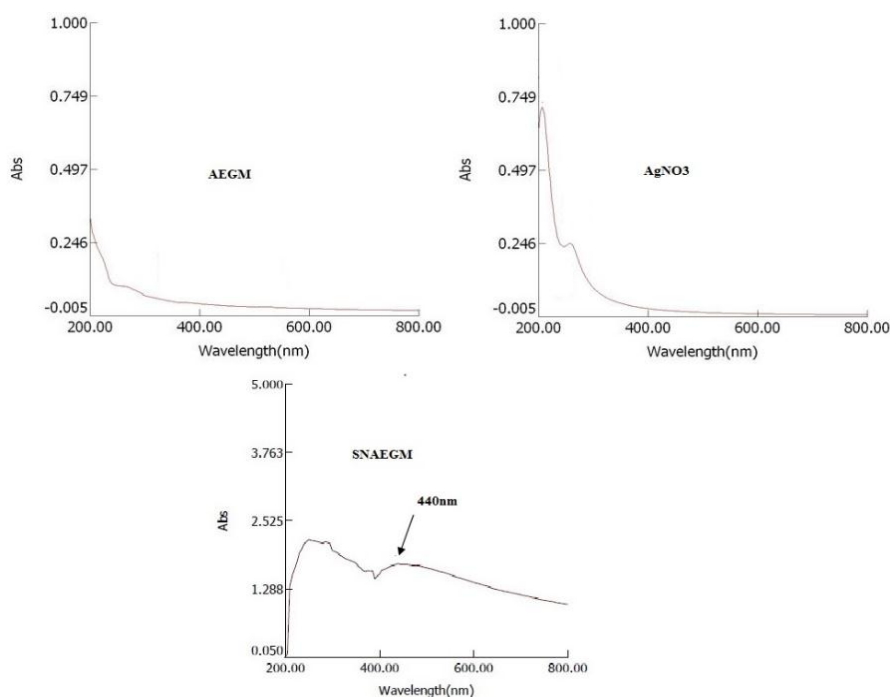


Fig. 2: UV-Vis spectrum of AgNO_3 , AEGM & SNAEGM, 440nm peak was shown in SNAEGM spectra.

Various functional groups playing a role in the formation of silver nanoparticles were explored by FTIR analysis and represented in (**Fig. 3**). Spectrum of AEGM and SNAEGM shows a shift in following peaks 3267-3274, 1623-1615, 1583-1591, 1454-1541, 1408-1455, 1316-1361, 1273-1279, 1118-1120 and 1041-1034 cm^{-1} . Absorption peak in AEGM at 3267 cm^{-1} corresponds to -O-H stretching of alcohols and phenols shifted to higher frequency of 3274 cm^{-1} in SNAEGM, peak at 1623 cm^{-1} in AEGM was due to asymmetrical carboxylate stretching shifted to lower frequency (1615 cm^{-1}) in SNAEGM, band at 1583 cm^{-1} (AEGM) was due to -C-O stretch of carboxylic acid group shifted to higher frequency of 1591 cm^{-1} (SNAEGM), 1454 cm^{-1} band in AEGM corresponds to -O-H stretching in phenol shifted

to higher frequency of 1541 cm^{-1} in SNAEGM, band at 1408 cm^{-1} (AEGM) corresponds to -O-H bend of polyphenol shifted to higher frequency of 1455 cm^{-1} (SNAEGM), peak at 1316 cm^{-1} in AEGM -N=O stretching of nitro groups shifted to 1361 cm^{-1} in SNAEGM, AEGM showed a band at 1273 cm^{-1} corresponds to -C-O stretching of carboxylic acid shifted to 1279 cm^{-1} in SNAEGM, 1118 cm^{-1} band of AEGM corresponds to C-O stretching of phenolic compounds was shifted to 1120 cm^{-1} in SNAEGM spectra and band at 1041 in AEGM corresponds to -O-H stretching in polyphenol shifted lower frequency to 1034 cm^{-1} in SNAEGM spectra. FTIR spectra of SNAEGM clearly showed the functional groups extract phytoconstituents involved in synthesizing AgNPs. Peak observed at

