Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2019, 11(3):351-363. DOI: 10.15835/nsb11310473
Original Article

Acute and Sub-acute Toxicity of *Ganoderma applanatum* (Pres.) Pat. Extract Mediated Silver Nanoparticles on Rat

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Abstract

Ganoderm applanatum is a polypore macrofungus and traditionally used as medicine rather than fodder. Silver nanoparticles mediated by *G. applanatum* extract were synthesized, characterized and toxicity impacts were investigated for their pharmacological and medicinal uses. The synthesized silver nanoparticles were of average 58.78 nm in diameter with -13.8 mV zetpotential, analysed by dynamic light scattering method. Fourier transform infrared spectroscopy analysis of synthesized nanoparticles confirmed the capping and stabilizing with mycochemicals, that showed transmittance at 3606 cm^{-1} corresponds to O-H stretch for phenol, 2430 cm^{-1} corresponds to N-H stretch for primary and secondary amines. 2000 mg kg^{-1}, 200 mg kg^{-1} and 400 mg kg^{-1} doses of silver nanoparticles showed insignificant ($p<0.05$) increase in body weight and organ weight. Insignificant increase in RBC indices were observed and significant ($p<0.05$) increase in WBC count ($7.28\pm0.41 \times 10^3$ μL^{-1} and $8.41\pm0.35 \times 10^3$ μL^{-1}) at 200 mg kg^{-1} and 400 mg kg^{-1} doses of silver nanoparticles were observed. Thus, *G. applanatum* extract mediated silver nanoparticles can be used pharmacologically and medicinally due to their nontoxic effect.

Keywords: fungi; haematology; medicine; nanoparticles; toxicity

Abbreviations: BAS: basophil; BW: body weight; DLS: dynamic light scattering; EOS: eosinophil; FTIR: Fourier - transform infrared spectroscopy; Hb-haemoglobin; HD: high dose; LD: low dose; LYM: lymphocyte; MCHC: mean corpuscles haemoglobin concentration; MCV: mean corpuscles volume; MON: monocyte; NEU: neutrophil; OW: organ weight; PCV: packed cell volume (haematocrit); Pt: platelet; RBC: red blood coruscels; SEM: scanning electron microscopy; SNPs: silver nanoparticles; UV-Vis: ultraviolet visible; WBC: white blood corpuscles; XRD: x-ray diffraction

Introduction

Human health is threatened by several diseases and disorders which are difficult to treat with systemically and delivered drugs because the absorption and therefore bioavailability of drugs use in conventional pharmacotherapy depends on many factors, such as solubility, pKa, molecular weight, number of bonds per hydrogen atom of the molecule, and chemical stability etc. and all of these can hinder the activity and therapeutic response of drugs (Vilar *et al.*, 2012). Nanotechnology includes the materials in nanoscale ranges 1 nm to 100 nm with their modified fundamental properties such as solubility, diffusivity, blood circulation half-life, drug release characteristics, and immunogenicity (Zhang *et al.*, 2008).

Recently application of nanotechnology for drug delivery systems mainly focus on spherical nanoparticles such as calcium phosphate, gold, silver, iron oxide, polymeric micelles, liposomes, hydrogel nanoparticles and dendrimers etc. which have at least two main components, one of which is a pharmaceutically active ingredient and the other is engineered nanoparticle which may be biological origin or non-biological origin such as metals (Athar and Das, 2014). Recently more attention has been paid in the synthesis of metal nanoparticles especially silver and gold nanoparticles because metallic nanoparticles can be easily synthesized and modified that would allow them to bind with ligands, antibodies, drugs and easily delivery (Prasad *et al.*, 2013). It has also been reported metallic nanoparticles can be easily conjugate with various biological agents such as peptides, antibodies and DNA/RNA to specifically target different cells (Sperling and Parak, 2010) with polymers and are biocompatible in vivo for drug and gene delivery

Received: 16 Mar 2019. Received in revised form: 04 Sep 2019. Accepted: 17 Sep 2019. Published online: 30 Sep 2019.

(Nishiyama, 2007). They can also transform light into heat, thus enabling thermal ablation of targeted cancer cells (Chen *et al*., 2010) for the imaging of tumor cells and delivery of anticancer drugs (Gibson *et al*., 2007).

Synthesis of nanoparticles can be done by different methods such as physical, chemical, physic-chemical and biological methods (Kuila *et al*., 2108). Biological method of synthesis of nanoparticles is also called green synthesis which is easy, less cost effective and eco-friendly (Makarov *et al*., 2014). Among the metal nanoparticles synthesis of silver nanoparticles is quite easy without using or formation of any toxic chemical in the synthesis protocol and biological method of synthesis of nanoparticles are mediated by plant extract, bacteria, fungi, yeast, blue green algae and biological particles such as viruses, proteins, peptide etc. (Korbekandi *et al*., 2012; Iravani *et al*., 2014).

Metallic nanoparticles shows toxicity due to their minute size and they penetrate the basic biological structures, disrupting their normal functions such as include tissue inflammation, increased production of reactive oxygen species and cell damage and death (Zoroddu *et al*., 2014). Two major risks of nanoparticles to produce toxicity are that they can cross tissue junctions and even cellular membranes and accumulate in specific organs, such as the liver, spleen, myeloid tissue etc. (Albanese *et al*., 2012). In recent decade use of silver nanoparticles has explored due to its powerful anti-microbial and light-weight, small size and other characteristics however its safety is continuously debated for therapeutic use in drug delivery system (Holsapple *et al*., 2005; Shin *et al*., 2015). It has also been reported that silver is used as therapeutic agent for long time and recent researches reported silver nanoparticles induce toxicity in various cancer cell lines (Sambale *et al*., 2015).

Ganoderma applanatum is a polypore macrofungi with hard and woody fruiting body (Niemela and Miettinen, 2008). Macrofungi belong to genus *Ganoderma* has been traditionally used as medicine rather than fodder in China, Japan and India for therapy of various diseases (Wasser, 2011).

Present study was carried out to synthesis of SNPs mediated by *G. applanatum* extract, their characterization and toxicity impacts on body weight (BW), organ weight (OW) and blood profile of normal albino rats because toxicity impact of SNPs on mammalian model has been least explored.

