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Study of Impacts of *Ganoderma applanatum* (Pres.) Pat. Extract on Hepatic and Renal Biochemical Parameters of Rats

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ABSTRACT

Traditionally, *Ganoderma applanatum* was used as a medicinal supplement for treatment of different diseases. In this study, biochemical screening, acute toxicity, and impact of *G. applanatum* aqueous extract on liver and renal parameters were studied. Qualitative screening of *G. applanatum* aqueous extract showed presence of various biochemicals such as tannin, phenolics, proteins, flavanoids and other biochemicals. FTIR analysis also showed spectrum transmission peaks for different mycochemicals such as 3248.13 cm⁻¹ for phenol O-H stretch, 1597.06 cm⁻¹ for primary amine N-H stretch, 783.10 cm⁻¹ for aromatic (meta disub benzene) C-H stretch. The extract increased body weight (Initial body weight: 181.74g; final body weight: 185.08g) when compared to control group (Initial body weight: 178.61 g; final body weight: 181.14 g) in acute toxicity test dose (2000mg/kg). Similar insignificant in final body weight of animals of different groups were observed when compared to initial body weight of animals used for the study of live and renal profile. The extract had no significant effect on organ weight. Low dose (200mg kg⁻¹) of extract insignificantly decrease AST (51.71±0.61 mg dL⁻¹), ALT (146.07±0.89 mg dL⁻¹), ALP (174.68±0.65 mg dL⁻¹) and bilirubin (0.61±0.01 mg dL⁻¹) level and significantly elevated serum albumin level (6.63±0.22 g dL⁻¹) compare to control group. Low dose (200mg kg⁻¹) extract showed similar insignificant decrease in serum urea (61.30±1.05 mg dL⁻¹) and creatinine level (0.90±0.02 mg dL⁻¹) and significantly decreased serum uric acid level (19.52±1.14 mg dL⁻¹) compare to control group of rats. Thus, dose-oriented application of *G. applanatum* extract can be beneficial for treatment of hepatic and renal diseases.

Keywords: Fungus, Toxicity, Renal, Hepatic, Medicinal

INTRODUCTION

In recent decade, interest has been paid to natural and alternative therapies by the people of many countries, which expands the use of medicinal plants, mushrooms, and herbal medicines and the use of herbal medicines for the treatment of various diseases has expanded rapidly in both developed and developing countries because of affordability, accessibility, and efficacy (Salawu *et al.*, 2009).

Traditionally people have been used medicinal mushrooms as a food supplement for health maintenance and as a therapeutic drug for the treatment of diseases. It has also been reported mushrooms belong to genus *Ganoderma* have been used widely as alternative source of therapeutic agent for many diseases such as cancer, cardiovascular diseases, autoimmune diseases, etc. (Lull *et al.*, 2005).

Along with its beneficial effects, mushrooms also possess some negative impacts on body. Many workers reported mushroom poisoning leads to gastrointestinal discomfort, chronic toxicity

to the liver, and renal failure (Eren *et al.*, 2010) and extracts of mushroom-like *Pleurotus sajor-caju* affects the renal function and decreases glomerular filtration rate (Tam *et al.*, 2002).

The World Health Organization (WHO) has reported that inappropriate use and practices of traditional medicines can have negative side effects and research are required to study the safety efficacy and to diagnose the toxicity of medicinal plants and mushroom for validation of their medicinal impacts (Wihastuti *et al.*, 2015).

Ganoderma applanatum is a polypore macrofungi with hard, woody, less fan-shaped, semicircular, fruiting bodies with a dull, unvarnished outer surface having wrinkled zones of brownish to grayish-brown color on carp surface and white color pore surface (Niemela & 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 (Jeong *et al.*, 2008; Wasser, 2011).

In this work, short term impacts of *G. applanatum* aqueous extract on hepatic and renal biochemical parameters of rat were studied to validate the medicinal efficacy of *G. applanatum*.

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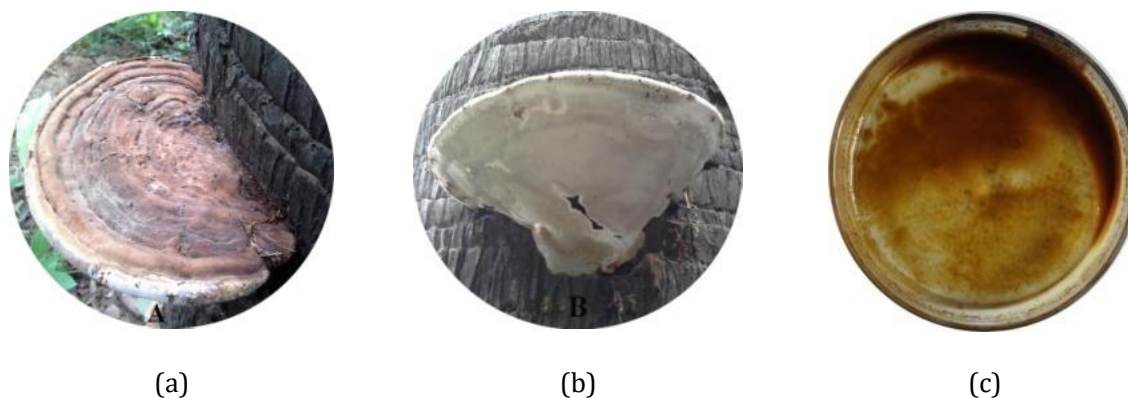


Figure 1. Fruiting body of *G. applanatum* ((a) and (b)) and extract (c).