1623cm^{-1} was due to asymmetrical stretching carboxylate group. Similarly, a very close peak at 1626cm^{-1} was reported in FTIR spectra of AgNPs synthesized by using seaweed *Gracilaria birdiae*.^[28] As we stated shift of band from 1583cm^{-1} to 1591cm^{-1} was due to -C-O stretch of carboxylic acid group, similarly a band very near to 1591cm^{-1} i.e., at 1595cm^{-1} was reported by Venkatanarasimha RC^[29] in 2016. 1454cm^{-1} and 1118cm^{-1} bands of AEGM spectra corresponds to -O-H stretching in phenol and polyphenols respectively, same -O-H stretching of phenols and polyphenol at 1460cm^{-1} and 1110cm^{-1} respectively, was reported in FTIR spectra of AgNPs synthesized by using *Datura stramonium* leaf extract.^[30] Bands at 3267cm^{-1} and 1408cm^{-1} corresponds to -O-H stretching of alcohols and phenols and -O-H bend of polyphenols respectively. Our results are in accordance with bands shown in FTIR spectra at 3270cm^{-1} and 1408cm^{-1} of AgNPs synthesized by using *Andean blackberry* fruit extract.^[31] -N=O stretching of nitro groups showed a band at 1361cm^{-1} in SNAEGM, for similar -N=O stretching band was shown at 1332cm^{-1} in FTIR spectra of AgNPs synthesized from *M.charantia* fruit extract.^[32] Bands at 1273cm^{-1} (AEGM) and at 1279cm^{-1} (SNAEGM) corresponds to -C-O stretching of carboxylic acid, similarly, -C-O stretching of carboxylic acid showed a peak at 1264cm^{-1} was reported by Jaganathan Umashankari.^[33]

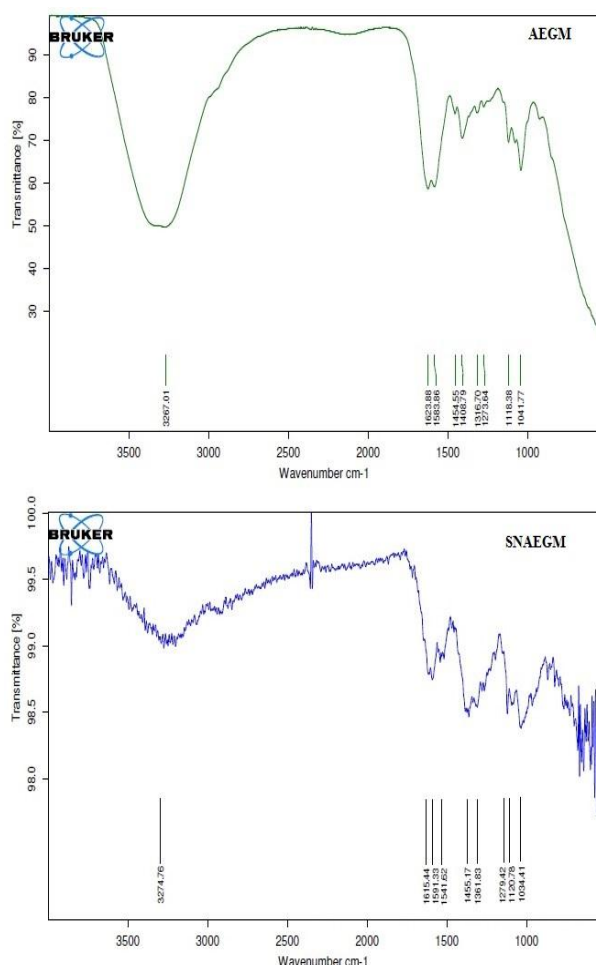


Fig. 3: FTIR spectra of AEGM & SNAEGM.

X-Ray Diffraction mediated assessment of SNAEGM

Fig. 4 depicts the XRD pattern of synthesized SNAEGM. 2θ peaks are observed at 28.79° , 33.56° and 46.76° corresponds to 111, 200 and 220 planes of Bragg's reflection of silver respectively. XRD studies confirmed the size of SNAEGM from 10nm to 30nm and average size of SNAEGM as 19.92nm. XRD spectra confirmed the nano range of synthesized SNAEGM, Similar results were also reported by Rau et al in 2009, and Silver nanoparticles showed size ranging from 10nm to 50nm and average size of 27nm.

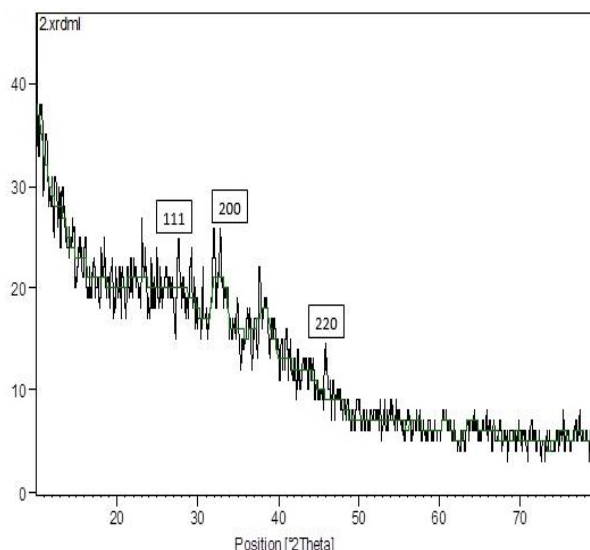


Fig. 4: XRD pattern of SNAEGM.

