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Assessment of behavioral changes and antitumor effects of silver nanoparticles synthesized using diosgenin in mice model

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ABSTRACT

Silver nanoparticles (AgNPs) are widely accepted for their physical, chemical and biological properties which could relate to their applications in medicine. Hence, these particles of the nano-regime could be a potential contender in search for novel medication with psychotherapeutic and antitumor potential. Considering this background, the objective of the present study was set to comparatively assess the anxiolytic, antidepressant and antitumor effects of AgNPs synthesized using diosgenin, a plant derived saponin of *Dioscorea oppositifolia*. The synthesized nanoparticles were characterized by using UV–Vis, FTIR, TEM, SEM, DLS and XRD. The AgNPs after characterization were tested at varying doses for their anxiolytic and antidepressant effects in Swiss albino mice. The mice spent more time in the open arm after treatment in elevated plus maze test (EPM). Significant dose dependent reduction in immobility time was observed in forced induced swimming test (FST). Dalton's ascites lymphoma (DAL) was induced *in vivo* for analysing the antitumor effect of AgNPs. After treatment with AgNPs, a significant reduction was observed in the body weight of the tumor-induced mice. Analysis of hematological parameters, lipid peroxidation marker, liver and kidney function tests indicate the oncosuppressive effects of the AgNPs. This was evidently observed via the effective revival of all these parameters to near-normal after treatment with AgNPs at a dose of 10 mg/kg. To conclude, the findings of the present study indicate that AgNPs synthesized using diosgenin could be considered as a valuable candidate for use in and as anxiolytic, antidepressant and antitumor medications.

1. Introduction

Diosgenin, a plant derived saponin of *Dioscorea oppositifolia* with several biomedical applications, is a precursor for several steroidal hormones such as progesterone and cortisone [1]. *Dioscorea oppositifolia* is a plant rich in phytochemicals, especially diosgenin, with potent antioxidant, anxiolytic and neuroprotective effects [2–4].

Anxiety is a phenomenon which is comorbid with mood disorders such as depression [5]. Depression is a common mood disorder among cancer patients and survivors [6–8]. More than half percentage of patients with anxiety is prone to depression as these two disorders co-occur. The quality of life among the affected individuals depends on several factors [9]. Subjects may experience mood swings ranging from happiness to sadness or both at the same time [10]. Duration of these disorders may vary in adults and have an inclined recurrence rate of 70% [11]. Patients suffering from these illnesses related to mood are at high risk for several metabolic disorders due to lack of physical activity and effects of the medication beyond desire [12]. Since the search for

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novel medication with psychotherapeutic potential to treat anxiety is the need of the hour, diosgenin could be an effective agent [13].

Nanoparticles are known to possess antidepressant and antitumor effects [14–18]. But there are no reports on the anxiolytic and anti-depressant effects of AgNPs. AgNPs are widely accepted for their physical, chemical and biological properties. They are widely used as antibacterial and anticancer agents. For an eco-friendly, large-scale and non-toxic approach, AgNPs is biosynthesized using plants and their products [19–21].

Therefore, the present study will be the first ever international report on the synthesis of AgNPs using diosgenin and analysis of its effects on mood disorders and DAL cells.

2. Materials and method

Diosgenin was purchased from Sigma Aldrich (St. Louis, MO, USA) and used for the study. Silver nitrate was purchased from Qualigens Fine Chemicals, Mumbai, India.

2.1. Synthesis and characterization of nanoparticles

AgNPs were synthesized using the protocol reports previously published, with the only change being the reductant involved, which was diosgenin, in place of plant extracts [22,23]. UV–Visible (UV–Vis, Hitachi double beam equipment model Lambda 35) and Fourier-transform infrared spectroscopies (FTIR, IR Affinity-1 equipment model, Shimadzu Corporation), transmission electron microscopy (TEM, Tecnai 12 instrument), Scanning electron microscopy (SEM, Carl Zeiss Evo 18 instrument), X-ray diffraction (XRD, Phillips PW 1830 model instrument) and zeta analysis (Nano Plus) were performed to characterize the synthesized AgNPs. Total antioxidant capacity was elucidated following the phosphomolybdenum method [24].

