



## Impact of Mushroom *Pleurotus tuber-regium* (Rumph. ex) Fr. Extract on Lipid Profile and Testosterone of Rat

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### ABSTRACT

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*Pleurotus tuber-regium* has been used as fodder and traditionally used as medicinal supplement. The aim of this study was to screen in vitro proximate biochemical composition and nutritional potentiality of *P. tuber-regium* extract for further in vivo analysis of impact of the extract on serum lipid and testosterone profile of rats. Total cholesterol, HDL cholesterol, LDL cholesterol were measured photometrically. Testosterone was measured by chemiluminescence immune assay. *P. tuber-regium* extract contains different biochemicals such as proteins, carbohydrates, tannins, flavonoids, phenols etc. and showed highly quantified calorific value (297.89±1.92 calori/100g). Acute toxicity test showed no mortality and toxic behavioural symptoms. Significant decrease in total cholesterol (75.52±0.39mg/dL), LDL cholesterol (34.58±1.69mg/dL) and triglyceride (81.31±1.25) were observed in rat group treated with 200mg/kg dose of extract. 400mg/kg dose of extract showed higher efficacy than 200mg/kg dose of extract and significantly decreased total cholesterol (72.25±1.11mg/dL), LDL cholesterol (26.37±1.21mg/dL) and triglyceride (69.42±0.72). The extract showed testosterone enhance efficacy. 400mg/kg dose of extract showed high level testosterone (178.96±0.68 ng/dL) enhance efficacy than 200mg/kg dose (174.64±0.64ng/dL). Due to hypocholesterolaemia and testosterone elevating properties of *P. tuber-regium* extract, it can be said that, mushroom *P. tuber-regium* can be consumed as healthy diet supplement to maintain cholesterol level and to maintain good male reproductive health.

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## Introduction

Lipids are large and diverse classes of biomolecules, associated with multiple biochemical functions such as fuelling energy production, and building blocks of plasma membrane and acts as signalling molecules during cellular communication (Tang, 2016). It has been reported that, high level of cholesterol is a major risk factor associated with cardiovascular diseases and according to the World Health Organization, cardio vascular diseases are the common causes of morbidity and mortality in the world by the year 2015 and Indian people are most affected with cardio vascular diseases amongst all ethnic population (Nag and Ghosh, 2013).

Mushrooms are called as macro-fungus with a distinctive fruiting body and they are important constituents of forest, they grow on the abundant biomolecule of hosts of this biosphere and synthesized their own biochemicals by the metabolic processes commonly called microchemical. Presently mushrooms are tremendously used as food and medicines besides their key ecological roles and they have been found effective against cancer, reduction hyper

cholesteromia, stress, insomnia, asthma, allergies and diabetes, antibacterial, oxidative stress, antiviral and other properties (Wani et al., 2010; Singh, 2017).

Mushrooms of *Pleurotus* genus are popularly consumed by the peoples of all over the world due to their high nutritional values and some medicinal properties. The *Pleurotus* mushrooms are rich in proteins, essential amino acids, polysaccharides and essential fatty acids, dietary fibers, minerals, some vitamins etc. (Khan and Tania, 2012).

*Plurotus tuber-regium* has been used as medicinal supplement for headache, stomach ailments, cold and fever, asthma, small pox, high blood pressure as well as for weight gain and malnourishment (Oso, 1997; Afieroho et al., 2013). Aim of this work was to study impact of *P. tuber-regium* extract on lipid profile and testosterone of rat because exploration and validation of medicinal impacts of *P. tuber-regium* on lipid profile and testosterone level on mammalian model were least explored.

## Materials and Methods

### Collection of Mushroom

Fresh fruiting bodies of *P. tuber-regium* were collected by the corresponding author from Manas National park of Assam, India (26° 39' 33.9264" N and 91° 0' 4.0644" E) and was match and identified on the basis of morphology with museum specimen by Plant Identification & Preservation Division of Department of Botany, Gauhati University, Assam, India where a voucher specimen (No. 832M) was deposited and rest of the fruiting bodies were brought to the Department of Zoology, Ranchi University, Ranchi, India for experimental work.

### Preparation of Extract

Fresh mushrooms were washed and disinfected by treating with ethanol and washed again. The mushrooms were dried in shade under room temperature for six to seven days, powdered and sieved. An aliquot of the fine powder (50 g) was subjected to Soxhlet extraction using distilled water for aqueous extraction. The obtained extract was filtered, concentrated and dried in a rotary flash evaporator maintained at 45°C (Dandapat and Sinha, 2015).

### Mycochemical Screening

Qualitative screening of tracable biochemicals present in the extract of *P. tuber-regium* was done following previous methods described by Arya et al. (2012). Presence of various biochemicals on the basis of function groups were detected by FTIR spectroscopy.

