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Research Article

Nutritional, Hypoglycemic, and Haematinic Potentiality of Edible Mushroom *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer

Sukumar Dandapat [1], Manoj Kumar [2], Rakesh Ranjan [1], Manoranjan Prasad Sinha [1]

[1] Department of Zoology, Faculty of Sciences, Ranchi University, Ranchi 834008, India
[2] Department of Zoology, Faculty of Sciences, St. Xavier's College, Ranchi 834001, India

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**Corresponding author:*
E-mail: dr.sukumar2018@gmail.com

ABSTRACT

Mycochemical composition, calorific value, the antioxidant activity of *Pleurotus tuber- regium* was analyzed and impact of *P. tuber- regium* extract on rat model especially glycemic, vitamins and blood parameters were explored to validate its medicinal importance. Mycochemical screening showed the presence of biochemicals such as phenols, flavonoids, proteins, carbohydrates etc. The extract showed good antioxidant activity (33.62% total antioxidant activity equivalent to 21.30 µg ascorbic acid). The extract showed dose -dependent hypoglycaemic activity by significant decreased average blood glucose level at high dose (100.62 ± 1.04mg/dL) compare to control (124.40 ± 1.45 mg/dL), haematinic activity by elevation of hemoglobin (14.75 ± 0.24 g/dL) at a high dose of extract compared to control (11.66 ± 0.21 g/dL). *P. tuber-regium* extract elevated vitamin B12 of rats at high dose of extract (449.60 ± 3.12 pg/mL) compared to control (420.00 ± 2.86pg/mL). *P. tuber-regium* extract showed a positive response to hypoglycemic, haematinic, and vitamin level of the body and posses high calorific value. Hence *P. tuber-regium* can be used as good fodder, medicinal and nutritional supplement.

Keywords: Vitamin, mycochemicals, medicinal, antioxidant, diabetes

Introduction

In recent decades population explosion and its burden is directly associated with burden of noncommunicable diseases especially chronic and nonchronic disease such as diabetes, renal, cardiovascular, respiratory, cancer and conditions associated with malnutrition [1]. Worldwide in past two decades more than 57 million deaths occurred, and more than 29 million deaths occurred due to noncommunicable diseases in a developing country [2]. Diabetes and malnutrition are two major factors in developing countries related to morbidity and approximately 6 million people die every year due to diabetes [3, 4]. It has been estimated and reported by WHO that, worldwide every year about 11 million death of population below 10 years age occurs due to nutritional deficiency diseases and disorders [5]. One of the major causes of diabetes is the production of free radicals (reactive oxygen and nitrogen species) impart deleterious effects and one of them is diabetes [6].

The leading causes of deficiency diseases in developing countries are due to low nutrient quality of food and deficiency to minerals, vitamins, and other nutrient proteins, fats [7]. In recent decades, annual population growth rate as well as the burden of disease in developing countries of the Middle East and Sub-Saharan Africa, Latin America, South Asia and Southeast Asia increasing continuously due to poverty, improper education, insufficient medicine and medical facility etc. [8]. The population growth in India for last 30 years have been increasing at the rate of 2.3% per year [9], and an emerging drastic environmental imbalance will lead wider spectrum of health risks, resource fulfilment such as nutritional diet, proper supply of medicine, etc. [10, 11].

Traditionally mushrooms have been used as

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medicine as well as excellent source of nutrient [12]. About 700 species of mushrooms have been reported for their significant therapeutic efficacy [13]. Medicinal mushrooms contain various mycochemicals such as tannins, alkaloids, flavonoids, phenolics, etc., which associated with the remedy of diseases and disorders. *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer, commonly called king oyster mushroom, has been traditionally consumed as medicine and nutraceutical food supplement [14].

The aim of the present work to study proximate biochemical composition with nutritional potentiality, antioxidant activity of *P. tuber-regium* and impact of *P. tuber-regium* extract on glycemic, vitamins and blood parameters of rat and to validate the nutritional potentiality of *P. tuber-regium* because the screening of medicinal effects of *P. tuber-regium* on mammalian model is least explored.

Material and Methods

Collection, identification of mushroom and preparation of extract

Fresh fruiting bodies of *P. tuber-regium* were collected from different sites of three National Parks namely Orang National Park, Kaziranga National Park and Manas National Park of Assam and were identified in Department of Botany, Gauhati University, Guwahati, Assam and brought to Department of Zoology, Ranchi University, Ranchi form preparation of extract.