2.2. Animal maintenance

Male Swiss albino mice (25–30 gm) were housed under controlled temperature (23 °C-25 °C), humidity (50%–60%) and 12-h light: 12-h dark cycle in polypropylene cages bedded with sterile paddy husk. The hormonal changes during different periods of life-cycle, its metabolic attributes, morphology and other associated practical problems makes a female mouse less desired to work with [25]. Due to such difficulties, male mice were preferred in this study instead of female mice. Institutional ethics committee of Bharathidasan University, India approved the study and CPCSEA guidelines were adhered during the experiment. Standard laboratory feed (22% crude protein, 4.12% crude oil, 2.79% crude fiber, 7.81% ash and 1.34% sand silica) was purchased from Sri Sai Durga feed, Bangalore, India. Drinking water was provided ad libidum. The animals were housed, acclimatized, fed and maintained under aseptic conditions.

2.3. Anxiolytic and antidepressant studies

The mice were divided into 4 groups of 6 animals each for both the EPM and FST. Group I was control (saline, 5 ml/kg). Group II animals in EPM test were treated using diazepam (2 mg/kg) as the standard, whereas, imipramine was used as the standard for FST (10 mg/kg). Both the standards used were administered intraperitoneally. Group III animals were treated with diosgenin (50 mg/kg). Group IV animals were treated with diosgenin-derived AgNPs (10 mg/kg).

2.4. Analysis of antitumor effect

In the antitumor assay, group I animals were normal. Ascitic cells were obtained under the courtesy of Amala Cancer Research Centre, Thrissur, Kerala, India and used for the study. After an adequate volume of cells develop in mice used to maintain the ascitic cells, the mice were euthanized and cells were collected from the peritoneal cavity. Group II animals were later injected intraperitoneally with the obtained ascitic cells at 1×10^6 cells/per fresh experimental mouse and maintained as tumor (DAL) control. Group III DAL-induced mice were treated with diosgenin (50 mg/kg). Group IV DAL-induced mice were treated with AgNPs synthesized using diosgenin (10 mg/kg). Each group contained six mice. After 3 days of fluid injection, the experimental animals were intra-peritoneally administered with test agents and euthanized at the end of experiment on day 13.

2.5. Analysis of blood parameters

Analysis of RBC, WBC and biochemical parameters such as LDH, SGPT, uric acid, creatinine and MDA were performed to determine the functioning of liver and kidney [20,26].

2.6. Statistical analysis

All data were expressed as mean ± SEM. SPSS version 17 was used to calculate the One-way analysis of variance (ANOVA) to determine the statistical significance. Duncan's Multiple Range Test (DMRT) was used to obtain the individual comparisons [27]. A value of $p < 0.05$ was considered to be statistically significant between the groups.

3. Results and discussion

3.1. Characterization of nanoparticles

The UV–Vis peak of the hydrosol was observed at 410 nm after 10 min of incubation of diosgenin with silver nitrate at 90 °C. The sample was kept as such as for several hours and the UV–Vis analyses were performed. The peaks obtained after 24 (413 nm), 48 (419 nm), 72 (414 nm) and 96 (416 nm) hours were presented in Fig. 1. Surface resonance peaks in the range of 410–450 nm are suggestive of spherical nanoparticles below 100 nm size [26,28–30]. We chose the particles synthesized after 24 h and used it for further analyses as it was the shortest duration taken for synthesis and the wavelength did not change much in solutions after varying time durations. FTIR is a critical tool to determine the functional groups in samples [31]. The major absorption bands obtained for AgNPs containing solution were observed at 680 cm^{-1} and 3422 cm^{-1}. The absorption bands for the blank solution were observed at 700 cm^{-1} and 3425 cm^{-1}. There were no considerable changes in peaks between the FTIR peaks of blank diosgenin and test AgNPs containing solution [Fig. 2A and B]. The observed bands can be correlated to alcohols and phenols corresponding to polyphenolic encapsulates [20]. Therefore, the steroidal sapogenin used, diosgenin could be responsible for nanoparticle synthesis and encapsulation [32,33].