### Qualitative Analysis of Mycochemicals

#### Test for carbohydrates

Presence of carbohydrate was determined by addition of few drops of Molisch's reagent to the test solutions (1mg/mL extract), this was then followed by addition of 1 mL concentrated H<sub>2</sub>SO<sub>4</sub> (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 appearance of purple violet ring.

#### Test for glycoside

Glycoside was determined by addition of 1mg/mL of extract to 3mL of an throne reagent and was mixed properly. The mixture was observed for appearance of green colour complex.

#### Test for proteins

Protein was estimated by addition of 0.5 mg/mL of the extract and 2mL of Bradford's reagent were left for few minutes. The mixture was observed for appearance of blue colour.

#### Test for alkaloid

Presence of alkaloid was determined by stirring of 1mg/mL 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 H<sub>2</sub>SO<sub>4</sub> (98%) with 2mg/mL of extracts was mixed vigorously. The mixture was observed for initially formation of red colour followed by blue and finally development of green colour.

#### Test for triterpene

Triterpene was estimated by addition of 1mg/mL extract with one drop chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> (98%). The mixture was observed for formation of red colour.

#### Test for phenol

Presence of phenol was estimated by phenolic -catechol method. Dilute aqueous extract (0.5 mL of 1:10 g/L) was pipette out in 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 colour.

#### Test for flavonoid

Flavonoid was estimated by dissolved 1mg/mL extracts in water and later addition of 2 mL of the 10% aqueous sodium hydroxide and then addition of dilute hydrochloric acid as an indicator. The mixture was observed for formation and disappearance of yellow colour.

#### Test for tannin

Tannin was estimated by stirring 0.5 mg/mL 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 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 concentrate HCl. Later few drops of 5% NaOH added drop wise and observed for froth formation.

#### Test for lipid

2 mL of extract was taken and iodine solution was added drop wise. Disappeared of original colour of iodine indicate the presence of lipid. The mixture was observed for disappearance of original colour of iodine.

#### FTIR spectra analysis

Fourier transform infrared (FTIR) spectra analysis was carried out IPResting-21 (Shimadzu Corp., Kyoto, Japan) in the diffuse reflectance mode operated at are solution of 4 cm<sup>-1</sup> in the range of 400 cm<sup>-1</sup> to 4,000 cm<sup>-1</sup> wave number and KBr as standard to identify the potential biomolecules present in fruiting body of *P. tuber-regium* extract which are responsible for reducing and capping the bio reduced silver nanoparticles. The FTIR machine was operated at 25±5°C, 60-70% humidity and 240V AC (IMUSG, 2002).

### Nutritional Potentiality

Calorific value of *P. tuber-regium* fruiting body was estimated on the basis of presence of total protein, total lipid and total carbohydrate in specific amount of eatable substance which together provided total metabolizable energy.

#### Determination of crude protein

The modified Lowry protein measurement was conducted according to the method described by Maehre (2018). The assay was carried out by diluting the extracts to 1 mL with H<sub>2</sub>O and adding 0.9 mL of solution A (2 g/L potassium sodium tartrate (KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub>.4H<sub>2</sub>O) and 100 g/L sodium

carbonate ( $\text{Na}_2\text{CO}_3$ ) in 0.5 M NaOH) before incubation for 10 min at 50°C. Following this, the samples were cooled down to room temperature, added 1 mL of solution B (0.2 g/L  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$  and 0.1 g/L copper sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in 0.1 M NaOH) and left for 10 min. Finally, 3 mL of solution C (Folin-Ciocalteu phenol reagent in  $\text{H}_2\text{O}$  (1:16 v/v)) was added before incubation for 10 min at 50°C. Total protein in the sample was measured by using a standard curve made of bovine serum albumin (BSA; 0, 0.0625, 0.125, 0.25, 0.5 and 1 g/L) and absorbance was recorded at 650 nm.

#### Determination of crude fat

1 g dry mushroom powder was subjected in petroleum ether (60-80°C) soluble fat was extracted in a soxhlet apparatus for about 16 hours. After extraction, the solvent was evaporated over the steam bath and the residue was oven dried at 70°C to a constant weight (Kaur et al., 2018). The total lipid content was determined using the below calculation.

Total Lipid = 100 (weight of soxhlet with extract-weight of dry soxhlet) / weight of mushroom sample.

#### Determination of total carbohydrate

The content of the crude carbohydrate was determined by the following equation (Kajendran et al., 2018).