METHODOLOGY

Collection of macrofungi

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

Preparation of extracts

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. As much as 50g of the fine powder was subjected to extraction chamber of Soxhlet and 300mL distilled water was taken in the 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 airtight containers at room temperature for further studies (Dandapat *et al.*, 2015).

Qualitative analysis of mycochemicals

The freshly prepared extract was used for mycochemical analyses. Presence of various biochemicals in the aqueous extract of *G. applanatum* was analyzed followed protocols described by Arya *et al.*, (2012) given below.

Test for carbohydrates

Presence of carbohydrate was determined by addition of few drops of Molisch's reagent to the test solutions (1mg mL⁻¹ extract), this was then followed by addition of 1 mL concentrated H₂SO₄ (98%) by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted by adding 5 mL of distilled water. The mixture was observed for the appearance of purple violet ring.

Test for glycoside

Glycoside was determined by addition of 1mgmL⁻¹ of extract to 3mL of anthrone reagent and was mixed properly. The mixture was observed for the appearance of the green color complex.

Test for proteins

Protein was estimated by addition of 0.5 mgmL⁻¹ of the extract and 2mL of Bradford's reagent were left for few minutes. The mixture was observed for the appearance of blue color.

Test for alkaloid

Presence of alkaloid was determined by stirring of 1mgmL⁻¹ extract with 5 mL of 1% HCl on hot water bath and then filtered. 1 mL of the filtrate was taken individually into 2 test tubes and few drops of Dragendorff's reagent were added into the test tube. The mixture was observed for appearance of dark brown colour.

Test for steroid

Presence of steroid was determined by addition of 2mL concentrated H₂SO₄ (98%) with 2mgmL⁻¹ of extracts was mixed vigorously.

The mixture was observed for initial formation of red color followed by blue and finally development of green color.

Test for triterpene

Triterpene was estimated by addition of 1mgmL⁻¹ extract with one drop chloroform and concentrated H₂SO₄ (98%). The mixture was observed for the formation of red color.

Test for phenol

Presence of phenol was estimated by phenolic -catechol method. Dilute aqueous extract (0.5 mL of 1:10 gL⁻¹) was pipette out in a series of test tubes and volume was made up to 3 ml with distilled water. Folin-Ciocalteu reagent (0.5mL) was added to each tube and incubated for 3 min. at room temperature and then sodium carbonate (20%; 2mL) solution was added, mixed thoroughly and the tubes were incubated for 1 minute in boiling water bath. The mixture was observed for the emergence of a blue-green color.

Test for flavonoid

Flavonoid was estimated by dissolving 1mgmL⁻¹ extracts in water and later adding 2 mL of the 10% aqueous sodium hydroxide as well as dilute hydrochloric acid as an indicator. The mixture was observed for formation and disappearance of yellow color.

Test for tannin

Tannin was estimated by stirring 0.5 mgmL⁻¹ of the extracts with 10 mL of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 mL of the filtrate.

The mixture was observed for the formation of yellow precipitate.

Test for saponin

Saponin was determined by heating 1mg/mL extracts with alcoholic KOH and boiled for 1 min and cooled, and then the mixture was acidified with 1mL of concentrated HCl. Later few drops of 5% NaOH added dropwise and observed for froth formation.

Test for lipid

The 2 mL of extract was taken and iodine solution was added dropwise. Disappeared of the original color of iodine indicates the presence of lipid. The mixture was observed for disappearance of original color of iodine.

FTIR spectra analysis

Fourier transform infrared (FTIR) spectra analysis was carried out IPRresting-21 (Shimadzu Corp., Kyoto, Japan) in the diffuse reflectance mode operated at a resolution of 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 extract of fruiting body of *G. applanatum*. The FTIR machine was operated at 25±5°C, 60-70%humidity and 240V AC (IMUSG, 2002).

Analysis of impacts of *G. applanatum* extract on a rat model

Equal numbers of male and female Wistar albino rats (175 to 200g) were obtained from the National Institute of Nutrition, Hyderabad, India. They were kept in a cage and maintained under standard laboratory conditions at ambient room temperature (22±3°C) and relative humidity (30%-65%), with dark-light cycle of 12 h for 5 days. Rats were fed with a commercial pellet 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 and vehicle (Distilled water) was performed at the test dose 2000mg/kg body weight (BW) with slight modification. 10 rats (males and females) were divided equally into two groups (Group-1 and Group-2) each group contains 5 rats. Rats were fasted (3-4hours) prior to dosing but were accessed with water *ad libitum*. Single-dose (2000mg kg⁻¹) of vehicle and *G. applanatum* extract were fed by gavage using stomach tube to single rat of each group and were provided with food and water *ad libitum* after 2hours. Similarly 4 other rats of each group were treated with *G. applanatum* extract and vehicle, and observed for 30 minutes, 4 hours and 24 hours for gross behavior and death due to toxicity (OECD, 2008; Saleem *et al.*, 2017). After treatment with single-dose all the animals were not treated further and observed till the end of 14th day for delayed occurrence of toxic effects such as behavioral changes, mortality and body, and organ weight. The rats were euthanized using diethyl ether as euthanize agent and finally cervical dislocation was done after 14 days. Liver and kidneys were removed carefully and weighed.