TEM and SEM techniques are similar in using electrons to observe the morphology and surface topography when used as a duo to observe a sample. They perform a localized analysis of the morphology of materials being tested at the nanoscale under different magnifications and can provide information on the particle size [34,35]. In this study, TEM and SEM analyses indicated the presence of spherical AgNPs. The particle size obtained using TEM was in the range of 13.23 nm [Fig. 3A and B].

Particle size is an important attribute for cellular uptake of nanoparticles which was 13.5 nm in this study. Nanoparticles of size less than 400 nm can accumulate in the tumor capillary which is 400–600 nm wide due to increased permeability [Fig. 4] [36,37]. The particle size observed in this report is suggestive of the efficacy of particles in targeting a tumor. XRD pattern of AgNPs indicate intense Bragg's reflections at 2θ values of 38.23°, 44.23°, 64.54° and 77.19°. The peaks could be assigned to the (111), (200), (220), and (311) typical planes of face-centered, cubic, and crystalline silver [38–40]. Unassigned peaks did occur, which could possibly be due to the organic phytoconstituent diosgenin used for the purpose of reducing the precursor silver nitrate

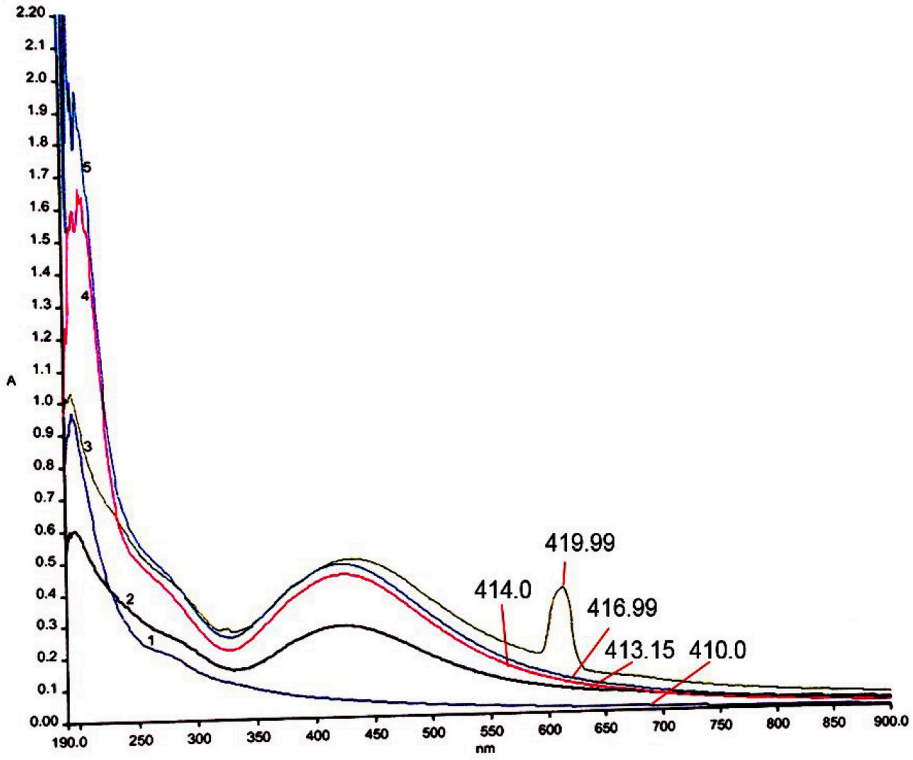

Fig. 1. UV spectrum of the synthesized AgNPs.