Scanning Electron Microscopy mediated assessment of SNAEGM

Fig. 5 depicts the topography and surface morphology of SNAEGM. SEM analysis revealed various sizes of SNAEGM ranging from 47.3nm to 81.1nm with an average size of 58.68nm falling within the nano range and exhibits different surface morphologies. SEM micrographs of SNAEGM reported the average size of nanoparticles as 58.68nm; our results are in accordance with the results of Narayanaswamy Krithiga^[34], where SEM micrographs revealed spherical AgNPs of size ranging from 10nm to 50nm. Results are also comparable with the *Calliandra Haematocephala* leaf extract mediated biosynthesis of AgNPs showed average size of 70nm.^[35]

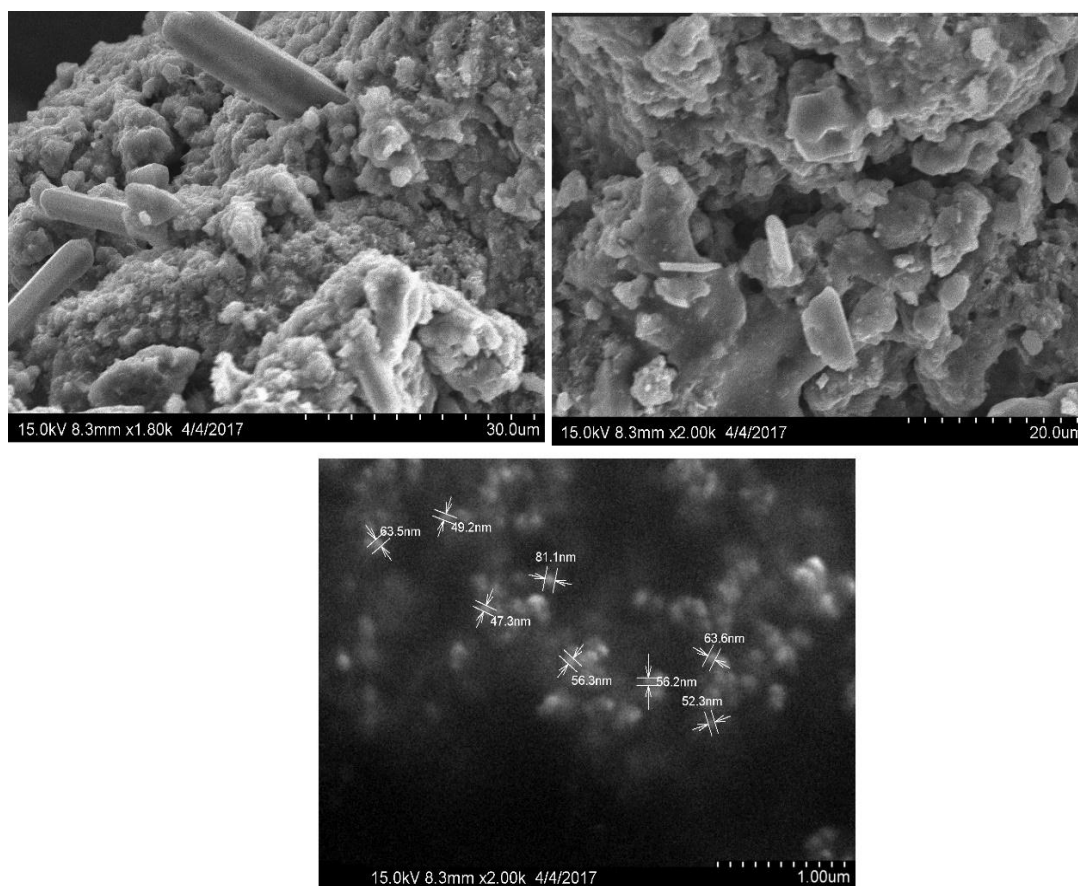


Fig. 5: SEM images of SNAEGM at 30.00µm, 20.00µm and 1.00µm.