Preparation of extract

Fresh mushrooms were dried in the shade under room temperature for six to seven days, powdered and sieved. As much as 50 g of the fine powder was subjected to the extraction chamber of soxhlet using distilled water. The extracts obtained were filtered, concentrated dried in the rotary flash evaporator and the dried extract was stored in air tight containers at room temperature for further study.

Mycochemical screening

Qualitative analyses of proximate biochemical present in the extract of *P. tuber-regium* were determined following Arya et al. [15]. Presence of various biochemicals based on function groups were detected by FTIR spectroscopy (IPRresting-21, Shimadzu). Quantitative estimation of traceable biochemicals was done following *Dandapat et al.* [16].

Nutritional potentiality

Nutritional potentiality of *P. tuber-regium* fruiting body was estimated on the basis of total protein, fat and carbohydrate using methodology proposed by Nile and Cobragade [17].

Antioxidant activity

Antioxidant activity of *P. tuber-regium* extract was determined on the basis of total antioxidant activity [18], free radical scavenging and hydroxyl radical scavenging activity [19] using standard methods.

Impact on rat model

Animals

Wistar albino rats of 175 to 200 g 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 hours. 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).

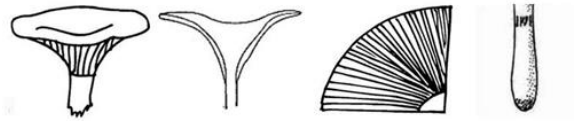
Acute toxicity studies

Single doses of *P. tuber-regium* extract (2000 $mg.kg^{-1}$ body weight) was administered orally by oral feeding gavage to rats. The animals were observed for gross behavior, neural, and autonomic toxicity as described on OECD guidelines [20].

Experimental design

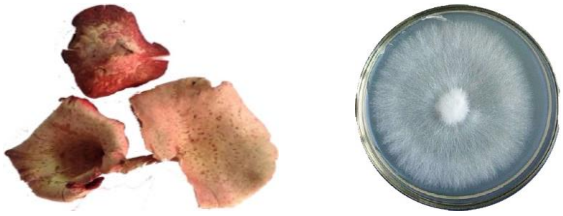
Fifteen fresh animals were acquired and equally distributed among three treatment groups (Group: 1, 2 and 3) each group contain 5 animals. Low dose and a high dose of extract was calculated as per the guideline of OECD [20] and Oghenesuvwe *et al.* [21]. At the end of the experiment (8^{th} day) animals were anesthetized and blood was collected by orbital sinus blood sample collection method.

Group-1 (Control): rats were served as a control and were not treated with mushroom extract and received 1mL of distilled water orally throughout the entire period of the experiment.



(a) (b) (c) (d)

Figure 1. Diagrammatic morphological representation of *P. tuber-regium*: fruiting body (a), decurrent gill (b), crowded lamellae (c), and stipe (d)



(a) (b)

Figure 2. Photograph of collected *P. tuber-regium* fruiting bodies and grown mycelia from *P. tuber-regium* fruiting body

Figure 3. Aqueous extract of *P. tuber-regium* fruiting bodies

Group-2 (low dose): Rats were received 200 mg.kg^{-1} body weight considered as low dose of *P. tuber-regium* extract orally for 7 days. Group-3 (high dose): Rats were received 400 mg.kg^{-1} body weight considered as high dose of *P. tuber-regium* extract orally for 7 days.

Estimation of HbA1c and average blood pressure (ABG)

Percentage of HbA1c was estimated by using fully automated HPLC (Model – TOSOH G8). Average blood glucose was estimated from derived values from HbA1c [22].

Estimation of vitamin D and vitamin B12

The concentration of vitamin D and vitamin B12 in the serum samples were estimated by using fully automated chemiluminescent immune assay (25-OH Vitamin D IR (chemi luminescence immune assay) CLIA kit [23] and AccuBind Vitamin B-12 CLIA kits of Tosoh Bioscience's) and Tosoh Bioscience's AIA-360 automated immunoassay analyzer [24].

Hematologial analysis

For haematological indices, blood samples were collected into sterile tubes containing EDTA and immediately analyzed for total white blood corpuscle count (TWBC), total red blood corpuscle count (TRBC), Hb, and mean corpuscles volume of RBC (MCV) using Sysmex automated blood analyzer - KX 21 Kobe, Japan.