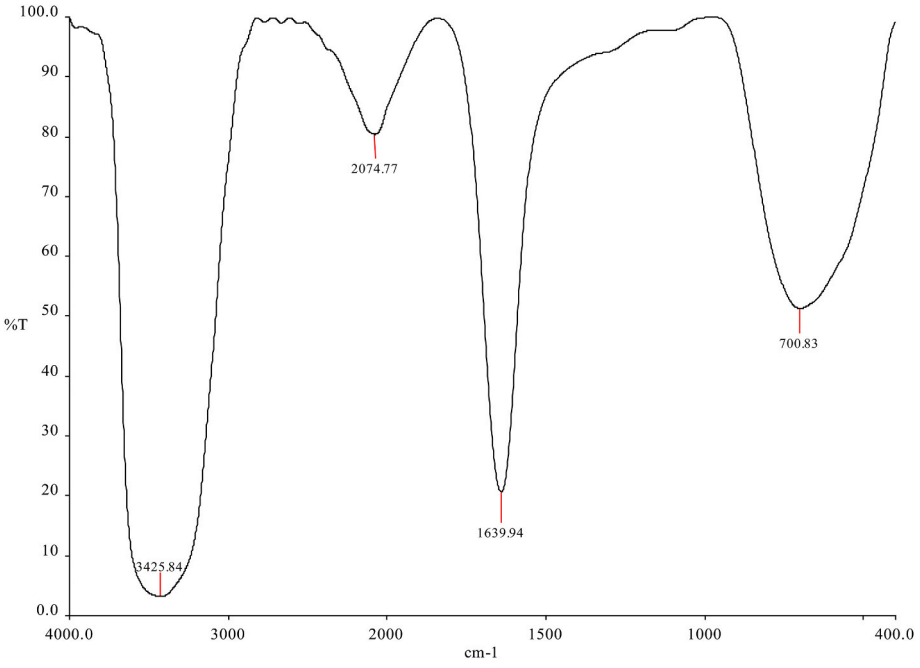

Fig. 2A. FTIR spectrum of the diosgenin control.

[41,42] [Fig. 5].

3.2. Anxiolytic and antidepressant effects

EPM is a simple, widely accepted assay for behaviour and anxiolytic effects because it uses the aversion concept of rodents towards height and spaces. It utilizes a natural stimuli that can induce anxiety in humans [43]. The outline of results obtained using this task can be reproduced in other class of species, anxiety/affective behavioral measures, research works and laboratories [44]. In EPM, anxiety is characterized by avoidance of open arms, increased duration of stay in the closed arms and a significant decrease in rearing [45,46]. The stay of the animal in central platform determines its decision-making ability. The total arm entries are determined by the measure of changes in anxiety or general activity [47]. The treatment with diosgenin nanoparticles indicates efficient anxiolytic effects with maximum time spent in open