Zeta potential analysis of SNAEGM

Zeta potential was found to be -12.9mV. Negative value justifies the stability and also prevents the agglomeration of synthesized SNAEGM. Results are depicted in **fig. 6**. Stability of SNAEGM was confirmed by measuring zeta potential (-12.9mV) which is more close to the zeta potential (-11.9mV) of AgNPs synthesized from *Phyllanthus amarus*.^[27] Capping of biomolecules present in AEGM on AgNPs may be the cause for negative value of zeta potential.

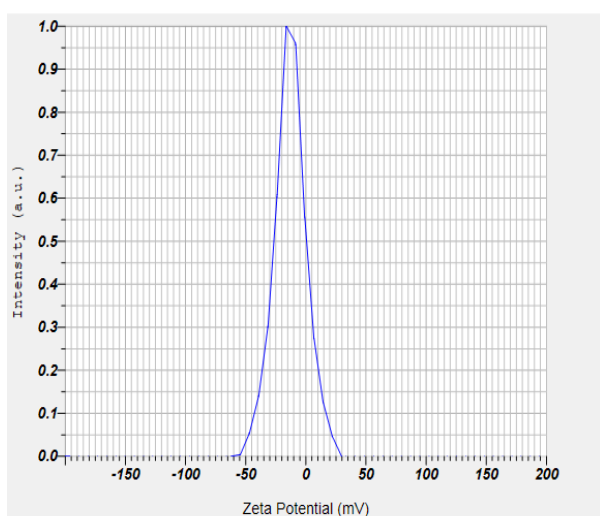


Fig. 6: Zeta potential of SNAEGM.

Effect of SNAEGM and AEGM on FST induced depression in wistar albino rats

Negative control group animals remained immobile for 44 sec indicating the induction of depression. Lower dose of both AEGM (100mg/kg) and SNAEGM (80mg/kg) does not produce any significant decrease in immobility time as compared to control group. Whereas amitriptyline (20mg/kg), AEGM at 200mg/kg and SNAEGM at 160mg/kg produced highly significant ($p < 0.0001$) decrease in immobility time as compared to control group, thus proving their effectiveness in the treatment of depression. Immobility time decreased significantly in SNAEGM 160mg/kg as compared to AEGM 200mg/kg (**Table 1**). Anti-depressant effect by decreasing immobility time in FST was produced by both AEGM and SNAEGM. Effect was more significant with SNAEGM as compared to AEGM. FST is well known paradigm for evaluating anti-depressant activity. Parameter recorded in this test is immobility time of rats which is similar to behavioral despair symptom occurring with patients suffering with depression.^{[36],[37]} Treatment with amitriptyline, AEGM and SNAEGM decreased immobility time significantly as compared to negative control group. Our results are supported by the study of Ashok KBS et al^[38], who reported similar decrease of mobility time in rats on treatment with Methanolic Extract of *Amaranthus spinosus*. Soya bean was found to contain different biologically active constituents like proteins, oils, carbohydrates,

isoflavones, phytosterols and saponins.^[39] Based on the literature survey, it may be concluded that these constituents are responsible to produce anti-depressant activity. Presence of these constituents even in the synthesized SNAEGM was confirmed by the results of FTIR. Many researchers separately publicized the anti-depressant activity of above mentioned constituents.

Role of linolenic acid and beta-sitosterol from *Sargassumhorneri* in treatment of depression was reported by Nicolas B et al^[40] and Donghai Z et al^[41] respectively. Likewise, anti-depressant activity of flavonoids and saponins was reported by butterweck et al^[42] and Xiang H et al^[43] respectively.

Table 1: Effect of SNAEGM and AEGM on FST induced depression in wistar albino rats.

Experimental group	Dose mg/kg	Duration of Immobilization (sec)
Negative Control	-	49.94±3.213
Positive Control (Amitriptyline)	20mg/kg	29.13±4.148***
SNAEGM	80mg/kg	41.61±1.33 ^{ns}
SNAEGM	160mg/kg	24.77±2.638****
AEGM	100mg/kg	44.73±4.158 ^{ns}
AEGM	200mg/kg	29.56±0.3706****

All values are expressed as Mean ± SEM, n=4, analysed by One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 as compared to control group; ns= non-significant.

We successfully reported the green synthesis of AgNPs from AEGM and screened its anti-depressant activity by FST. Further studies should be carried to discover new safer drug molecules for the treatment of depression.

CONCLUSION

The nanoparticles of aqueous seed extract of *Glycine max*, demonstrated potential antidepressant properties and less toxicity in the experimental animals at the doses used. In recent years, ethno botanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. Based on this, we may conclude that linolenic acid, beta-sitosterol, flavonoids and saponins present in SNAEGM are responsible for its anti-depressant activity. Further studies are required to evaluate the mechanism of action at molecular level of these different constituents.

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