Carbohydrate (g/100g sample) = [100 - weight of (Protein + Fat + Ash + Fibre + Moisture)]

#### Determination of Calorific value

Metabolizable energy is calculated using the below formula (Schakel et al., 1997):

Metabolizable energy (Kcal/100g) = [(4 × Total protein) + (9 × total fat) + (4 × Total carbohydrate)]

### Impact of *P. Tuber-Regium* Extract on Rat Model

#### Animals

Wistar albino rats of 175 to 200g were obtained from the National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at ambient room temperature and relative humidity, with dark-light cycle of 12 h. Animals 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) for Ph.D. research project (Memo No. B/1195/18), Department of Zoology, Ranchi University, Ranchi, Jharkhand, India.

#### Acute toxicity studies

According to OECD (Organisation for Economic Co-operation and Development) test guideline 425 (Up and Down procedure) limited test for *P. tuber-regium* extract was performed at the single test dose 2000mg/kg body weight (BW) on male albino rat to observe death, behavioural changes due to toxicity and decrease in BW. 5 rats were taken and were fasted (3-4hours) prior to dosing but were provided with water *adlibitum*. Single dose of 2000mg/kg BW of *P. tuber-regium* extract was fed to single rat through gavage using stomach tube and rats were provided with food and water *adlibitum* after 2hours. Similarly, 4 other rats were treated with same dose of extract (OECD, 2008; Saleem et al., 2017). The treated rats were further not received any treatment for 7 days.

Experimental design for impact of *P. tuber-regium* extract on lipid profile

Fifteen fresh animals were acquired and equally distributed among three treatment groups (Group: 1, 2 and 3) each group contain 5 animals. Two successive doses 200 mg/kg BW and 400 mg/kg BW of extract were taken per the guideline of OECD (Oghenesuvwe et al., 2014). At the end of experiment (8<sup>th</sup> day) animals were anaesthetized and blood was collected by orbital sinus blood sample collection method.

Group-1: This group was considered as control group and rats were not treated with mushroom extract and received single dose of 1mL of distilled water daily throughout the entire period of the experiment (7days).

Group-2: This group was considered as low dose (LD) group and rats were fed single dose (200 mg kg<sup>-1</sup> BW) of *P. tuber-regium* extract daily for 7 days.

Group-3: This group was considered as high dose (HD) group and rats were fed single dose (400 mg kg<sup>-1</sup> BW) of *P. tuber-regium* extract daily for 7 days.

All the animals were sacrificed on 8<sup>th</sup> day under light ether anaesthesia. 5 mL blood was collected from animals by cardiac puncture using sterile needle and syringe. The blood sample was blood was collected in evacuated voiles (SRL Diagnostic Pvt. Ltd., India) and allowed to clot for 30 min at 37°C. The clear serum was separated by centrifuge (Wisperfuge 1384 Samson, Holland) at 2500 rpm for 10 min and lipid and hormonal assay were carried out.

Table 1. Microchemical screening of *P. tuber-regium* aqueous extract (+ = present, - = absent).

Microchemical	Present / Absent
Carbohydrate	+
Glycosides	+
Protein	+
Alkaloid	+
Steroid	+
Triterpene	+
Flavonoid	+
Tannin	+
Lipid	+
Saponin	+
Phenol	+

Study of impact of *P. tuber-regium* extract on lipid profile

Estimation of different parameters of cholesterol was studied photometrically (Rifa and Warnick, 2006). Estimation of serum cholesterol parameters was done on a semi-automatic chemistry analyser: 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). Total cholesterol was measured by using Cholesterol FS\* kit (Cat. No. 113009910023), high density lipoprotein (HDL) was measured by HDL-C Immuno FS\* kit (Cat. No. 13521 9910 023), low density lipoprotein (LDL) was measured by LDL-C Select FS\* kit (Cat. No. 1412199 10 026) and triglyceride was measured by Triglycerides FS\* kit (Cat. No. 157109910021).

Study of impact of *P. tuber-regium* extract on Testosterone

Serum testosterone level was measured by chemiluminescence immune assay (Anna et al., 2013) by using semi-automated chemiluminescence (CLIA) plate analyzer (Semi Auto Chemilumi Basic CLIA 2096 plus, Analytical Technologies Limited, Gujarat, India) operated at 100-240VAC  $\pm$ 10%, 10°C to 40°C within 15%-75% humidity. The assay was performed by using Acculite CLIA microwells kit (Product code: 5375-300, Monobind Inc. Lake Forest, California, USA).

### Statistical Analysis

Entire statistical works were done using full proof statistical software WinSTAT (R. Fitch Software, Canal Park, Cambridge, Massachusetts, USA). Data were taken N=5 and results were expressed as a mean  $\pm$  standard error of mean. Statistical analysis was performed by one-way ANOVA with post-hoc student's t-test,  $P \leq 0.05$  was considered as statistically significant.