Analysis of *G. applanatum* extract on hepatic and renal profile of rats

To study the short term medicinal effect of *G. applanatum* extract on liver and kidney, seven days experimental period was chosen following the previous method of Garba *et al.*, (2009). Therefore, therapeutic efficacy of *G. applanatum* extracts on hepatotoxic and nephrotoxic rats can be studied further. To study the impact of extract on hepatic and renal profile, again 15 fresh rats (males and females) were taken and were distributed equally into three treatment groups each group contains 5 rats. The experiment designed for study of impact of extract is described below. Dose determination and feeding of extract were performed following previous method of Oghenesuvwe *et al.*, (2014).

Group-1: Rats were served as control and were not treated with extract and received 1mL of distilled water daily orally throughout the entire period of the experiment (7 days).

Group 2: Rats of this group were received daily single LD of extract orally daily for 7 days.

Group 3: Rats of this group were received daily single HD of extract orally daily for 7 days.

At the end of the experimental period (8th Day) animals were lightly euthanized using diethyl ether and blood was collected from rats by retro-orbital sinus blood collection method into evacuated voiles (SRL Diagnostic Pvt. Ltd.) containing clotting activator (Kumar M, 2017). Collected blood samples were allowed to clot and then centrifuged at 2000-2500 rpm and the serum was collected within 45 minutes of collection of blood samples. Serum samples were kept at 4 °C for analyses of hepatic and renal profile. After collection of blood the rats were finally sacrificed by cervical dislocation and Liver and kidneys were removed carefully and weighed.

Estimation of serum hepatic and renal parameters was done on a semi-automatic chemistry analyzer: SACA-19100 (MRC Ltd., Israel) operated at 0-40 °C, ≤85% relative humidity and 110V/220V±11V/22V alternative current using the diagnostic reagent kit by DiaSys international Pvt. Ltd. (Holzheim, Germany). Quantification of hepatic parameters such as total bilirubin, serum transaminases, alkaline phosphatase, and albumin were done by standard method of Reitman and Frankel, Kingsley and Frankel and Bessey *et al.*, respectively and renal profile such as serum urea and creatinine uric acid will be estimated by Mod. Berthelot method and Mod. Jaffer's method respectively with modification following Agrawal and Gupta (2013).

Analysis of hepatic profile Alkaline phosphatase (ALP)

In vitro quantification of alkaline phosphatase was done by a kinetic photometric test using alkaline phosphatase FS* DGKC kit (Cat. No.104019990314). A mono reagent was prepared by adding 20ml of R1(Diethanolamine 1.2 molL⁻¹, pH 9.8 and Magnesium chloride 0.6 mmol/L) and 5 mL of R2 (p-Nitrophenylphosphate 50 mmolL⁻¹). The system was calibrated by using universal calibration serum (TruCal U calibrator, No.591009910063) at 420nm wavelength, 1cm optical path, 37°C against air. 20µL sample serum and calibrator serum were taken in cuvettes and 1000µL monoreagent was added, mixed and absorbance was recorded after 1, 2 and 3 min. Amount of ALP in sample serum was calculated by using formula given below-

$$\text{ALP } \left[\frac{\text{U}}{\text{L}} \right] = \frac{\text{Absorbance / min of sample}}{\text{Absorbance / min of calibrator}} \times \text{Conc. calibrator [U/L]}$$

Aspartate aminotransferase (AST/SGOT)

Aspartate aminotransferase (ASAT/AST), formerly called Glutamic oxalacetic transaminase (GOT). Quantitative *in vitro* determination of AST (SGOT) in serum was done photometrically by using ASAT (GOT) FS* (IFCC mod.) kit (Cat. No.126019990314). Monoreagent mixture was prepared by mixed 20mL R1 [TRIS 110 mmolL⁻¹ (pH 7.65), L-aspartate 320 mmolL⁻¹, MDH (malate dehydrogenase) ≥ 800 U/L, LDH (lactate dehydrogenase) ≥ 1200 UL⁻¹] and 5mL R2 [2-Oxoglutarate 85 mmolL⁻¹, NADH 1 mmolL⁻¹, pyridoxal-5-phosphate F8, good's buffer (pH 9.6) 100 mmolL⁻¹, Pyridoxal-5-phosphate 13 mmolL⁻¹]. The system was calibrated by using universal calibration serum (TruCal U calibrator, No. 5 9100 99 10 063) at 365nm wavelength, 1cm optical path, 37°C against air. 100µL sample serum and calibrator serum were taken in cuvettes and 1000µL monoreagent was added, mixed and absorbance was recorded after 1, 2 and 3 min. Amount of AST in sample serum was calculated by using formula given below-

$$\text{AST [U/L]} = \frac{\text{Absorbance / min of sample}}{\text{Absorbance / min of calibrator}} \times \text{Conc. calibrator [U/L]}$$

Alanine aminotransferase (ALT/SGPT)

Alanine aminotransferase (ALAT/ALT), formerly called glutamic pyruvic transaminase (GPT). Quantitative *in vitro* determination of ALAT

(GPT) in serum was done photometrically by using ALAT (GPT) FS* (IFCC mod.) kit (Cat. No.127019910023). Monoreagent mixture was prepared by mixed 20mL of R1 [TRIS (pH 7.15) 140 mmolL⁻¹, L-alanine 700 mmolL⁻¹, LDH (lactate dehydrogenase) ≥ 2300 U/L] and 5mL R2 [2-oxoglutarate 85 mmolL⁻¹, NADH 1 mmolL⁻¹, pyridoxal-5-phosphate F8 good's buffer (pH 9.6) 100 mmolL⁻¹, pyridoxal-5-phosphate 13 mmolL⁻¹]. The system was calibrated by using universal calibration serum (TruCal U calibrator, No. 591009910063) at 340 nm wavelength, 1cm optical path, 37°C against air. 100µL sample serum and calibrator serum were taken in cuvettes and 1000µL monoreagent was added, mixed and absorbance was recorded after 1, 2 and 3 min. Amount of ALT in sample serum was calculated by using the formula given below:

$$\text{ALT } \left[\frac{\text{U}}{\text{L}} \right] = \frac{\text{Absorbance / min of sample}}{\text{Absorbance / min of calibrator}} \times \text{Conc. calibrator [U/L]}$$