Statistical analysis

Data were taken 5 times and results were expressed as a mean ± standard error of the mean. Statistical analysis was performed using Student's t-test, $p < 0.05$ was considered as statistically significant. Entire statistical analyses were performed using full proof software WinSTAT.

Results and Discussion

Morphological of P. tuber-regium

The fruiting body of the mushroom is cup-shaped. Pileus is deeply infundibulate, incurved margin with deeply decurrently gills, smoky dark at center, pale and interrupted white villous form towards the margin presented in Figure 1. The pileus of the fruiting bodies is 3.4 to 7.2 cm in diameter presented in Figure 2. Stem or stipes is central, attached with gills, without annulus, sub cylindrical, slightly thick at base having underground tuber sclerotium (Figure 1). The stipeses are 2.5 cm to 6.3 cm long and 6 mm to 11 mm in diameter, minutely smoky or pale in color presented in Figure 2. Morphological identification of *P. tuber-regium* was done from collected fruiting body and matched with the description mention given in fungi database Mycobank and Encyclopedia of life [25, 26]. 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 ± 1.75 cm, SD: 5.23 ± 1.53 cm) and variable length of stipe (HS: 6.72 ± 1.44 cm, MSHS: 5.83 ± 0.47 cm, SD: 4.83 ±0.77 cm) [27]. Jonathan and Adeoyo also reported the morphological characteristics of *P. tuber-regium*. They reported, the collected fruiting bodies were creamy in colour, the caps were 5.6 cm in diameter and stalks were 5.6 cm in

Table 1. Qualitative biochemical analysis of *P. tuber-regium* extract

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

length [28].

Mycochemical screening

The result of the qualitative screening of biochemical present in *P. tuber-regium extract* (Figure 3) presented in Table 1. The biochemical screening of extract shows the presence of different mycochemicals such as carbohydrates, glycosides, proteins, tannins, saponins, alkaloids, steroids, and lipids. Confirmation of presence of mycochemicals in the extract was done by FTIR spectroscopy analysis. The FTIR spectrum of *P. tuber-regium* is presented in Figure 4. The result of FTIR analysis shows major absorption peaks at 3,290 cm^{-1}, represents O-H stretch for alcohol and phenols, 2,935 cm^{-1} represents N-H and N-H stretch for amino acids, carboxylic acid and its derivatives, 1,658 cm^{-1} represents C=C stretch for alkane, 1,600 cm^{-1} represents C= stretch for aromatic compounds, 1,392 cm^{-1} stretch for O-H represents phenolic compound, 1,041 cm^{-1} represents C-X stretch for chloroalkanes, 945 cm^{-1} represents C-H and P-OR stretches for aromatic and phosphate group respectively and 520 cm^{-1} represents C-Br or C-I or S-S stretch. Result of qualitative biochemical analysis of traceable mycochemical is presented in Figure 5. Among the mycochemicals alkaloid was found significantly ($p < 0.05$) high quantity (28.14 ± 0.32 mg/100 g) and tannin significantly ($p < 0.05$) low quantity (2.74 ± 0.26 mg/100 g) among all the studied biochemicals.

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 [29]. FTIR analysis provides the confirmation about functional groups different mycocheicals [30-32]. 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 3,600 cm^{-1} and 3,200 cm^{-1} for O-H corresponds sugar residue, 2,980 – 2,840 cm^{-1} represents C–H stretching, 1,200 – 900 cm^{-1} corresponds to of carbohydrates and stretching vibrations of C–C, C–O–C for glucopyranose and C–O, 1,640 cm^{-1} and 1,530 cm^{-1} for amides for the confirmation of presence of biochemicals such as saponins, tannins, glycosides, reducing sugar, alkaloid, flavonoid, terpenoid etc. [33].