Figure 1. Fruiting bodies of *P. tuber-regium* and aqueous extract of *P. tuber-regium*

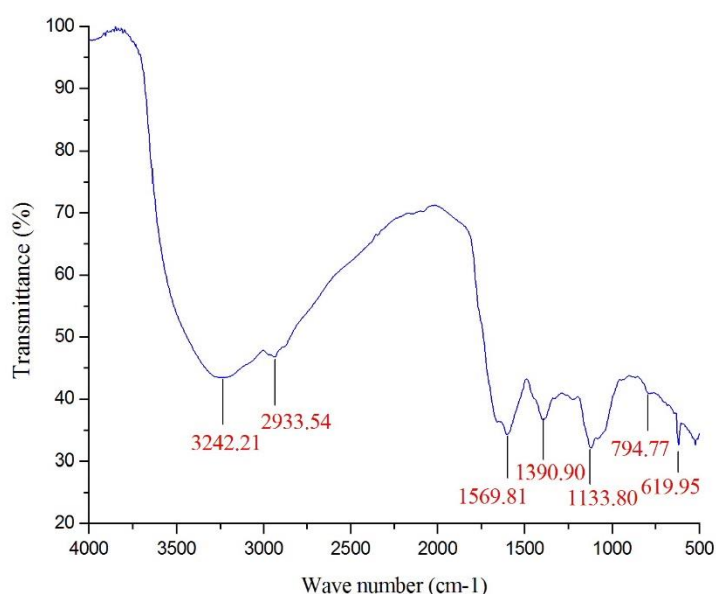


Figure 2. FTIR spectra analysis of *P. tuber-regium* extract

## Results

### Mycochemical screening

The fruiting body of the mushroom is cup-shaped. Pileus is deeply infundibulate, incurved margin with deeply decurrently gills, smoky dark at centre, pale and interrupted white villous form towards the margin. The pileus of the fruiting bodies are 3.4 to 7.2 cm in diameter. Stem or stipe is central, attached with gills, without annulus, sub cylindrical, slightly thick at base having underground tuber sclerotium. The stipeses are 2.5 cm to 6.3 cm long and

6mm to 11mm in diameter, minutely smoky or pale in colour presented in figure-1. 38.5g% water soluble extractive was obtained at the end of extraction. The result shows the extract contain different mycochemicals such as carbohydrate, lipid, protein, tannin, saponin, tannin, flavonoid, phenol etc. Mushroom contains various types of biochemicals such as phenols and flavonois, tannins, proteins, polysaccharides etc. presented in table-1. Confirmation of presence of mycochemical in extract was

done by FTIR spectroscopy analysis. The FTIR spectrum of *P. tuber-regium* is presented in figure-2. Fouriertransform infrared (FTIR) spectroscopy analysis of *P. tuber-regium* extract is presented in Fig. 4. The result showed major transmission broad peak at 3242.21 cm<sup>-1</sup> for O-H stretch of H-bond of alcohol and phenol. Peak at 2933.54 cm<sup>-1</sup> represented to C-H stretch of alkanes. Peak at 1569.81 cm<sup>-1</sup>

corresponded to N-H stretch for primary amines and N=O stretch for nitro methane. Peak at 1390.90 cm<sup>-1</sup> represented to C-H stretch corresponded to alkane. A major peak at 1133.80 cm<sup>-1</sup> represented to C-N stretch or C-O stretch for aliphatic amine or ester respectively. Peak at 794.77 cm<sup>-1</sup> and 619.95cm<sup>-1</sup> corresponded to C-Cl stretch for halo alkanes.

Table 2. Nutritional value of *P. tuber-regium*, n = 5 ± SE, \* = P < 0.05.

Nutritional components				Nutritional value
Crude protein (g%)	Crude carbohydrate (g%)	Crude fat(g%)	Crude fibre(g%)	Calorific value (Kcal/100g)
10.54±0.70*	58.24±2.89*	2.53±0.37*	8.64±0.66*	297.89±1.92

Table 3. Observation toxic behavioural patterns of rats in different groups treated with *P. tuber-regium* extract.

Behaviour patterns	Treatment groups of rats and observation period											
	Control			200 mg/kg of Extract			400 mg/kg of Extract			2000 mg/kg of Extract		
	30 min	24 Hrs.	7th Day	30 min	24 Hrs.	7th Day	30 min	24 Hrs.	7th Day	30 min	24 Hrs.	7th Day
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	N	N	I	N	N	I	N	N
Somatomotor activity & behavior pattern	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	Y	N	N	Y	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

N=nontoxic effect, I=increase, NF=not found, Y=Found

Table 4. Effect of *P. tuber-regium* extract of body weight of rats in different treatment groups.