Total bilirubin (BIL)

Quantitative *in vitro* determination of total bilirubin in serum was done photometrically by using Bilirubin Auto Total FS* kit (Cat. No.108119910023). The system was calibrated by using universal calibration serum (TruCal U calibrator, No. 591009910063, Diyasys Pvt. Ltd.) at 560 nm, 1cm optical path, 37°C against reagent blank (distilled water and NaCl solution 9 gL⁻¹). 25µL distilled water, calibrator serum and sample serum were taken in cuvettes and 1000 µL R1 [Phosphate buffer 50 mmolL⁻¹ and NaCl 150 mmolL⁻¹] was added, mixed and incubated for 5 minutes at 37 °C and then absorbance (A1) was taken. After taken A1, R2 [2,4-Dichlorophenyl-diazonium salt 5 mmolL⁻¹, HCl 130 mmolL⁻¹] was mixed with previous samples and incubate for 5 minutes at 37 °C and then absorbance (A2) was taken. Final absorbance was calculated (FA = F2-F1). Total bilirubin in the test sample was calculated by using formula given below-

$$\text{Bilirubin [mg/dL]} = \frac{\text{FA of sample}}{\text{FA of calibrator}} \times \text{Conc. calibrator [mg/dL]}$$

Total protein (TP)

Quantitative *in vitro* determination of total protein in serum was done photometrically by using Total protein FS* kit [Cat. No.123119910023 and 123009910030 (standard)]. The system was calibrated by using universal calibration serum (TruCal U calibrator, No. 591009910063,

Diyasys Pvt. Ltd.) at 540 nm, 1cm optical path, 37°C against reagent blank (distilled water and NaCl solution 9 gL⁻¹). A monoreagent was prepared by mixed R1 [Sodium hydroxide 100 mmolL⁻¹, Potassium sodium tartrate 17 mmolL⁻¹] and R2 [Sodium hydroxide 500 mmolL⁻¹, Potassium sodium tartrate 80 mmolL⁻¹, Potassium iodide 75 mmolL⁻¹, Copper sulphate 30 mmolL⁻¹]. 20µL standard (bovine serum albumin), calibrator serum and sample serum were taken in cuvettes and 1000 µL monoreagent was mixed with samples and incubate for 5 minutes at 37 °C and then absorbance was taken. Total protein in the test sample was calculated by using the formula given below:

$$\text{Total protein [g/dL]} = \frac{\text{FA of sample}}{\text{FA of calibrator}} \times \text{Conc. calibrator [g/dL]}$$

Albumin (ALB)

Quantitative *in vitro* determination of albumin in serum by photometrically using Albumin FS * kit [Cat. No.102209910023 and 102009910030 (standard)]. The system was calibrated by using universal calibration serum (TruCal U calibrator, No. 591009910063, Diyasys Pvt. Ltd.) at 600 nm, 1cm optical path, 37°C against a reagent blank (distilled water and NaCl solution 9 gL⁻¹). 10µL standard (bovine serum albumin), calibrator serum and sample serum were taken in cuvettes and 1000 µL monoreagent was mixed with samples and incubate for 5 minutes at 37 °C and then absorbance was taken. Serum albumin in the test sample was calculated by using formula given below:

$$\text{Albumin [g/dL]} = \frac{\text{FA of sample}}{\text{FA of calibrator}} \times \text{Conc. calibrator [g/dL]}$$

Analysis of renal profile

Serum urea

Quantitative *in vitro* determination of urea in serum was done by photometrically using Urea FS* kit (Cat. No.131019910023). The system was calibrated by using 340nm 1cm optical path, 37°C against reagent blank (distilled water and NaCl solution 9 gL⁻¹). A mono reagent was prepared by mixed 20 mL of R1 [TRIS pH 7.8 150 mmol/L, 2-Oxoglutarate 9 mmolL⁻¹, ADP 0.75 mmolL⁻¹, Urease 3 7 kUL⁻¹, GLDH (Glutamate dehydrogenase, bovine) 3 1 kUL⁻¹] and 5 mL of R2 [NADH 1.3 mmolL⁻¹]. 10µL serum was taken in cuvettes and 1000 µL monoreagent was mixed with samples and incubated for 30 – 40 seconds at 37 °C and

Table I. 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	+

then absorbance was taken (A1). The absorbance (A2) of the sample was taken again exactly after another 60 seconds. Final absorbance was calculated (FA = A2-A1) and serum urea was measured using the formula given below:

$$\text{Urea [mg/dL]} = \frac{\text{FA of sample}}{\text{FA of calibrator}} \times \text{Conc. calibrator [mg/dL]}$$

Serum creatinine (CREA)