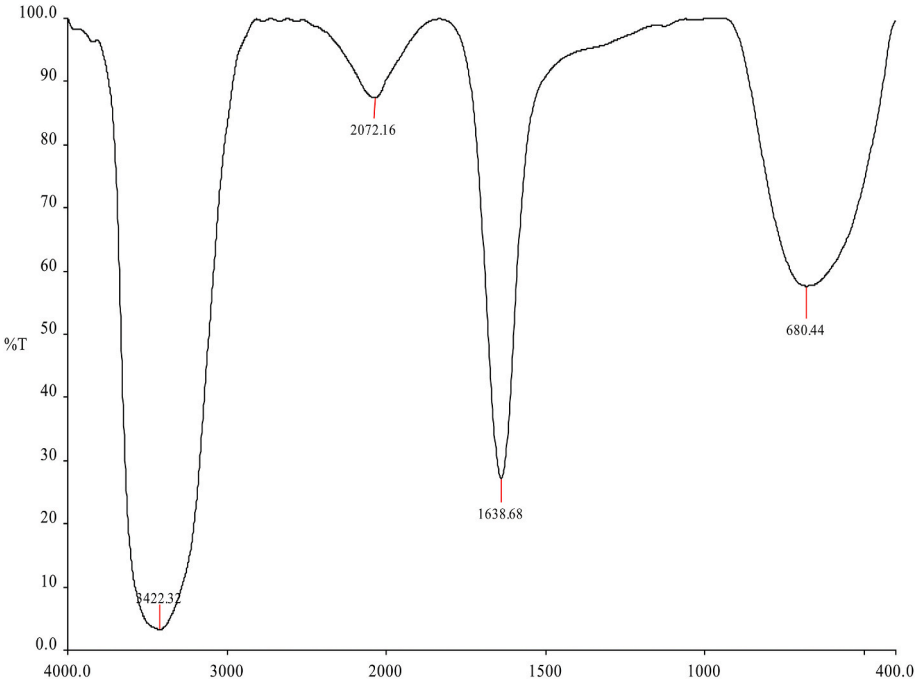

Fig. 2B. FTIR spectrum of the synthesized AgNPs.

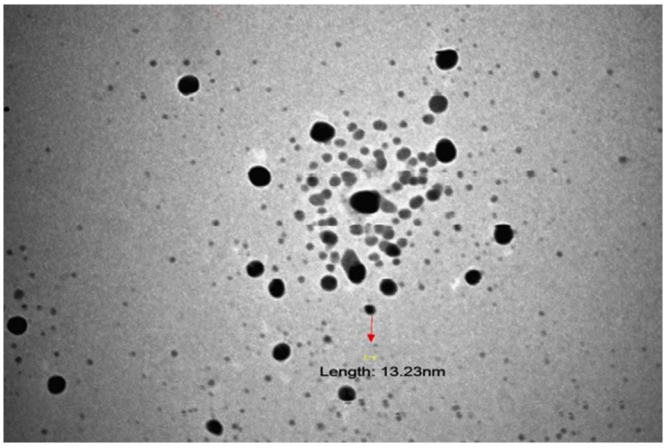

Fig. 3A. TEM image of the synthesized AgNPs at 200 nm.

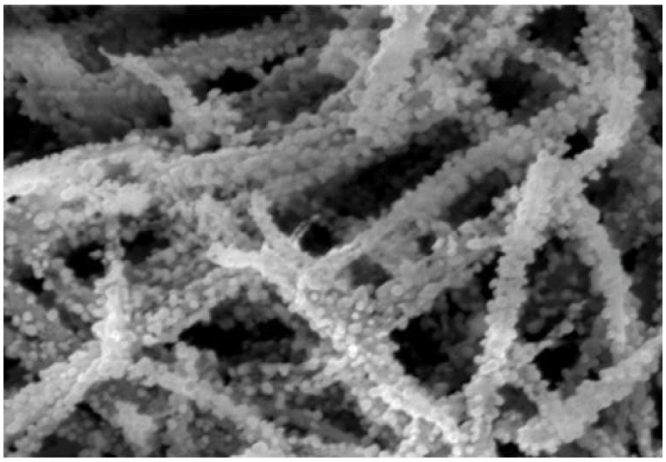

Fig. 3B. SEM image of the synthesized AgNPs at 200 nm.

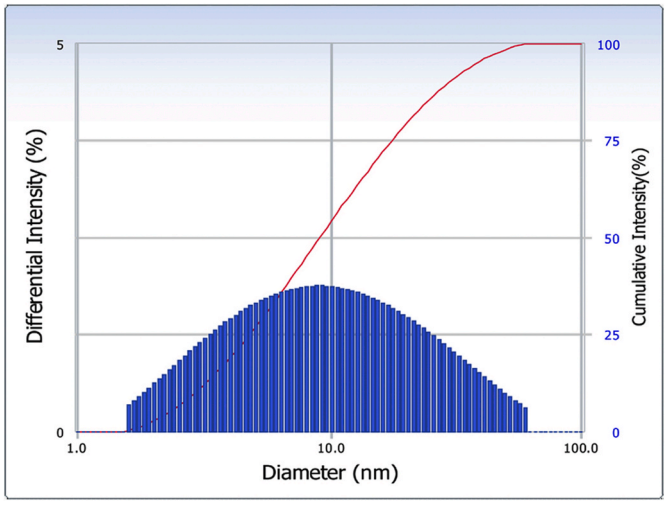

Fig. 4. Particle size analysis of the synthesized AgNPs.

arms, number of entries and limited time spent in closed arms [Table 1].