Materials and Methods

Collection of macrofungi

Fresh fruiting body of *G. applanatum* (presented in Fig. 1) was collected from Kaziranga National Park of Assam (26° 30' N -26° 45' N to 93° 08' E- 93° 36' E) and was match and identified on the basis of morphology with museum specimen by Plant Identification and Preservation Division of Department of Botany, Gauhati University, Assam where a voucher specimen (No. 833 M) was deposited and another fruiting body of *G. applanatum* brought to Department of Zoology, Ranchi University, Ranchi for further studies.

Preparation of extrac

The fresh fruiting bodies of *G. applanatum* were initially washed by distilled water and then by absolute ethyl alcohol (99.8%) to avoid microbial contamination. The mushrooms were dried in shade under room temperature for six to seven days, powdered and sieved. Fifty (50) g of the fine powder was subjected to extraction chamber of Soxhlet and 300 mL distilled water was taken in boiling flask as extraction solvent for aqueous extraction. The extract obtained was filtered, concentrated and dried in rotary flash evaporator maintained at 45 °C for proper dehydration and the dried extracts were stored in air tight containers at room temperature for further studies (Dandapat *et al*., 2015a).

Mycochemical analysis

Freshly prepared extract was used for mycochemical analyses. Presence of various biochemicals in the aqueous extract of *G. applanatum* was analysed followed protocols described by Arya *et al*. (2012) and co-worker.

Biosynthesis of nanoparticles

The synthesis of silver nanoparticles (SNPs) was done slight modification of previous method of Dandapat *et al*. (2014), Kumar and Sinha (2017). Synthesis of nanoparticles were done by mixed 3 mL (41 mg/mL) of *G. applanatum* fruiting body aqueous extract and 197 mL of 0.1M silver nitrate (169.87 g/mol) solution (i.e., 3.35 g $AgNO_3$ in 197 mL of distilled water) and incubated by using hot magnetic stirrer bar at 80 °C, until the light yellow colour of the solution was changed to dark brown.

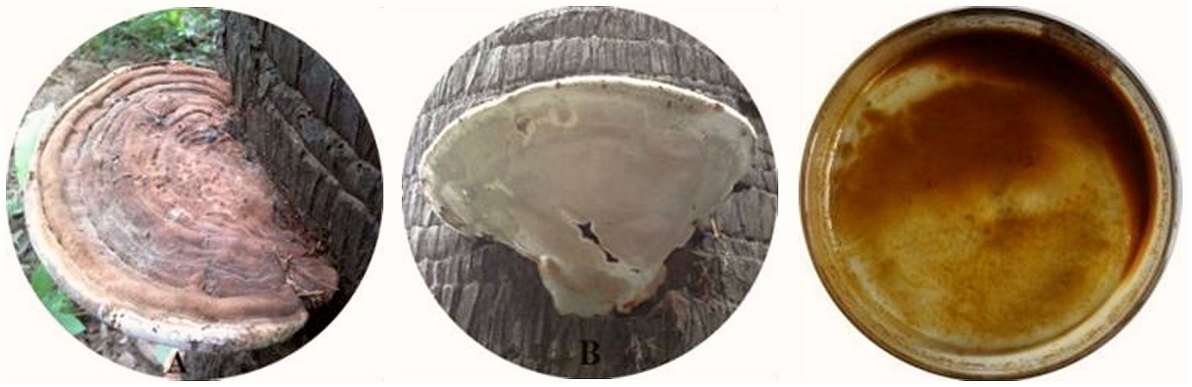


Fig. 1. Fruiting body of *G. applanatum* (A and B) and extract (C)

Then the solution was cooled to room temperature and centrifuged at 15000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with distilled water and was dried in the incubator at room temperature for characterization.

Characterization of synthesized SNPs

Synthesized SNPs were characterization by UV-Visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), X-Ray diffraction analysis (XRD), dynamic light scattering (DLS) analysis and Fourier transform infrared spectroscopy (FTIR) described below.

UV-Vis spectra analysis of SNPs

SNPs sample for UV-Vis spectra analysis was prepared by dilute 1 mL of pure SNPs solution in 4 mL of deionized water and 1 mL of diluted sample was taken in standard quartz cuvette and placed in sample compartment. UV-Visible spectra analysis was done by using Parkin Elmer Lambda-25 UV-Visible spectrophotometer (PerkinElmer Inc., USA). The UV-Visible spectrophotometer was operated at 240 V, 20±2 °C, 60-70% humidity and light test specification at 200-800 nm wave length (Kumar *et al*., 2018).

SEM analysis of SNPs

Scanning electron microscopy was done using JEOL JSM-6390 LV (Japan) machine provided with Vega TC software. Thin layer of nanoparticles powder sample (1 mg) was prepared on glass slide and then press on a carbon taped copper grid for SEM. Excess powder on surface of carbon taped copper grid was blown away with compressed air and the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min and was coated with platinum using ion sputter (Bini *et al*., 2018).

XRD analysis of SNPs

XRD analysis of the prepared sample of nanoparticles was done using a Rigaku-smartlab powered diffraction XRD machine with 40 kV operating voltage and 15 mA current, Cu-Kα X-rays of wavelength (λ)=1.54056 Å and data was taken for the 2θ range of 10° to 90° with a step of 0.02°. The particle size was calculated by considering the peak at degrees and by using Debye-Scherrer formula (Kumar and Rao, 2013).

$$D = \frac{0.9\lambda}{\beta cos\theta}$$,

Where, 'λ' is wave length of X-Ray (0.1541 nm), 'β' is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter size.

DLS analysis of SNPs

The sample was diluted, filtered and 0.1 mg/mL concentration of SNPs colloidal solution was ultra-sonicated at 20% sonication amplitude with continuous mode during 882 second to avoid agglomeration and for proper dispersion of nanoparticles in the solution. The dynamic light scattering for particle size and zeta potential analysis of nanoparticles was carried out using Malvern Nano ZS green badge) ZEN3500 (U.K.) zeta sizer provided with zetasizer Nano software (ZNUM, 2013).

FTIR spectra analysis of SNPs

FTIR spectra analysis was carried out IPR resting-21 (Shimadzu Corp., Kyoto, Japan) in the diffuse reflectance mode operated at 4 cm^{-1} in the range of 400 cm^{-1} to 4 000 cm^{-1} wave number and KBr as standard to identify the potential biomolecules present in fruiting body of *G. applanatum* extract which are responsible for reducing and capping the bio reduced *G. applanatum* extract mediated SNPs. The FTIR machine was operated at 25±5 °C, 60-70% humidity and 240 V AC (IMUSG, 2002).