Quantitative in vitro determination of creatinine in serum was done photometrically by using Creatinine FS* kit (Cat. No.117119910023). The system was calibrated by using 492 nm, 1cm optical path, 37°C against reagent blank (distilled water and NaCl solution 9 gL⁻¹). A mono reagent was prepared by mixed 20 mL R1 [Sodium hydroxide 0.2 molL⁻¹] and 5 mL R2 [Picric acid 20 mmolL⁻¹]. 50µL serum sample was taken in a cuvette and 1000 µL mono reagent was mixed with samples, mixed properly and incubated for 60 seconds at 37 °C and then absorbance was taken (A1). The absorbance (A2) of sample was taken again exactly after another 120 seconds. Final absorbance was calculated (FA = A2-A1) and serum creatinine was measured using the formula given below:

$$\text{Creatinine [mg/dL]} = \frac{\text{FA of sample}}{\text{FA of calibrator}} \times \text{Conc. calibrator [mg/dL]}$$

Serum uric acid (SUA)

Quantitative in vitro determination of creatinine in serum was done photometrically by using Uric acid FS* kit (Cat. No.117119910023). The system was calibrated by using 520 nm, 1cm optical path, 37°C against reagent blank (distilled water and NaCl solution 9 gL⁻¹). A mono reagent was prepared by mixed 20 mL of R1 [Phosphate buffer (pH 7.0) 100 mmolL⁻¹, TBHBA (2,4,6-Tribromo-3-hydroxybenzoic acid) 1.25 mmolL⁻¹

and 5 mL of R2 [Phosphate buffer (pH 7.0) 100 mmolL⁻¹, 4-Aminoantipyrine 1.5 mmolL⁻¹, K4{Fe(CN)6} 50 µmolL⁻¹, Peroxidase (POD) ≥ 10 kUL⁻¹, Uricase ≥ 150 UL⁻¹]. 20µ serum was taken in the cuvette and 1000 µL mono reagent was mixed with samples, mixed properly and incubated for 30 minutes at 37 °C and then absorbance was taken (A1). The absorbance (A2) of sample was taken again exactly after another 60 minutes. Final absorbance was calculated (FA = A2-A1) and serum uric acid was measured using the formula given below-

$$\text{Uric acid [mg/dL]} = \frac{\text{FA of sample}}{\text{FA of calibrator}} \times \text{Conc. calibrator [mg/dL]}$$

Statistical analysis

Entire statistical works were done using full-proof statistical software WinSTAT. Data were taken N=5 and results were expressed as a mean ± standard error of mean. Statistical analysis was performed using Student's t-test, p ≤ 0.05 was considered as statistically significant.

RESULT AND DISCUSSION

Result

Biochemical screening

The identified mushroom and the extract obtained from a dry powder of mushroom (Figure 1). Result of biochemical analysis of crude extract of *G. applanatum* (Table I). 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*.

Furior transform infrared (FTIR) spectroscopy analysis of *G. applanatum* extract

FTIR spectroscopy of *G. applanatum* extract (Figure 2). FTIR spectroscopy analysis of *G. applanatum* extract showed major transmittance

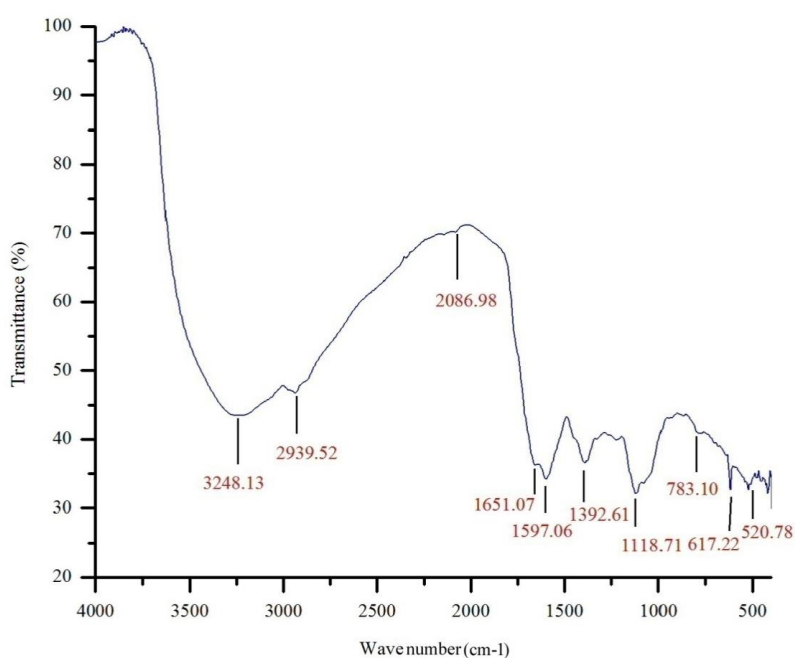


Figure 2. FTIR spectra of *G. applanatum* extract.

Table II. Behavioural patterns of rats treated with vehicle and *G. applanatum* extract in acute toxicity test.

Parameters	Group-1				Group-2			
	30 Min.	4 Hrs.	24 Hrs.	14Day	30 Min.	4 Hrs.	24 Hrs.	14Day
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	N	I	N	N
Convulsions & tremors	NF	NF	NF	NF	NF	NF	NF	NF
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

Note: N=normal, NF= not found, I=increase

peaks at 3248.13cm⁻¹ for phenol O-H stretch, 2939.52cm⁻¹ for alkyl C-H stretch, 2086.98cm⁻¹ for terminal alkyne C≡C stretch, 1651.07cm⁻¹ for amide C=O stretch, 1597.06cm⁻¹ for primary amine N-H stretch, 1392.61cm⁻¹ for fluoro alkane C-F stretch, 783.10cm⁻¹ for aromatic (metadisub benzene) C-H stretch, 617.22cm⁻¹ for chloro alkane C-Cl stretch and 520.78cm⁻¹ for bromo alkane C-Br stretch.