Diosgenin nanoparticles induced significant recovery as indicated by a decrease in their immobility time, gradual increase in swimming and significant increase in climbing time in comparison to the control [Table 2]. Diosgenin and its nanoparticles act as effective antidepressants which were proved through FST, a widespread method used to identify antidepressants [48].

Previous reports on phytochemicals and their pharmacology elucidate that they are indeed effective against CNS disorders [49]. Substances with antioxidant activity can result in anxiolytic effect as impediment of antioxidant defence is an important parameter in development of anxiety [50]. The total antioxidant capacity of diosgenin was 430.55 mg/g in ascorbic acid equivalents. Silver nanoparticles are known to possess antioxidant activity [51]. Therefore, the anxiolytic and antidepressant effects of diosgenin and its nanoparticles can be attributed to their antioxidant property.

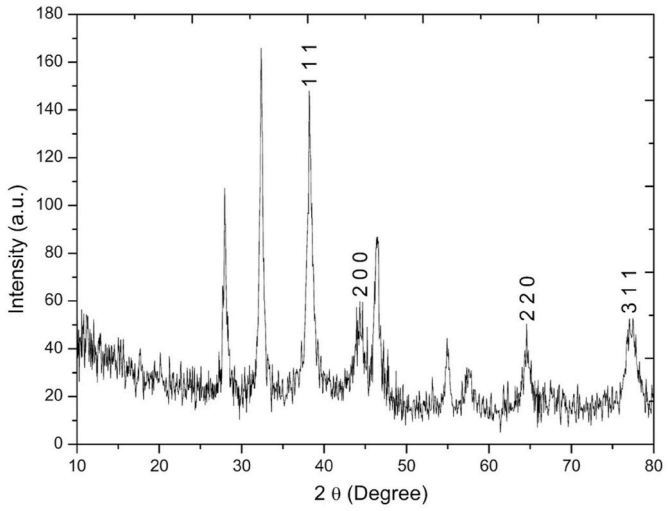

Fig. 5. XRD pattern of the synthesized AgNPs.

Table 1
Data of elevated plus maze test expressed as mean ± SEM (n = 6).

Groups	Time spent		Entries in Closed arm
	Open arm (sec)	Closed arm (sec)	
Control (5 ml/kg)	60.4 ± 1.38^{d}	20.20 ± 2.54^{a}	4.38 ± 2.04^{a}
Diazepam (2 mg/kg)	72.4 ± 1.38^{c}	18.18 ± 2.32^{a}	4.86 ± 2.12^{a}
Diosgenin (50 mg/kg)	76.12 ± 1.1 ^{b}	20.02 ± 2.08^{a}	3.26 ± 2.28^{a}
AgNPs (10 mg/kg)	80.18 ± 1.22^{a}	13.16 ± 2.28 ^{b}	3.20 ± 2.16^{a}

Table 2
Forced swimming test. Data are expressed as mean ± SEM (n = 6).

Groups	Immobility (sec)	Swimming (sec)	Climbing (sec)
Control (5 ml/kg)	42.0 ± 2.39^{a}	14.2 ± 4.32 ^{b}	50.0 ± 1.4^{a}
Imipramine (10 mg/kg)	38.28 ± 2.12^{a}	18.2 ± 3.92 ^{b}	32.02 ± 1.2 ^{b}
Diosgenin (50 mg/kg)	12.12 ± 1.98 ^{b}	26.02 ± 3.42^{a}	30.12 ± 1.02 ^{b}
AgNPs (10 mg/kg)	15.12 ± 1.61 ^{b}	28.02 ± 4.12^{a}	25.12 ± 1.02^{c}

3.3. Antitumor activity

Dalton's lymphoma originated in the thymus of murine models as a transplantable ascitic non-Hodgkin's T-cell lymphoma and is widely used for cancer research [52–54]. In DAL-induced animals, ascitic fluid is characterized as a nutritional source. Extension of life span, decrease in body weight and reduction of ascitic fluid accumulation are critical parameters in determining the efficacy of an oncosuppressive drug [55,56]. Considering these criteria, the following analyses were performed.