It has been reported other mushroom also contain 64.12 ± 1.2 mg/g phenols, 0.016 ± 0.001 mg/g flavanoid, 0.28 ± 0.04 mg/g saponins, 0.1 ± 0.04% alkaloids and 0.014 ± 0.003 % tannins in *Tricholoma nudum* and 6.012 ± 0.91 mg/g phenols, 0.031 ± 0.02 mg/g flavanoids, 0.27 ± 0.008 mg/g saponins, 2.0 ± 0.01% alkaloids and 0.014 ± 0.001 % tannins in *Psalliota campestris* and the biochemicals possess therapeutic efficacy [34]. Biochemicals such as phytophenols, flavonoids, tannins, saponins, etc. are associated with the reduction of free radicals and decrease the risks of disease and disorders associated with oxidative stress [35, 36]. In the present study FTIR analysis (Figure 4) of *P. tuber-regium* extract also confirmed mycochemicals such as phenols, amines, carbohydrates, etc.

Antioxidant activity

Antioxidant activity of *P. tuber-regium* was determined on the basis of free radical scavenging, hydroxyl radical scavenging capacity and total antioxidant activity of the mushroom extract. Results of antioxidant activity of *P. tuber-regium* are presented in Figure 6, 7, and 8. BHA (Butylated Hydroxy Anisole) is a synthetic reducing agent and its free radical scavenging activity is quite higher than the extract presented in Figure 6. However, the extract also shows good free radical scavenging activity.

As much as 100 µg/mL of extract showed significantly ($p < 0.05$) highest free radical scavenging activity (8.98 ± 1.02%) among the tested con-

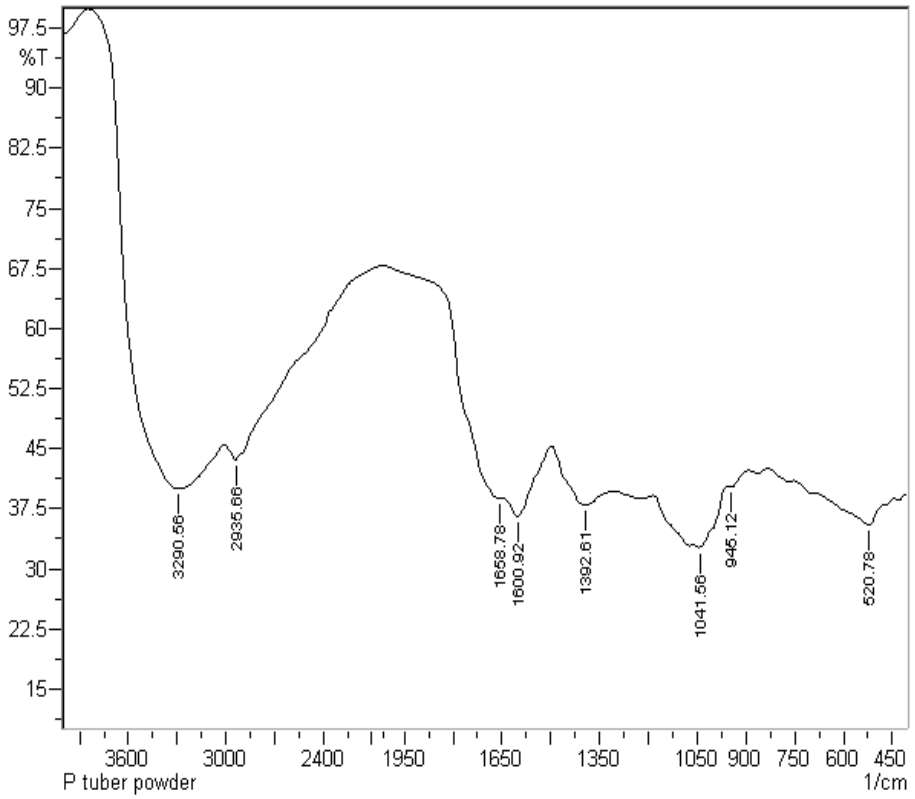


Figure 4. FTIR spectra analysis of P. tuber-regium extract

Table 2. Nutritional value of *P. tuber-regium*, n = 5 ± SE, *= p < 0.05, ***= < 0.0005

Nutritional components (g%)				Nutritional value
Crude protein	Crude carbohydrate	Crude fat	Crude fiber	Calorific value (Cal/100g)
$10.54 \pm 0.70^{*, ***}$	$58.24 \pm 2.89^{***}$	$2.53 \pm 0.37^{*, ***}$	$8.64 \pm 0.66^{***}$	297.89±15.92

centrations of the extract. Hydroxy radical scavenging activity of the extract was compared with ascorbic acid (presented in Figure 7) and found 100 µg/mL of the extract showed significantly ($p < 0.05$) highest (10.85 ± 0.73%) hydroxyradical scavenging activity among the tested extract but the ascorbic acid showed significantly ($p < 0.05$) more effective scavenging activity (68.11 ± 2.46%). Total antioxidant capacity of the crude extract (presented in Figure 8) showed significantly ($p < 0.05$) very effective result. As much as 100 µg/mL extract showed 21.50 ± 1.3% antioxidant activity equivalent to ascorbic acid when compared to the same concentration of BHA (65 ± 1.5% antioxidant activity). However, 10 µg/mL extract did not show any activity.