Acute toxicity study of *G. applanatum* extract

Results of acute toxicity of *G. applanatum* extract (Table II and Table III). The results showed no mortality and behavioural changes such as convulsions, tremors, itching, shivering, somatomotor activity etc. due to toxicity till 14 days in rats treated with vehicle and 2000mg/kg dose of extract. Slight elevation in breathing rate and sleeping only observed for first 30 min in rats

Table III. Body weight and organ weight of rats treated with vehicle and *G. applanatum* extract in acute toxicity test.

Treatment groups		
	Group-1	Group-2
Body weight and organ weight (in g)		
Initial body weight	181.65±1.49	181.58±1.09
Final body weight	182.98±1.27	183.76±0.75
Weight of liver	5.52±0.38	5.53±0.36
Weight of kidneys	1.60±0.13	1.58±0.11

Table IV. Behavioural patterns of different treatment groups of rats used to study impact of *G. applanatum* extract on hepatic and renal profiles.

Groups of rats	Group-1				Group-2				Group-3			
	30 Min.	4 Hrs.	24 Hrs.	7Dy	30 Min.	4 Hrs.	24 Hrs.	7Dy	30 Min.	4 Hrs.	24 Hrs.	7Dy
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	N	I	N	N	N	I	N	N	N
Somatomotor activity & behavior pattern	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	I	N	N	N	I	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

Note: N=normal, NF= not found, I=increase

of both groups (Table II). The result of acute toxic impact of extract on body weight and organ weight is in Table III. The result showed insignificant increase in final BW of rats treated with vehicle (182.98±1.27g) and *G. applanatum* extract (183.76±0.75g) compared to initial BW of animals treated with vehicle (181.65±1.49g) and *G. applanatum* extract (181.58±1.09g). No significant differences in weight of liver (5.53±0.36g) and kidneys (1.58±0.11g) were also observed in rats treated with 2000mg/kg dose of extract compared to weight of liver (5.52±0.38g) and kidneys (1.60±0.13g) of rats treated with vehicle.

Effect of 200mg/kg and 400mg/kg *G. applanatum* extract on body and organ weight and behavioral changes

Effects of *G. applanatum* extract (200mg/kg and 400mg/kg) on behavioral changes, body, and organ weight (Table IV and V). Mortality and behavioral changes such as convulsions, tremors, itching, shivering, somatomotor activity, etc. Were

not observed in any treatment group. Insignificant increase in final body weight was observed in rats of group-2 (185.82±2.17g) and group-3 (187.04±2.44g) compared to group-1 (184.70±2.77g). Both 200mg/kg and 400mg/kg extract showed insignificant change in weight of kidney in group-2 (1.64±0.10g) and group-3 (1.64±0.13g) compared to group-1 (1.64±0.12). Similarly impact of extract in weight of liver also observed among the treatment groups.

Effect of *G. applanatum* extract on hepatic profile

Result of *G. applanatum* extracts on hepatic profile of rat is Table VI. The 200 mg kg⁻¹ dose of extract showed insignificant decrease in ALP (174.68±0.65 mgdL⁻¹), AST (51.71±0.61 mg dL⁻¹), ALT (146.07±0.89 mg dL⁻¹), total bilirubin (0.61±0.01 mg dL⁻¹) and showed insignificant increase in total protein (9.02±0.17 gdL⁻¹) compared to ALP (176.24±0.57 mgdL⁻¹), AST (52.32±0.70 mg dL⁻¹), ALT (146.63±0.78 mg dL⁻¹), total bilirubin (0.62±0.01 mg dL⁻¹) and total

Table V. Body and organ weight of different treatment groups of rats used to study impact of *G. applanatum* extract on hepatic and renal profiles.

Groups of rats	Group-1	Group-2	Group-3
Body weight and Organ weight (in g)			
Initial body weight	182.44±2.72	182.46±2.16	184.74±2.52
Final body weight	184.70±2.77	185.82±2.17	187.04±2.44
Weight of liver	5.47±0.22	5.47±0.23	5.49±0.24
Weight of kidneys	1.64±0.12	1.64±0.10	1.64±0.13

Table VI. Effect of *G. applanatum* extract on hepatic profile; N=5±SE of mean; *=p<0.05, **=p<0.025, ***=p<0.0005.

Groups of rats	Group-1	Group-2	Group-3
Parameters			
ALP (mgdL ⁻¹)	176.24±0.57	174.68±0.65	173.79±0.98*
AST(mg dL ⁻¹)	52.32±0.70	51.71±0.61	50.62±1.04
ALT(mg dL ⁻¹)	146.63±0.78	146.07±0.89	145.32±0.89
Bilirubin total (mg dL ⁻¹)	0.62±0.01	0.61±0.01	0.60±0.01
Protein(g dL ⁻¹)	8.57±0.24	9.02±0.17	9.51±0.30**
Albumin(g dL ⁻¹)	4.23±0.31	6.63±0.22***	7.32±0.42***

Table VII. Effect of *G. applanatum* extract on renal profile of rat; N=5±SE of mean; *=p<0.05, **=p<0.005; ***=p<0.0005.