Animal group	Initial Body weight	Final Body weight	Mortality
Acute toxicity groups	179.46 ± 1.48	181.14 ± 1.30	NO
Control	178.61 ± 1.49	181.76 ± 1.38	NO
200mg/kg treatment group	179.92 ± 1.54	181.84 ± 1.33	NO
400mg/kg treatment group	179.26 ± 1.42	183.22 ± 1.26	NO

#### Acute toxicity study of *P. tuber-regium* extract

Results of acute toxicity of *P. tuber-regium* extract are presented in table-3 and table-4. Acute toxicity test of *P. tuber-regium* extract revealed no behavioural changes and death of animals during the experimental period. However, insignificant increase in the final body weight of the animals treated with 200mg/kg, 400mg/kg and 2000mg/kg were observed compared to animal group treated with vehicle.

#### Impact of *P. tuber-regium* extract on lipid profile and testosterone of rats

Results of impact of *P. tuber-regium* extract on lipid profile and testosterone level of rats is presented in figure-3 to 7. The results showed that, 200mg/kg dose of extract *P. tuber-regium* extract significantly (P<0.05) decreased total cholesterol (75.52±0.39 mg/dL) and 400mg/kg dose of extract significantly (P<0.05) decreased total cholesterol (72.25±1.11 mg/dL) compare to control treatment group of rats (79.37±0.66 mg/dL) presented in Figure-3. 200mg/kg dose of extract *P. tuber-regium* extract significantly (P<0.05) increased HDL cholesterol (55.23±0.87 mg/dL) and 400mg/kg dose of extract significantly (P<0.05) increased HDL cholesterol (61.23±1.36 mg/dL) compare

to control treat group of rats (46.35±0.93 mg/dL) presented in Figure-4. 200mg/kg dose of extract *P. tuber-regium* extract significantly (P<0.05) decreased LDL cholesterol (26.37±1.21 mg/dL) and 400mg/kg dose of extract significantly (P<0.05) decreased LDL cholesterol (34.58±1.69 mg/dL) compare to control treat group of rats (39.80±1.49 mg/dL) presented in Figure-5. 200mg/kg dose of extract *P. tuber-regium* extract significantly (P<0.05) decreased triglyceride (69.42±0.72 mg/dL) and 400mg/kg dose of extract significantly (P<0.05) decreased triglyceride (81.31±1.25 mg/dL) compare to control treat group of rats (119.50±1.39mg/dL) Figure-6). However, 200mg/kg dose of extract *P. tuber-regium* extract significantly (P<0.05) increased testosterone level (174.61±0.64 ng/dL) and 400mg/kg dose of extract also significantly (P<0.05) increased testosterone level (178.96 ±0.68ng/dL) compare to control treat group of rats (170.77±1.36 ng/dL) presented in Figure-7.

#### Discussion

Morphological identification of *P. tuber-regium* was done from collected fruiting body and match with the description mention given in fungi database Mycobank and

Encyclopedia of life (Mycobank, 2015, EOL, 2015). In the present study sclerotiums were not collected and presented. Previously it has been reported *P. tuber-regium* (Singer.) grown on humus soil (HS), mixture of sawdust and humus soil (MSHS), sawdust (SD) had variable diameter of the pileus (HS: 7.74±2.18 cm, MSHS: 8.65±1.75cm, SD: 5.23

±1.53cm) and variable length of stipe (HS: 6.72±1.44 cm, MSHS: 5.83±0.47cm, SD: 4.83 ±0.77cm) (Onuoha and Obi-Adumanya, 2010). Present findings of morphology of *P. tuber-regium* correlates with the previous studies. Jonathan and Adeoyo (Jonathan and Adeoyo, 2011) describe the similar morphological description of *P. tuber-regium*.