Toxicity of silver nanoparticles

Animals

Wistar albino rats (*Ratus norvegicus*) of mass between 175 to 200 g were obtained from the National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at ambient temperature of 25 ± 2 °C and relative humidity at 50 ± 15 %, with dark-light cycle of 12 h. Animals were fed with a commercial pallet diet (Sadguru Shri Shri Industries Pvt. Ltd. Pune, India) and water. The experiment was performed after prior approval of the Ethics committee of Ranchi University, Ranchi (Proceeding no. 46, page no. 137).

Acute toxicity study

According to OECD test guideline 425 (Up and Down procedure) limited test for *G. applanatum* extract mediated SNPs was performed at the test dose 2000 mg/kg. Ten (10) rats were equally divided into two groups (A and B) and were fasted (3-4 hours) prior to dosing but were accessed with water *ad libitum*. Single dose (2000 mg/kg) of *G. applanatum* extract mediated SNPs and vehicle (distilled water) were administrated to single animals of each group by gavage using stomach tube and rats were provided with food and water *ad libitum* after 2 hours. Similarly 4 other rats of each group were treated with *G. applanatum* extract mediated SNPs and vehicle (OECD, 2008a; Saleem *et al*., 2017). Animals were observed for clinical symptoms of toxicity and mortality during the treatment period (8 days), the initial body weight (IBW), final body weights (FBW) and weight of vital organs (OW) of animals of both the groups were recorded.

Sub-acute toxicity of silver nanoparticles

Sub-acute toxicity of the SNPs was evaluated on Wistar albino rats as per the Organization for Economic Co-operation and Development (OECD) guidelines with slight modification (Njoya *et al*., 2008; OECD, 2008b). Fifteen fresh male rats were distributed among three treatment groups (Group: 1, 2 and 3) and each group contains 5 animals. The animals were received single dose vehicle, high and low of SNPs daily for 7 days as described below.

Group-1: Rats of this group serve as control and were received 1 mL distilled water orally for 7 days.

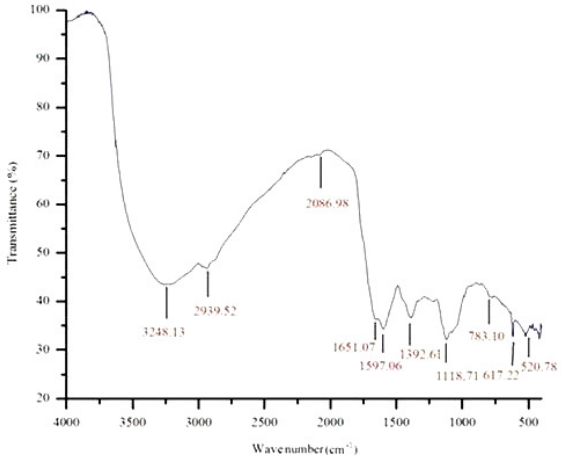
Group-2: Rats of this group were received 200 mg kg^{-1} body weight (low dose: LD) of *G. applanatum* extract mediated SNPs orally for 7 days.

Group-3: Rats of this group were received orally 400 mg kg^{-1} body weight (high dose: HD) of *G. applanatum* extract mediated SNPs orally for 7 days.

Animals were observed for clinical symptoms of toxicity during the treatment period (for 7 days), and the body weights of animals were recorded before blood collection by retro-orbital sinus blood collection method. At the end of the treatment (8th day), animals were fasted overnight, but allowed free access to water. These animals were anesthetized with ether and were sacrificed by cervical dislocation for the collection vital organs such as heart, liver, kidney, heart and testis. OWs of treatment groups were calculated and compared with the OW of the control group animals.

Impact of silver nanoparticles on blood profile rat

For hematological indices, blood samples were collected into sterile tubes containing EDTA and immediately analysed for TWBC, RBC, HB, PCV, MCV, MCH, MCHC, PLT and LYM using Sysmex automated blood analyser - KX 21 Kobe, Japan.



Results

The collected fruiting body of *G. applanatum*, cultured mycelia and extract obtained is presented in Fig. 1. The collected basediocarp was semi-circular in shape, 13 cm in diameter. Outer surface of carp having wrinkled zones of brownish to greyish-brown colour and the lower surface is white.

Mycochemical screening

Result of mycochemical screening is presented in Table 1. In the present study different mycochemicals such as carbohydrate, protein, alkaloid, flavonoid, saponins, steroid, phenolics etc. were found in the aqueous extract of fruiting body of *G. applanatum*.

FTIR spectra analysis of G. applanatum extract

FTIR spectroscopy spectra of *G. applanatum* extract is presented in Fig. 2. FTIR spectroscopy analysis of *G. applanatum* extract showed major transmittance peaks at 3248. 13 cm^{-1} for phenol O-H stretch, 2939.52 cm^{-1} for alkyl C-H stretch, 2086.98 cm^{-1} for terminal alkyne C≡C stretch, 1651.07 cm^{-1} for amide C=O stretch, 1597.06 cm^{-1} for primary amine N-H stretch, 1392.61 cm^{-1} for fluro alkane C-F stretch, 783.10 cm^{-1} for aromatic (metadisub bengene) C-H stretch, 617.22 cm^{-1} for chloro akane C-Cl stretch and 520.78 cm^{-1} for bromo alkane C-Br stretch.

Table 1. Screening of proximate mycochemicals present in aqueous fruiting body extract of *G. applanatum*

Mycochemicals	Present(+) or Absent (+)
Carbohydrate	+
Glycosides	+
Protein	+
Alkaloid	+
Steroid	+
Triterpene	+
Flavonoid	+
Tannin	+
Lipid	+
Saponin	+

Synthesis and characterization of SNPs

Synthesis of silver nanoparticles mediated by aqueous fruiting body extract solution is presented in Fig. 3. Result showed the change of pale yellow colour of mixed solution of extract and $AgNO_3$ solution turns into dark brown as the temperature and incubation time period increase, which indicates the formation of nanoparticles.

UV-Visible spectroscopy analysis

The absorption spectrum of nanoparticles obtained from UV-visible absorption spectroscopy is presented in Fig. 4, which shows peak at 400 nm corresponds to the surface plasmon resonance.