Groups of rats	Group-1	Group-2	Group-3
Parameters			
Serum urea(mg dL ⁻¹)	62.32±1.17	61.30±1.05	57.62±0.74**
Serum creatinine(mg dL ⁻¹)	0.93±0.01	0.90±0.02	0.86±0.008**
Serum uric acid(mg dL ⁻¹)	22.5±0.70	19.52±1.14*	14.24±0.73***

protein (8.57±0.24 g dL⁻¹) of control group. Only significant (p ≤ 0.05) in albumin of group-1 (6.63±0.22 g dL⁻¹) was observed compared to control group (4.23±0.31 g dL⁻¹). 400 mg kg⁻¹ dose of extract showed high efficacy than 200 mg kg⁻¹ dose of extract (Table V). Significant (p ≤ 0.05) decrease in ALP (173.79±0.98) and increase in protein (9.51±0.30 g dL⁻¹) and albumin (7.32±0.42 g dL⁻¹) were observed in rats treated with 400 mg kg⁻¹ dose of extract compared to control group. 400 mg kg⁻¹ dose of extract had no significant effect on total bilirubin, AST, ALT compared to values of the control group.

Effect of *G. applanatum* extract on renal profile of rat

Result of *G. applanatum* extracts on hepatic profile of rat (Table VII). The LD of extract (200 mg kg⁻¹) significantly (p<0.05) decreased serum uric acid (19.52±1.14 mg dL⁻¹) in rats of group-2 compared to control (22.5±0.70 mg dL⁻¹). 400 mg kg⁻¹ dose of extract showed high efficacy than low

dose (200 mg kg⁻¹) and significantly (p<0.05) decreased serum urea (57.62±0.74 mg dL⁻¹), creatinine (0.86±0.02 mg dL⁻¹) and uric acid (14.24±0.73 mg dL⁻¹) in rats of group-3 compared serum urea (62.32±1.17 mg dL⁻¹), creatinine (0.93±0.01 mg dL⁻¹) and uric acid (22.5±0.70 mg dL⁻¹) in rats of control group.

Discussion

Fruiting bodies of *Ganoderma* spp. have been used in traditional medicine and used in variety of products in the form of coffee, powder, dietary supplements, spore products, drinks, syrups, toothpastes, soaps and lotions (Lai *et al.*, 2004) because of their various therapeutic biochemicals (mycochemicals) composition such as reducing sugars, amino acids, phenolic compounds, flavonoids, glycosides terpenoids etc. (Singh *et al.*, 2014). In the present study mycochemical screening of *G. applanatum* extract (Table I) showed presence of similar mycochemicals as previously reported

mycochemical screening of *Ganoderma* spp. Previous FTIR analysis showed *Ganoderma lucidum* extract contains high protein and carbohydrates with spectra transmission peaks of 1800 to 1400 cm^{-1} and 889 cm^{-1} respectively (Keonget *et al.*, 2012). In the present study FTIR analysis of *G. applanatum* extract (Figure 2) also provided transmission spectra for phenols, amines and other compounds.

The World Health Organization and Food and Drug Administration have recommended that acute and chronic toxicological evaluation of herbal medicines (medicinal plants and fungal origin) must be done for the appropriate ethnomedicinal uses (Subramanion *et al.*, 2011; Saleem, U., 2017). General behavioral patterns and body weights are one of the critical indicators for the evaluation of early signs of acute toxicity of drugs (Sireeratawong *et al.*, 2008). In the present study mortality and adverse behavioral changes and toxic outcome on the body were not observed in the animal groups during the acute toxicity experimental period (Table II) and in treatment rat groups (Table IV) used for the study to see the medicinal impact *G. applanatum* extract on liver and renal profile.

Proper food and water intake did not produce a concomitant decrease in the bodyweight but change in the body weights due to intake of any medicinal supplement is one of the first critical signs of toxicity (Sireeratawong *et al.*, 2008). It has also been reported that adverse interaction of the herbal extract with the major organs cause cellular constriction and inflammation, which usually reflects in the significant changes in weight of vital organs such as liver and kidney (Devaki *et al.*, 2012). In the present study body weight of rats increased, used in acute toxicity test and rats (Table III) used to study to see the medicinal impact *G. applanatum* extract on liver and renal profile (Table V). The *G. applanatum* extract showed insignificant variation in the weight of liver and kidneys after treatment. The increase in body weight of rats showed insignificant differences between initial and final body weight. Thus, it can be said that the biochemicals of *G. applanatum* extract are associated with normal function of liver and kidneys and proper processing of lipids, carbohydrates and protein metabolism inside body because these nutrients play a major role in different physiological functions of the body (Kuatsienu *et al.*, 2017; Saleem *et al.*, 2017).

Analysis of certain serum biochemical parameters for specific organs such as kidneys and the liver, provide useful information regarding the mechanisms of toxicity and safe therapeutic use of

drugs (Yamthe I *et al.*, 2012). The liver is the main organ of body plays biotransformation and disposition of xenobiotics and liver underexposure of large concentrations of exogenous substances such as drugs and their metabolites modulate the properties of hepatotoxicant by either increasing its toxicity or by detoxification (Kedderis, 1996; Singh *et al.*, 2011). Liver function tests conducted through serological assays provide information about the status of liver and its proper function, cellular integrity and its link with the biliary tract (Agbaje *et al.*, 2009).