Antioxidants are chemicals mainly derivative of plants or mushrooms that reduces free radicals such as O_2^-, OH^-, Fe^{2+} or Fe^{3+} etc. [37]. Previously antioxidant activity of *Pleurotus florida* and *Calocybe indica* was studies and reported hydroxyl radical scavenging of extracts of *P. florida* and *C. indica* 65.41 ± 0.65% at 1,000 µg/mL and 46.99 ± 2.58% at 1,000 µg/mL respectively [38]. It has been reported antioxidant activity of plant and mushroom extracts are concentration dependent, and the antioxidant activity depends upon the concentration of bioactive mycochemicals such as alkaloids, tannins, saponins, flavonoids, phenols, etc. present in the fruiting body of mushrooms [39]. It has also been reported bioactive chemicals including primary and secondary metabolites of plant and mushroom origin possess reducing power and reduces the reactive oxygen and nitrogen species produced in the human body during pathogenic infections, so that they can act as a source of good and safe antioxidants [40, 41].

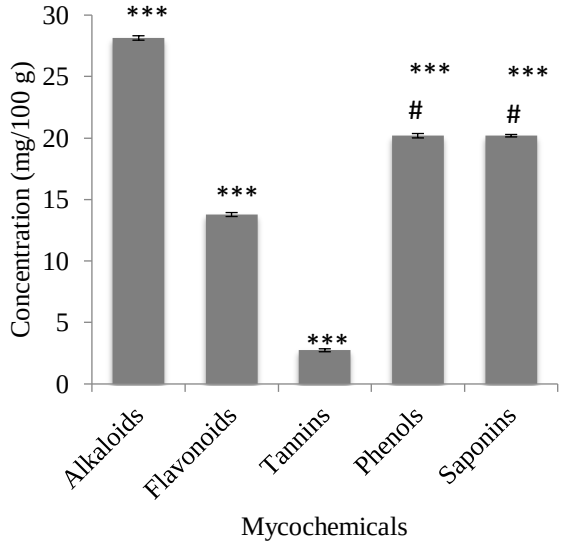


Figure 5. Qualitative analysis of traceable biochemical of *P. tuber-regium*, n = 5 ± SE, *** = p < 0.0005, # = No significant differences between phenol and saponin

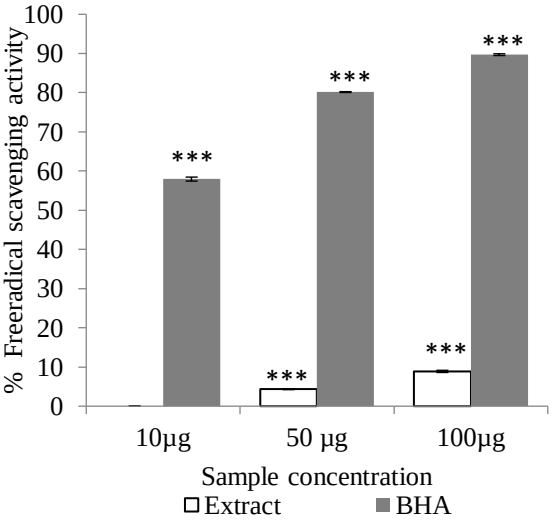


Figure 6. Free radical scavenging activity of *P. tuber-regium* extract n = 5 ± ES, ***= <0.0005