Scanning electron microscopy (SEM) analysis

Scanning electron microscopy provided the confirmation about the morphology of synthesized green nanoparticles. Result of SEM analysis is presented in Fig. 5, which showed the synthesized nanoparticles are spherical shaped and size ranges from 70 nm to 120 nm in diameter.

X-ray diffraction analysis

The information pertaining to phase formation, translational symmetry present and size and shape of the unit cell are obtained from peak positions in the diffraction pattern of a sample. The X-ray diffraction pattern of the *G. applanatum* extract mediated synthesized SNPs are shown

in Table 2 and Fig. 6. The result showed particles of 60.60 nm to 121.19 nm were formed with average particle size 102.08 nm. The diffraction pattern has been analysed and refined using open source full proof analysis software. It consists of the major peaks of silver nanoparticles with fcc type lattice and some additional unassigned peaks, which may be attributed to the formation of bio-organic phase acting as surfactant for the silver nanoparticles.

Table 2. Average size estimation of *G. applanatum* extract mediated nanoparticles using X-ray diffraction analysis of and using Scherrer formula

Copper K radiation: Wavelength λ (nm) = 0.154

2θ of the major peaks (deg.)	θ of the peak s (deg.)	d-spacing (Å)	Intensity (cps)	FWHM of major peaks (β: deg.)	FWHM of the major peaks (β: rad.)	Size (Å)	Size (nm)	Avg. Size (nm)
35.51	17.75	2.52581	9732.26	0.0796	0.0013	1093.7	109.37	102.08
29.66	14.83	3.00943	1690.34	0.0732	0.0012	1171.7	117.17	
21.73	10.86	4.08588	1652.76	0.1393	0.0024	606.0	60.60	
75.09	37.54	1.26401	1411.44	0.0863	0.0015	1211.9	121.19	

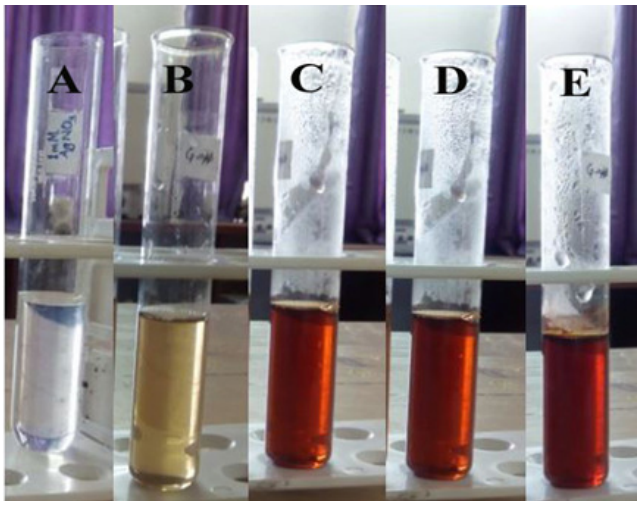


Fig. 3. Change in colour of mixed solution: (A) $AgNO_3$ solution; (B) $AgNO_3$ solution and extract at room temperature; C, D and E-mixed solution after heat stirring of 30 minutes, 1 hour and 2 hours respectively

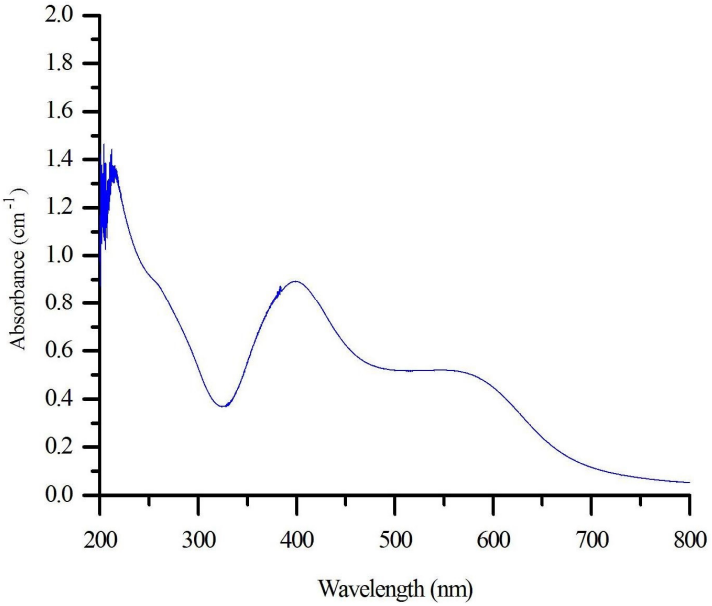


Fig. 4. UV-Visible spectrum of synthesized SNPs mediated by aqueous fruiting body extract of *G. applanatum*

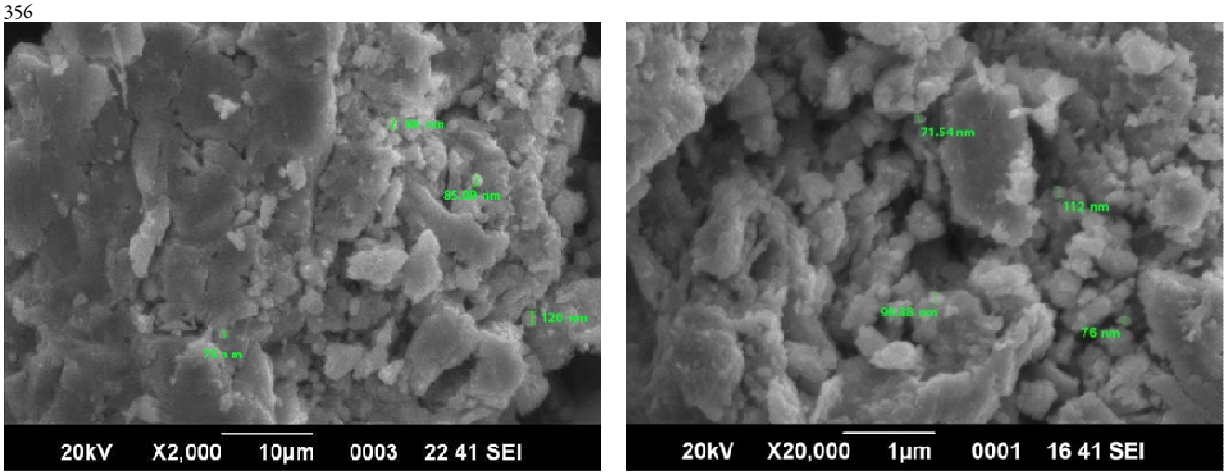


Fig. 5. Scanning electron microscopy photograph of SNPs mediated by aqueous fruiting body extract of *G. applanatum*