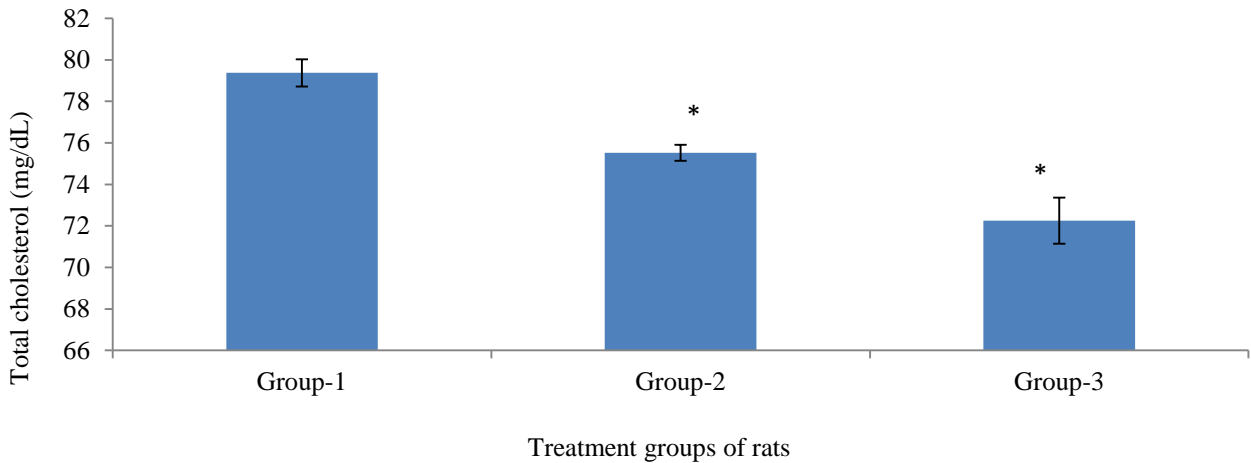


Figure 3. Impact of *P. tuber-regium* extract on total cholesterol of rat; Data were presented N=5±SE of mean, \*= P≤0.05 compared to the group-1

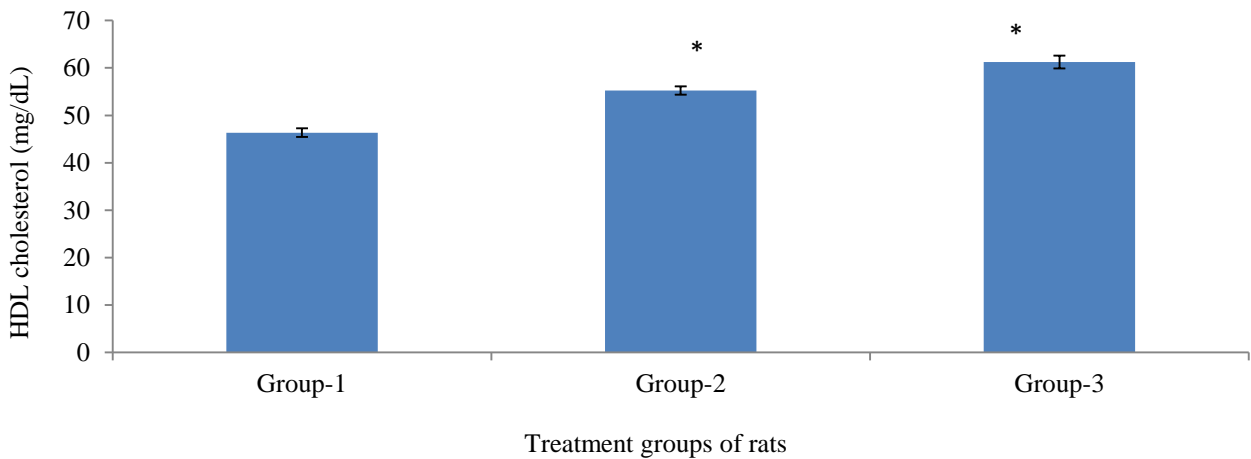


Figure 4. Impact of *P. tuber-regium* extract on HDL cholesterol of rat; Data were presented N=5±SE of mean, \*=P≤0.05 compared to the group-1.

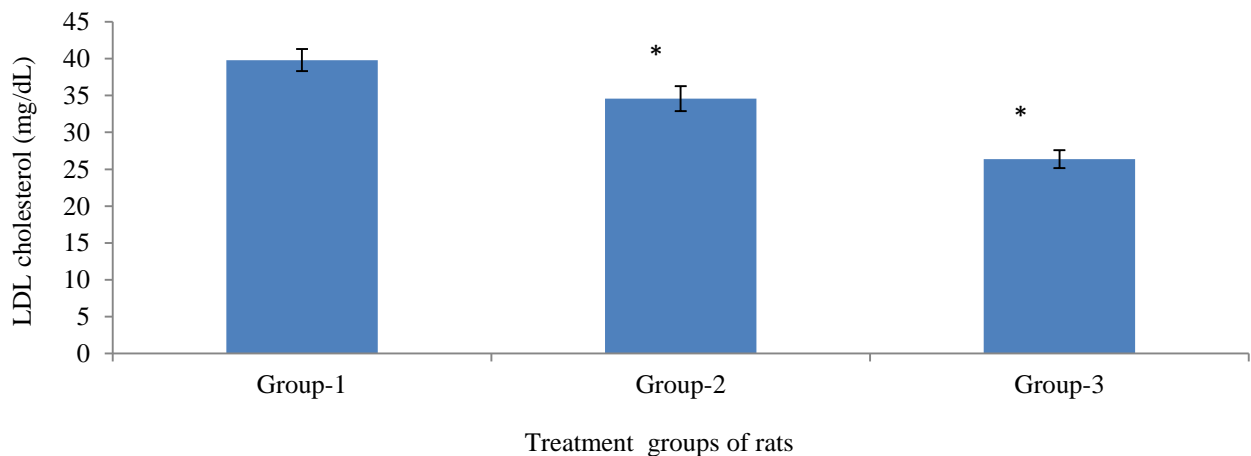


Figure 5. Impact of *P. tuber-regium* extract on LDL cholesterol of rat; Data were presented N=5±SE of mean, \*= P≤0.05 compared to the group-1.