Levels of hepatic enzymes such as AST, ALT, ALP and total protein, albumin, and bilirubin are widely used as sensitive markers for assessment of toxic effects in the liver (Mukinda & Syce, 2007). AST and ALT are two major hepatic enzymes increase the in the serum because of damage of hepatocytes (Ali *et al.*, 2008; Ogunlana *et al.*, 2013). However, ALP is the major standard biomarker of biliary tract obstruction (Saleem *et al.*, 2017). It has been reported that low levels of ALP occur in several diseases such as hypothyroidism, pernicious anaemia, zinc deficiency and congenital hypophosphatasia (Gowda *et al.*, 2009). Bilirubin is produced by the metabolism of heme derived from damaged red blood corpuscles and increase level of bilirubin in serum represents chronic liver injuries, but not in mild liver injuries (Bigoniya *et al.*, 2009). Estimation of total proteins in the body is helpful in differentiating between a normal and damaged liver function because the major plasma proteins like albumins are produced in the liver (Thapa & Walia, 2007). Previous researches reported that increased protein and albumin level indicate liver dysfunctions which impair protein synthesis (Rajesh SV, 2009). However, according to clinical biochemistry hepatic damage leads to decrease in albumin production and decrease total protein level of blood plasma (Thapa & Walia, 2007; Singh *et al.*, 2011). In the present investigation LD of *G. applanatum* extract in significantly decrease ALT, AST, ALP and total bilirubin in serum (Table VI). However, HD of *G. applanatum* extract significantly modulated total protein and albumin level and helps elevated total albumin level. Hence, both LD and HD of *G. applanatum* extract showed non-toxic impact to liver but increase the efficiency of liver. Previous studies reported that, mycochemicals of edible medicinal mushrooms and *Ganoderma* spp. extract improve status of damaged liver in hepatotoxicated rats by suppressing the high serum ALT, AST, ALP and total bilirubin level and elevate total protein and albumin level (Mishra & Singh, 2013; Eroglu & Beytut, 2018). Result of present study correlates with previous studies and

supports the hepato tonic potentiality of *G. applanatum*.

Protein and nitrogen metabolism is necessary for normal health and they are derived from dietary protein intake, which is necessary for protein synthesis and maintenance of muscle, and excess of unwanted amino acids are degraded and converted to urea in the liver and removed through excretion by the kidneys (Weiner *et al.*, 2015). It is important to know about urea functions and metabolism because urea is the major circulating source of nitrogen-containing compounds and it plays important roles in regulating kidney function and uremic symptoms occurs due to the accumulation of ions and toxic compounds in body fluids and improper renal function (Mitch & Fouque, 2012; Weiner *et al.*, 2015). Creatinine is formed endogenously in the muscle during muscle protein metabolism by non-enzymic action on creatine phosphate (Mayes, 1988; Nwankpa *et al.*, 2018). Creatinine is excreted along with urea in the glomerulus of the kidney and its assessment is a useful tool to assess the functionality of the kidney (Ifeanacho, 2017). In present investigation LD and HD of *G. applanatum* extract insignificantly and significantly decreases serum urea level but both doses of the extracts insignificantly decrease creatinine level (Table VII). The previous study reported that natural substance having high antioxidant activity and free radical scavenging activity effectively eliminates the urea and creatinine through excretion and decreases the serum urea and creatinine level (Mehrddad *et al.*, 2007). It has also been reported that mushroom belongs to *Ganoderma* have high antioxidant capacity and their supplements reduced renal pathology by decreasing serum urea and creatinine concentration (Zhong *et al.*, 2015; Abdullah *et al.*, 2018). Uric acid is the end product of endogenous purine metabolism and its production and metabolism involving various factors that regulate hepatic production, renal and gut excretion of this compound (Simao *et al.*, 2012). Endogenously uric acid is mainly produced from the liver, intestines and other tissues like muscles, kidneys and the vascular endothelium (Chaudhary *et al.*, 2013; Maiuolo *et al.*, 2016). It has been reported that elevated serum levels of uric acid play an important role to promote many disease such as gout, articular degenerative disorders, vascular inflammation, and atherosclerosis and adversely change renal vasculature to promote chronic renal disease (Isekiet *et al.*, 2001; Maiuolo *et al.*, 2016;). Previous studies have been reported that, mushroom carbohydrates especially polysaccharides, flavonoids, polyphenolic and other mycochemicals decrease high serum uric

acid level by suppressing liver xanthine oxidase activity which is one of the major catabolic agents involves in the formation of uric acid and free radical generation (Ma *et al.*, 2014; Vanishree *et al.*, 2017). Previously it has also been reported that *Ganoderma* extract showed hypouricemic effects, mediated through renal organic anion transporter 1, glucose transporter 9, and uric acid transporter 1 and gastrointestinal concentrative nucleoside transporter 2 which elevate urine uric secretions and decline in the absorption of purine in the gastrointestinal tracts lead to decline serum uric acid level (Yong *et al.*, 2018). In present study LD of *G. applanatum* extract showed insignificant decrease serum creatinine level. The LD and HD of extract insignificantly and significantly decrease serum urea level. Both the doses of *G. applanatum* extract showed high hypouricemic activity (Table IV), correlates and supports previous studies.

CONCLUSION

G. applanatum extract showed presence did not show any acute toxic impact and mortality and associated with significant elevation of body weight. The extract also showed a negative sub-acute toxic impact on hepatic and renal biochemical parameters. The extract of *G. applanatum* served as hepato tonic activity by elevation of serum albumin level and the extract also showed nephroprotective activity by decrease serum urea and uric acid level. Thus, dose oriented application *G. applanatum* extract can prevent the liver and kidney toxicity and the extract can be used in chronic renal and hepatic diseases.

CONFLICT OF INTEREST

Authors declared that there is no conflict of interest is associated with this publication.

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