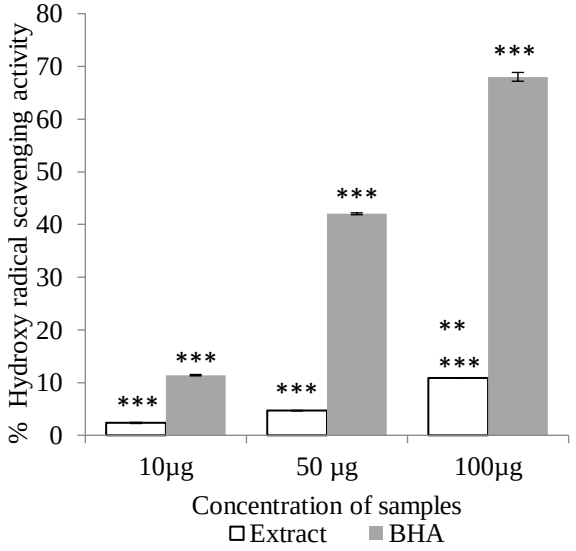


Figure 7. Hydroxyl radical scavenging activity of *P. tuber-regium* extract n = 5 ± ES, ** = p < 0.0025, *** = < 0.0005

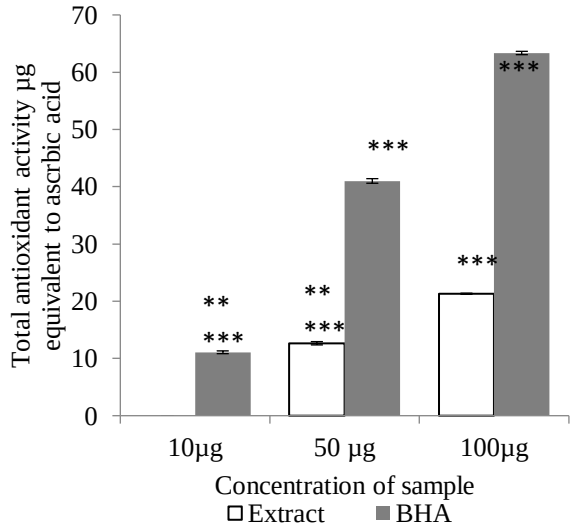


Figure 8. Total antioxidant capacity of P. tuber-regium extract, n=5 ± ES, **= p<0.005, ***= <0.0005

Nutritional potentiality

In the present study, nutritional value of *P. tuber-regium* is presented in Table 2. The result shows carbohydrate content significantly ($p < 0.05$) high and fat content is significantly ($p < 0.05$) low among the nutritional components. It has been reported that mushrooms are rich in nutritional constituents such as proteins, minerals, vitamins, fiber and carbohydrate with low-fat content but mushroom has twice higher protein content than vegetables and four times than cereals [42]. Thatoi and Singdevsachan [43] studied the nutritional composition of eight *Pleurots* spp. and reported carbohydrate content (32 – 63%) was higher than protein content (19 – 39%). They also reported the mushroom species to contain very low fat (1 – 3%) and good edible fibre (4 – 48%). Edible mushrooms contain almost all the essential and non-essential amino acids which are associated with maintenance of the antioxidant system of the body [44]. In a previous study, it has been reported edible mushrooms contains a high amount of protein and carbohydrates with very

low lipid content (Table 2).

Impact of the extract on rat

Rats were treated with extract of *P. tuber-regium* for acute oral toxicity test did not show any morbidity and toxic effects such as convulsion, hair loss, nausea etc. hence the extract of *P. tuber-regium* is safe for long term use. Results of impact of extract of *P. tuber-regium* on rat for evaluation nutritional potentially has been studied and reported under following sub-heads.

Impact of extract on the glycemic regulation

Impact of *P. tuber-regium* extract on glycemic parameters of rats is presented in Figure 9 and 10. The extract significantly ($p < 0.05$) decreased the HbA1c of high dose group rats (2.40 ± 0.43%) compare to low dose (4.03 ± 0.65%) and control group of rats (5.57 ± 0.67%). However, a non-significant decreased of HbA1c was observed in rats of low dose group compared to control group. *P. tuber-regium* extract showed good hypoglycaemic activity on both low and high dose groups of rats. The extract showed significant decrease ($p < 0.05$) in average blood glucose level of both low (109.63 ± 1.40 mg/dL) and high dose groups of rats (100.62 ± 1.04 mg/dL) compare to control group (124.40 ± 1.45 mg/dL).