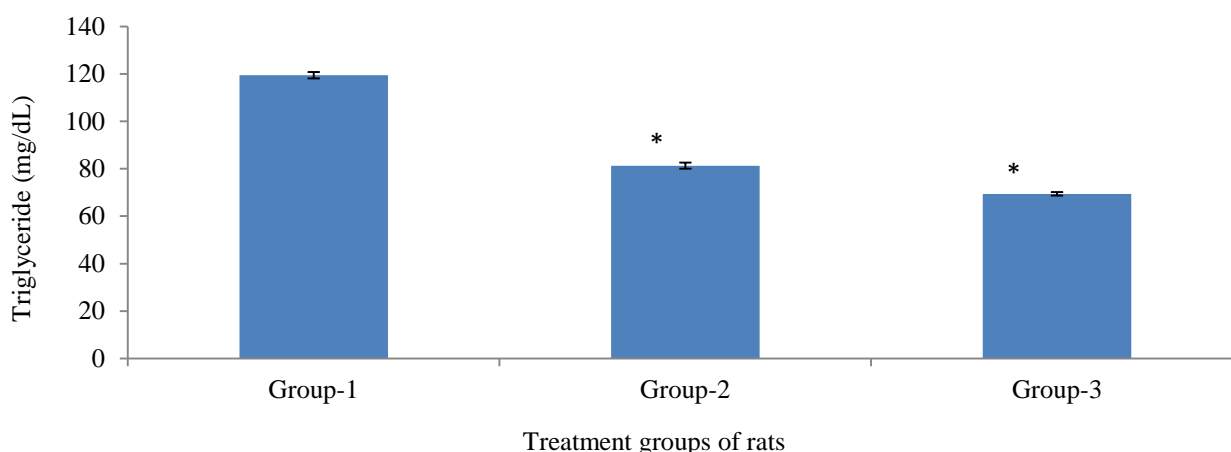


Figure 6. Impact of *P. tuber-regium* extract on triglyceride of rat; Data were presented N=5±SE of mean, \*= P≤0.05 compared to the group-1.

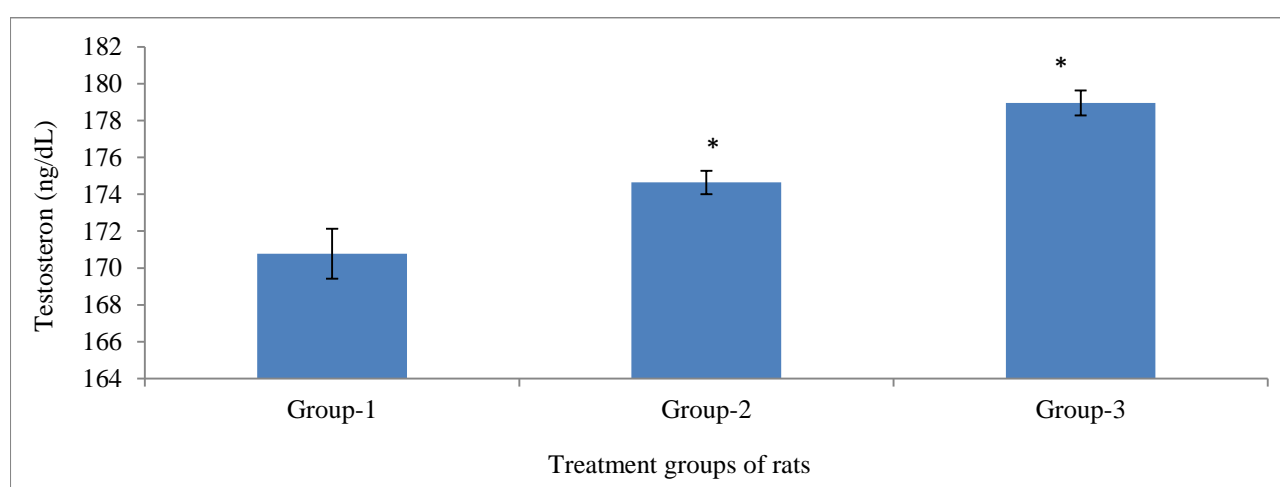


Figure 7. Impact of *P. tuber-regium* extract on testosterone of rat; Data were presented N=5±SE of mean, \*= P≤0.05 compared to the group-1.