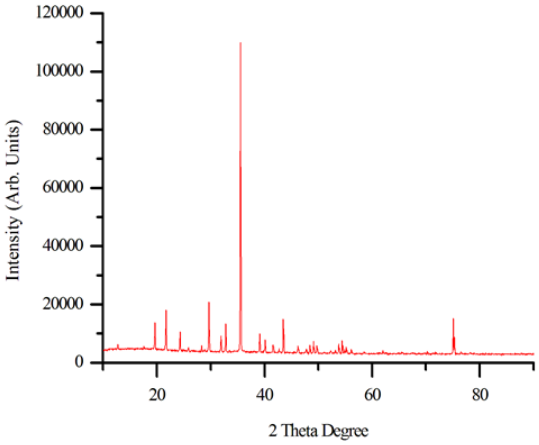


Fig. 6. X-Ray diffraction peaks of *G. applanatum* extract mediate nanoparticles powder

Dynamic light scattering (DLS) analysis

Size and distribution of nanoparticles play a fundamental role in quality control of nanoparticles synthesis. It also basically associated diffusivity and passage of nanoparticles through cell membranes in the field of nano biotechnology. In the present study size distributions of number, intensity, volume and zeta potential of synthesized *G. applanatum* extract mediated nanoparticles were analysed by DLS method. The results of DLS analysis are presented in Figs. 7, 8, 9 and Table 3. Result of zeta potential analysis if SNPs is presented in 10 and 11 (hypothetical figure).

Cumulants mean (Z-average) obtained by DLS analysis for the synthesized silver nanoparticle is 58.78 d.nm corresponds to average size in diameter. Fig. 7 (particle size distribution by intensity) represents high peak for nanoparticles of 77.25 nm diameters with 99.6% intensity and small peak for 5177 nm diameter nanoparticles with 3.4% intensity. Fig. 8 (particle size distribution by volume) represents high peak and low peak for nanoparticles of 88.4% and 11.3% size distribution by volume of 38.21 nm and 5282 nm diameter nanoparticles respectively. Fig. 9 (particle size distribution by number) represents single peak for nanoparticles of diameter of 23.64 nm with 100% size distribution by number. Total Number of SNPs with their respective size distribution also revealed that, the SNPs were distributed evenly in the in sample solution (Table 3). Zeta potential is the electrostatic charge distribution, develops in liquid layer or capping materials on surface presented in Fig. 10. In present study the zeta potential analysis of nanoparticles provides peak at -13.8 mV potential with 100% area distribution, presented in Fig. 11.

FTIR analysis of SNPs

In the present study FTIR analysis of silver nanoparticles synthesized from *G. applanatum* extract is presented in Fig. 12. The result represents absorption peaks at 3606 cm^{-1} corresponds to O-H stretch for alcohol and phenol, 2430 cm^{-1} corresponds to N-H stretch for primary and secondary amines, 2156 cm^{-1} corresponds to C≡ stretch for alkynes, 1681 cm^{-1} corresponds to C=N for amines or C=O stretch for unsaturated aromatic carboxylic acid, 1234 cm^{-1} corresponds to C-O stretch for aromatic compound, 1091 cm^{-1} corresponds to C-F stretch for fluroalkanes, 925 cm^{-1} corresponds to C=C stretch foralkanes and also stretch for O-H and 613 cm^{-1} corresponds to C-Cl or C-Br stretch for chloro and bromo alkanes.

Acute and sub-acute toxicity effect of SNPs

Results of acute toxicity of SNPs are presented in Tables 3 and 4. The results showed no mortality was observed in rats of vehicle and SNPs treated rats at 2000 mg/kg dose. Behavioral observation of the test animals after dosing showed elevated respiration rate and sleeping for first 30 min (Table 4). However, no other behavioural changes such as convulsions, tremors, itching, shivering, somatomotor activity etc. were observed in the animals group treated with SNPs (Table-4) for 4 hours, 24 hours and 7 days. Result acute toxic impact of SNPs on body weight and organ weight is presented in Table 5. The results showed that, final BW of rats significantly ($p<0.05$) increased compare to initial BW of rats treated with extract and final BW of rats treated with vehicle. A non-significant increase in weight of liver, kidneys, lungs and heart of rats treated with SNPs compare to vehicle treated group. However, significant ($p<0.05$) decrease in weight of testes was observed in SNPs treated groups compare to vehicle treated group.

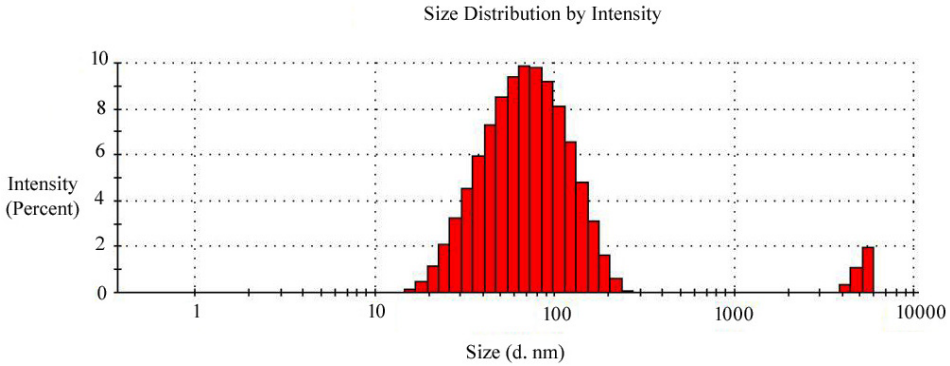


Fig. 7. DLS size distribution by intensity of *G. applanatum* extract mediate SNPs