Body weight was increased in DAL mice. It was decreased to near-normal values via nanoparticle treatment. Reduction of body weight is a critical parameter in analysis of antitumor effect [57]. Myelosuppression that leads to anaemia and an elevation in WBC count, termed leukocytosis, are hematological parameters to identify an effective anticancer agent [58–62]. The results of this study indicate that the RBC and WBC levels altered significantly in comparison to tumor-induced group. Analysis of liver function by estimation of serum enzymes such as SGPT and cellular metabolite LDH are indicators of hepatic injury [63,64]. Renal function is determined by estimating the levels of uric acid and creatinine [65,66]. MDA is a toxic metabolite and marker for lipid peroxidation [67]. All these biochemical parameters were significantly brought back to near normal levels in nanoparticle-treated group in comparison to the mice of tumor-induced group [Table 3].

Table 3
Biochemical parameters *in vivo*. Data are expressed as mean ± SEM (n = 6).

Parameters	Normal	Tumor-induced	Diosgenin	AgNPs
Body weight (gm)	24.6 ± 2.88 ^{b}	30.0 ± 1.92^{a}	25.4 ± 0.54 ^{b}	26.6 ± 0.54 ^{b}
RBC (cells/cu. mm)	5.12 ± 0.022^{a}	2.52 ± 0.044 ^{b}	3.2400 ± 0.884 ^{b}	4.2600 ± 0.568^{a}
WBC (cells/cu. mm)	6000 ± 15.81^{c}	12281 ± 181.61^{a}	9368 ± 503.95 ^{b}	5870 ± 17.39^{c}
LDH (IUdL^{-1})	912.8 ± 2.58 ^{b}	2915.4 ± 0.54^{a}	903.86 ± 29.83 ^{b}	924.98 ± 55.6 ^{b}
MDA (nmoles/gm)	1.216 ± 0.39 ^{b}	2.354 ± 0.81^{a}	1.792 ± 0.55 ^{ab}	1.026 ± .028 ^{b}
SGPT (IU/l))	38 ± 0.47^{d}	120.4 ± 0.54^{a}	51.66 ± 0.80 ^{b}	46.62 ± 3.70^{c}
Uric acid (mg/dl)	3.54 ± 0.054 ^{b}	6.14 ± 0.114^{a}	5.74 ± 0.391^{a}	3.2 ± 0.158 ^{b}
Creatinine (mg/dl)	0.42 ± 0.044 ^{bc}	0.76 ± 0.054^{a}	0.3 ± 0.1^{c}	0.45 ± 0.05 ^{b}

Depression is a comorbid disorder that is prevalent among cancer patients especially elderlies [68–70]. Anxiety and depression may deteriorate the quality of the life in cancer patients. This may occur because of a delay in regaining the normal health status based on several factors including age [71]. Inflammation, hypothalamic pituitary adrenal axis, and excitotoxicity caused by neurotransmitters such as glutamate contribute predominantly to cancer-induced depression [72]. The communication between these physiological processes are intricate to analyse and requires effective research to prevent cancer-related depression [69].

4. Conclusion

AgNPs were synthesized using diosgenin as a reductant. The EPM, FST and biochemical parameters showed that the AgNPs synthesized using diosgenin were effective in the anxiolytic, antidepressant and antitumor effects as evidenced through this first ever report. Hence, AgNPs could be considered a valuable candidate for future medications related to mood disorders and cancer.

Author statement

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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