Mushroom contains various types of biochemicals such as phenols and flavonoids, tannins, proteins, polysaccharides etc. Previously preliminary biochemical screening of edible white button mushroom *Agaricus bisporus* was done and presence of biochemical such as saponins, tannins, glycosides, reducing sugar, alkaloid, flavonoid, terpenoid etc. were reported (Singh et al., 2017). FTIR analysis provides the confirmation about functional groups different mycochemicals (Reusch, 2018; Libertext, 2018; Wikipedia, 2018). In previous study FTIR analysis of crude extract of *Lentinula edodes*, *Pleurotus ostreatus* and *Agaricus blazei* were done and reported stretches of absorption spectra of  $3600\text{cm}^{-1}$  and  $3200\text{cm}^{-1}$  for O-H stretching,  $2980\text{--}2840\text{cm}^{-1}$  represents C-H stretching,  $1200\text{--}900\text{cm}^{-1}$  corresponds to of carbohydrates and stretching vibrations of C-C, C-O-C for glucopyranose and C-O,  $1640\text{ cm}^{-1}$  and  $1530\text{ cm}^{-1}$  for amides for the confirmation of presence of biochemicals such as saponins, tannins, glycosides, reducing sugar, alkaloid, flavonoid, terpenoid etc. (Radzki and Kalbarczyk, 2010). In present study FTIR analysis of *P. tuber-regium* extract (Figure-2) also provided transmission peaks for phenols, amines and other bioactive mycochemicals which correlates with the previous FTIR analysis of other mushroom extracts.

It has been reported that mushrooms are rich in nutritional constituents such as proteins, minerals, vitamins, fibre and carbohydrate with low fat content but mushroom has twice higher protein content than vegetables and four times than cereals (Okwulehie and Odunze, 2004). Thatoi and Singdevsachan (Thatoi and Singdevsachan, 2014) studied the nutritional composition of eight *Pleurotus* spp. and reported carbohydrate content was higher than protein content and very low fat and good edible fibre. In previous study it has been reported edible mushrooms contains high amount of protein and carbohydrates with very low lipid content and in the present findings (Table-2) similar results were obtained which revealed mushroom *P. tuber-regium* is nutritionally beneficial for health.

It has been reported that mushroom powder increases the excretion of total lipids and cholesterol. Mevinolin a polysaccharide present in fruiting bodies of *Pleurotus* spp. possess hypocholesterolemic activities (Hossain et al., 2003). It has also been reported that, *Pleurotus* spp. reduce cholesterol absorption and reduce the activity of reduced HMG-CoA reductase in the liver associated with cholesterol metabolism (Bobek and Ozdin, 1996). Previously it has been reported that, powder of *P. ostreatus*, *P. citrinopileatus*, *P. pulmonarius* and *P. salmoneostramineus* significantly decreases plasma,

triglyceride, low-density lipoprotein, total lipid of hypercholesterolemic rats (Alam et al., 2011; Yoon et al., 2012). It is reported that higher HDL level is associated with better cardiovascular health and no significant effect in cardiovascular system was found further increase in HDL level after gaining its optimum level (Cesare, 2006). It has also been reported that, significant decrease in HDL level and increase level of LDL increases 10-fold and 3-fold risk and of heart disease (Barter et al., 2007). In the present investigation *P. tuber-regium* extract significantly decreases the serum total cholesterol, LDL cholesterol and triglycerides level (Table- 4) and correlates with the previous studies. Normal male reproductive function depends on testosterone and proper function of interstitial cells of testicular follicles. It has been reported that, endogenous testosterone level gradually decreases with aging in men and detrimentally affects health status (Jones, 2010). Low levels of endogenous testosterone are associated with an increased risk of atherosclerosis in men, and serum lipids are essential for atherosclerosis development due to deficiency of testosterone (Traish et al., 2009; He et al., 2013; Ruige et al., 2013). Several clinical and epidemiological studies have reported that serum testosterone levels are inversely correlated with total cholesterol and LDL cholesterol levels and it has been demonstrated markedly increased serum cholesterol levels in testosterone-deficient male mice (Hatch et al., 2012; Zhang et al., 2014). These findings indicate that testosterone metabolism is associated with regulation of serum cholesterol metabolism and in our study *P. tuber-regium* significantly correlates with the previous studies.

## Conclusion

*P. tuber-regium* extract contains different types of biochemicals and the extract of *P. tuber-regium* significantly decreases total cholesterol, LDL cholesterol, and triglyceride and increase HDL cholesterol and testosterone level of rats. The mushroom *P. tuber-regium* also possesses high calorific value. Thus, consumption of *P. tuber-regium* is beneficial for health and its consumption can prevent diseases associated with hypercholesterolemia and maintain good male reproductive health. Further, isolation and screening of specific biochemicals from *P. tuber-regium* and their path associated activity studies in animal model must be done for discovery of specific compound associated with anti-hypercholesterolemic activity.

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## Conflict Ofinterest

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

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