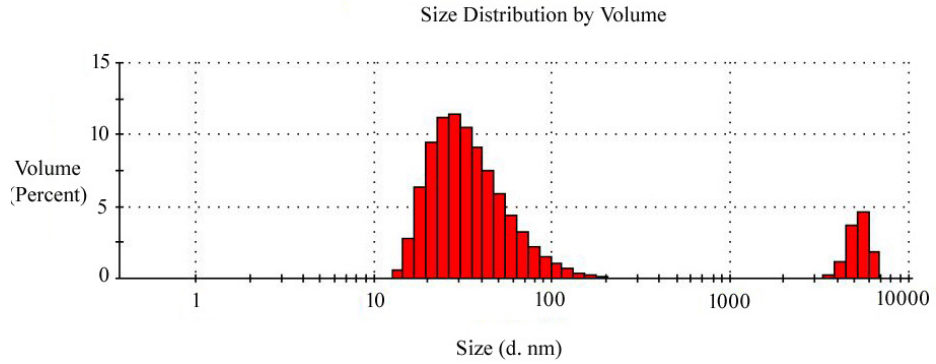


Fig. 8. DLS size distribution by volume of *G. applanatum* extract mediate SNPs

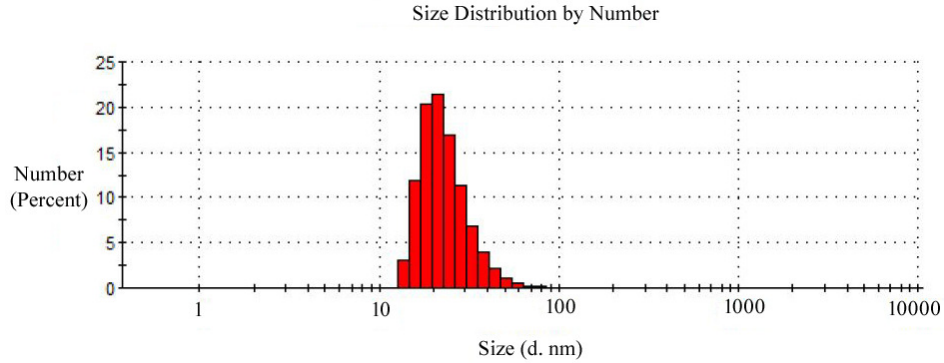


Fig. 9. DLS size distribution by number of *G. applanatum* extract mediate SNPs

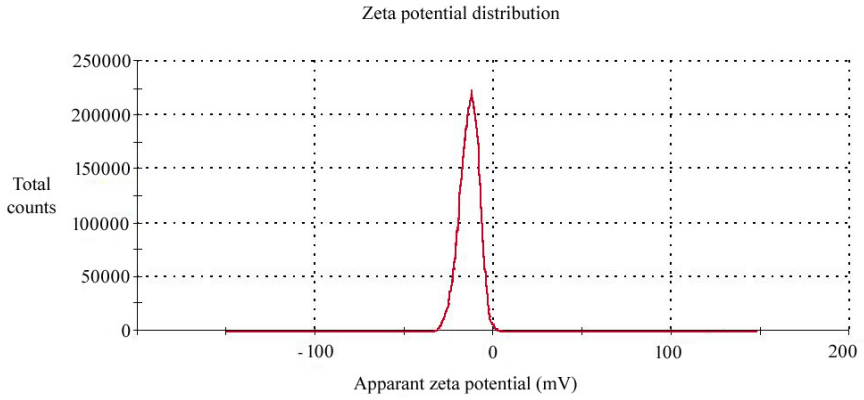


Fig. 10. DLS zetapotential of *G. applanatum* extract mediate SNPs

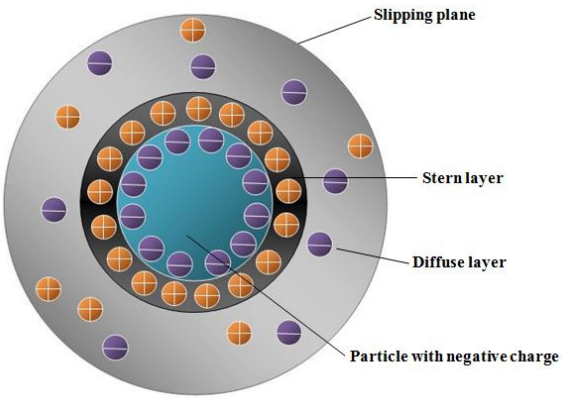


Fig. 11. Dielectric potential exists at the boundary of a SNP (Ztasizernano user manual, 2013; Haider and Kang, 2015)

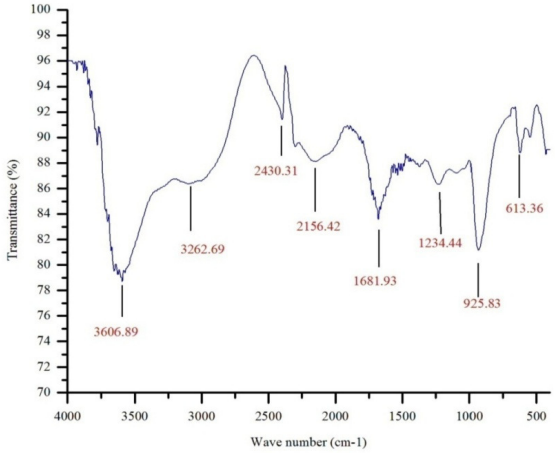


Fig. 12. FTIR sperta of *G. applanatum* extract mediated silver nanoparticles

Table 3. Size distribution report of synthesized nanoparticles by intensity, by volume and by number

Size distribution by intensity				
Peaks	Size (d. nm)	% Intensity	St Dev. (d. nm)	Z-Average (d. nm)
1	77.25	96.6	40.37	58.78
2	5177	3.4	487.1	
Size distribution by volume				
Peaks	Size (d. nm)	% Volume	St Dev. (d. nm)	Z-Average (d. nm)
1	38.21	88.4	23.11	58.78
2	5282	11.6	711.5	
Size distribution by number				
Peaks	Size (d. nm)	% Number	St Dev. (d. nm)	Z-Average (d. nm)
1	23.64	100	8.379	58.78

Table 4. Behavioural symptoms of rats treated with vehicle and SNPs 2000 mg/kg. N=nontoxic effect, I=increase, NF=not found

Groups of rats / Parameter	Group-A				Group-B			
	30 Min.	4 Hrs.	24 Hrs.	7 Days	30 Min.	4 Hrs.	24 Hrs.	7 Days
Fur & skin	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N
Breathing	I	N	N	N	I	N	N	N
Somatomotor activity & behavior pattern	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	I	N	N	N
Convulsions & tremors	NF	NF	NF	NF	N	N	N	N
Itching	NF	NF	NF	NF	NF	NF	NF	NF
Coma	NF	NF	NF	NF	NF	NF	NF	NF
Death	NF	NF	NF	NF	NF	NF	NF	NF

Table 5. Organ weight and body weight of rats treated with vehicle and SNPs 2000mg/kg. n=5±SE of mean, a=p<0.05, b=p<0.025, c=p<0.005

Groups of rats / BW and OW (g)	Group-A	Group-B
Initial BW	178.61±1.58	181.14±1.47
Final BW	181.58±1.16	185.10±0.90[a,b]
Liver	5.52±0.38	4.87±0.37
Kidneys	1.60±0.13	1.56±0.12
Heart	0.78±0.04	0.79±0.04
Lungs	0.93±0.09	0.93±0.09
Testes	2.42±0.11	1.94±0.05[c]

Effect of LD and HD of SNPs on body weight, organ weight and behavior

Results of *G. applanatum* extract mediated SNPs on BW, OW and behaviour are presented in Table 6 and 7. The results showed final BW of rats treated with extract significantly (p<0.05) increased compare to initial BW of rats treated with LD and HD of SNPs.

Effect of LD and HD of SNPs on hematological parameters

Effect of *G. applanatum* extract mediated SNPs on hematological profile of rats is presented in Table 8. The results showed SNPs non-significantly elevated RBC, Hb, PCV, MCV and MCHC. SNPs significantly (p<0.05) elevated WBC, neutrophil and lymphocyte count in LD and HD treatment groups compare to control group. *G. applanatum* extract mediated SNPs significantly (p<0.05) elevated platelet count in HD treatment group compare to control group.

Discussion

Mushrooms are also called as macrofungi possess high antioxidant activity and therapeutic efficacy due to their synthesized antioxidant compounds such as phenolics, organic acids, alkaloids etc. derived by saprophytic metabolism and the mushrooms can be used as a fodder and in the pharmaceutical industry (Menaga *et al*., 2012;

Table 6. Behavioural symptoms of rats treated with vehicle, SNPs 200 mg/kg and 400 mg/kg. N=nontoxic effect, I=increase, NF=not found

Groups of rats / Parameter	Group-1				Group-2				Group-3			
	30 Min.	4 Hrs.	24 Hrs.	7 Days	30 Min.	4 Hrs.	24 Hrs.	7 Days	30 Min.	4 Hrs.	24 Hrs.	7 Days
Fur & skin	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Breathing	I	N	N	I	I	N	N	I	I	N	N	I
Somatomotor activity & behavior pattern	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Convulsions & tremors	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Itching	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Coma	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
Death	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF

Table 7. Organ weight and body weight of rats treated with vehicle and SNPs 200 and 400 mg/kg. n=5±SE of mean, a=p<0.05

Groups of rats / BW and OW (g)	Group-1	Group-2	Group-3
Initial BW	180.36±1.38	186.06±2.62	184.74±2.52
Final BW	183.76±1.51	189.42±2.62[a]	187.86±2.33[a]
Liver	5.47±0.24	6.40±0.54	6.14±0.55
Kidneys	1.64±0.12	2.07±0.24	1.89±0.24
Heart	0.77±0.01	0.91±0.11	0.96±0.11
Lungs	1.08±0.13	1.38±0.18	1.24±0.12
Testes	2.47±0.11	2.63±0.09	2.50±0.15

Table 8. Effect of SNPs on hematological profile rats treated with vehicle and SNPs 200 and 400 mg/kg. n=5±SE of mean, a=p<0.05, b=p<0.01, c=p<0.005, d=p<0.0025, e=p<0.0005

Groups of rats / BW and OW (g)	Group-1	Group-2	Group-3
RBC X $10^6 \mu L^{-1}$	4.21±0.02	4.42±0.13	4.46±0.09
Hb g dL^{-1}	11.66±0.21	11.70±0.31	11.83±0.32
PCV %	26.34±0.45	26.66±0.46	26.64±0.62
MCV fL	93.00±0.15	93.01±0.15	93.04±0.16
MCHC g dL^{-1}	23.71±0.16	23.86±0.18	24.02±0.15
WBC X $10^3 \mu L^{-1}$	6.80±0.20	7.28±0.42^{da}	8.56±0.35^{a}
NEU %	23.27±0.17	24.46±0.32^{b}	25.04±0.17^{e}
MON %	7.21±0.32	7.52±0.37	7.91±0.52
LYM %	32.88±0.29	33.23±0.23^{ac}	33.65±0.25^{c}
EOS %	4.43±0.03	4.51±0.24	4.33±0.23
BAS %	0.73±0.003	0.74±0.007	0.75±0.009
Pt %	339.40±0.74	340.84±0.55	340.85±1.35^{b}

Dandapat and Sinha, 2015b). Previous FTIR analysis showed *Ganoderma lucidum* extract contains high protein and carbohydrates with spectra transmission peaks of 1800-1400 cm^{-1} and 889 cm^{-1} respectively (Keong *et al*., 2012). It was previously reported that *Ganoderma* spp. contains biochemicals such as alkaloids, flavonoids, reducing polysaccharides, tannins, glycosides, phenolics and steroids etc. possess medicinal values in healthcare drug delivery system and medicines (Usman *et al*., 2012). In present study *G. applanatum* extract possess good numbers of biochemical (Table 1 and Fig. 2) which are associated with bioactivity and various medicinal properties and reflects the previous studies.

Mycochemicals such as phenols, flavonoids and tannin etc. possess high antioxidant activity and reduces free radicals (Dandapat *et al*., 2018). In this study change of pale yellow colour of mixed solution of extract and $AgNO_3$ solution turns into dark brown as the temperature and incubation time period increase (Fig. 3), which indicates the formation of nanoparticles due to reduction of silver ions into nanoparticles by the active mycochemicals (Firdhouse *et al*., 2012). The UV-visible absorption spectrum of nanoparticles showed peak at 400 nm (Fig. 4) corresponds to the surface plasmon resonance and according to the previous studies, formation and stability of silver nanoparticles mediated by extracts from biological sources such as plants and fungi show maximum absorption 400-500 nm correspond to the surface plasmon resonance (SPR) for silver nanoparticles (Khan *et al*., 2013; Gujral, 2015). SEM of synthesized SNPs (Fig. 5) matched with previous study report of *Boswellia ovalifoliolata* extract mediated SNPs of spherical shaped, 30-40 nm diameter SNPs (Gurunathan *et al*., 2012). Result of present study correlates with previous study of *B. ovalifoliolata* extract mediated SNPs. X-ray diffraction pattern of a powder sample provides the information pertaining to phase formation, translational symmetry present and size and shape of the unit cell are obtained from peak positions in the diffraction pattern of a sample (Brady *et al*., 1995). Synthesized nanoparticles mediated by from *Ganoderma sessiliforme* extract of 45.26 nm has been studied by XRD and previously it has also been reported nanoparticles synthesized using *Ganoderma lucidum* mycelia extract of average size 6 nm were analysed by XRD (Kumar *et al*., 2017). In present study *G. applanatum* extract mediated SNPs (Fig. 6 and Table 2) of average 102.08 nm consists of the major peaks of silver nanoparticles with fcc type lattice and some additional unassigned peaks, which may be attributed to the formation of bio-organic phase acting as surfactant for the silver nanoparticles (Kumar and Rao, 2013). Dynamic light scattering (DLS) is also known as photon correlation spectroscopy (PCS) and has been widely used for analysis of nanoparticles size in liquid phase, particle shape, colloidal stability, and surface coating (Lim *et al*., 2013). The result of DLS analysis of nanoparticles distribution by intensity in the colloidal solution depends upon the rate of fluctuation of intensity of the laser beam by the particles of different size bean due to Brownian motion (ZNUM, 2013; Nanocomposix, 2015). Although, an intensity distribution is the fundamental size distribution generated by DLS which is further converted to a volume distribution and the volume distribution further converted to a number distribution by inbuilt software of nanozetasizer (ZNUM, 2013). The DLS size distribution by number analysis of nanoparticles represents the total number of particles of different size bins was reported in previous study (Nanocomposix, 2015; Kumar and Sinha, 2017). Zeta potential is the electrostatic charge distribution, develops in liquid layer or capping materials on surface (stern layer) of the nanoparticles and diffuse layer present outside the stern layer which gives the potential stability of the particles in colloidal system presented in Figure 11 (ZNUM, 2013; Haider and Kang, 2015). It has been reported nanoparticles dispersion with ± 10 to 20 mV are moderately stable (Tucker *et al*., 2015). Zeta potential of nanoparticles within -25 mV provides efficiency of the capping material to stabilize the nanoparticles in colloid solution and their evenly distribution (Bhattacharjee, 2016). Results of DLS analysis of *G. applanatum* extract mediated SNPs of average size 58.78 d.nm and -13.8 mV correlates with the stability and highly distribution of SNPs in colloidal solution (Fig. 7 to 11 and Table 3). FTIR analysis provides confirmation of presence of biomolecules by analysis of functional groups and provides the confirmation of capping tendency of therapeutic molecules

of biological extracts present on the surface of synthesized nanoparticles (Khan *et al*., 2013). Synthesized gold nanoparticles (AuNPs) by extract of *G. lucidum* showed strong bands of FTIR spectra at 602,1096, 1201, 1388, and 1636 cm^{-1} correspond to the amide polypeptides or proteins which served as capping agents in AuNPs and make them stable in colloidal solution (Gurunathan *et al*., 2014). In present study FTIR transmission spectra (Fig. 12) provided confirmation of capping biochemical present in *G. applanatum* extract and our results also correlates the previous studies.

Previous many studies reported that, metal nanoparticles especially silver nanoparticles are potentially toxic materials and has been used today in numerous consumer products including drug development (Imani *et al*., 2015). Acute oral toxicity study is necessary to determine the safer dose range to manage the clinical signs and symptoms of the drugs (Saleem *et al*., 2017) and the toxic outcomes of drugs such as decrease body weight, clinical signs and symptoms which are principal observations among various toxicity indicators (Ogbonnia *et al*., 2010). In this study non-significant increase in BW and OW was observed except testicular weight in rats treated with 200 mg/kg, 400 mg/kg and 2000 mg/kg doses of *G. applanatum* extract mediated SNPs (Table 5 and 7). Various behavioural changes due to toxicity were not observed in rats treated with different dosed of SNPs (Table 4 and 6).

In modern medical practice, toxicity studies are essential for safety of extracts or drugs used in clinical medicine because of interaction of the compound possess toxicity or its metabolite bring significant changes in haematological parameters, rapid or slow changes in structure and function of the affected tissues (Arika *et al*., 2016). Therefore, assessments of haematological indices provide adverse effects of foreign compounds on the blood constituents to evaluate toxicity of compounds. In this study it was observed that, *G. applanatum* extract mediated SNPs non-significantly ($p<0.05$) elevated in RBC, Hb, MCHC, PCV and MCV indices and significantly ($p<0.05$) elevated WBC, NUT, LYM and Pt indices (Table 8). The results revealed increase Hb, MCH and MCHC due to increase in RBC count which is associated with non-adverse and slow boost effect of haematopoietic system body (Ambali *et al*., 2010) and it has been reported that non-significant increase in PCV and MCV does not affect the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of red blood cells (Kuatsienu *et al*., 2017). Mushrooms have been known to possess immunstimulatory properties and increase the WBC count (Mizuno, 1999). Significant elevation of the total WBC, NUT and LYM showed an improvement in the immune system of SNPs treated animals in the toxicity study (Adedapo *et al*., 2004). Lymphocytes are primarily responsible for humoral antibody formation (Schalm *et al*., 1975) and Neutrophils are known to express receptors that specifically recognize microorganisms and efficiently ingest and destroy these pathogens (Ishikawa and Miyazaki, 2005). In present study *G. applanatum* modulated the immune response of rats and showed significant surge of WBC, NUT and LYM count of rats.

Conclusions

Ganoderma applanatum extract effectively helps to synthesize SNps within nanoscale range and the extract contains different mycochemicals associated with therapeutic properties. SNPs synthesized using *G. applanatum* did not show adverse effects on BW, OW and haematological indices. However, the, SNPs enhance the immune system of body by significant increase in total WBC count. Thus, SNPs synthesized mediated by *G. applanatum* extract at minimum doses is nontoxic to animals. More studies on at molecular level will be required to investigate the toxicity impact of SNPs.

Acknowledgements

The authors acknowledge Department of Zoology, Ranchi University for providing the working and asset facilities. The authors also acknowledge Department of Botany for their support during the collection and identification of mushrooms.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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