It has also been reported that free radicals and oxidative stress directly associated with the development of diabetes [18]. HbAc1 and average blood glucose are two major indices to screening, diagnosis and prediction of progression of glycemia and there is a positive correlation between HbAc1 and blood glucose level [45, 46]. It has been reported medicinal mushrooms have been used as a traditional source of potential hypoglycemic and anti-diabetic agent and the mushrooms contain natural bioactive compounds such as polysaccharides, proteins, dietary fibers, and alkaloid, saponins, phenols, flavonoids, tannins, etc. [41, 47].

Several studies have been reported that, different biochemical have different mechanism to regulate and decrease blood glucose level [48]. Flavonoids either mimic as insulin or regulate the expression of enzymes involved in carbohydrate metabolism path way which enhance glucose uptake in tissues [49]. Odoh and Ezugwu [50] reported that, alkaloids increase glucose uptake in muscle by inhibiting expression of protein tyrosine phosphatase-1B of the insulin signaling pathway. Tannins are associated with glucose uptake from blood to muscles and other tissues by inducing translocation of the glucose transporter GLUT-4 and phosphorylation of the insulin receptors [51]. It has been also reported that saponins reduce serum glucose levels in old age diabetic patients [48, 52]. It has been reported mushroom belongs to genus *Pleurotus* possess antidiabetic activity [47]. Jayasuriya et al. [53] studied the oral hypoglycaemic activity of *Pleurotus ostreatus* (P.o.) and *P. cystidiosus* (P.c.) mushrooms on normal and diabetic rats and reported that, both the mushrooms significantly ($p < 0.05$) reduced the serum glucose levels of normal and diabetic rats. Khatun et al. [54] studied the hypoglycemic activity of *P. florida* in rat and reported the mushroom powder significantly decreases blood glucose level of rat without any toxic effect. In present *P. tuber-regium* also shows similar result.

Impact of extract on vitamin D and vitamin B12

Results of *P. tuber-regium* extract on vitamin D and vitamin B12 level of the rat are presented in figure- 11 and 12, respectively. *P. tuber-regium* extract significantly ($p < 0.05$) elevated the vitamin D level of high dose group of rats (33.05 ± 3.08 ng/dL) compare to low dose group (23.27 ± 2.46 ng/dL) and control group (18.18 ± 1.92 ng/dL). However, a non-significant increase in vitamin D was observed in low dose group of rats compared to control rat group. Mushrooms are one of the best sources of vitamins especially Vitamin B, C, and D [55, 56]. Vitamin D is an essential vitamin for human health. It has been reported that food with rich vitamin D and additional vitamin D supplementation reduces risks of cerebrovascular, chronic kidney disease and increase insulin sensitivity [57]. Jasinghe *et al.* [58] studied bioavailability of the vitamin D-enriched mushrooms Lentinula edodes on rat model and found bone mineral density and serum calcium concentrate of the experimental group of rats was significantly higher ($p < 0.01$) than the control group.

The extract also showed a good result of vitamin B12. A significant increase ($p < 0.05$) in vitamin B12 level of Rats treated with high dose of the extract (449.60 ± 3.12 pg/mL) was observed compare to low dose group (443.60 ± 2.37 pg/mL) and control group (420.00 ± 2.86 pg/mL) but a non-significant increase was observed in high dose

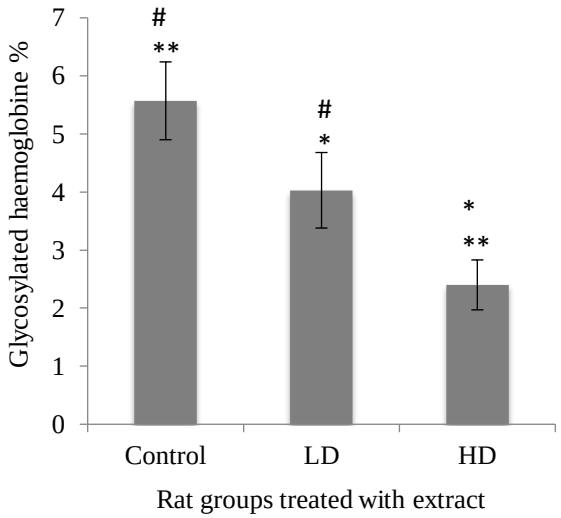


Figure 9. Impact of *P. tuber-regium* extract on glycosylated haemoglobin (HbA1c) %, n = 5 ± SE, # = No significant differences between control and low dose, * = p < 0.05, ** = p < 0.0025.

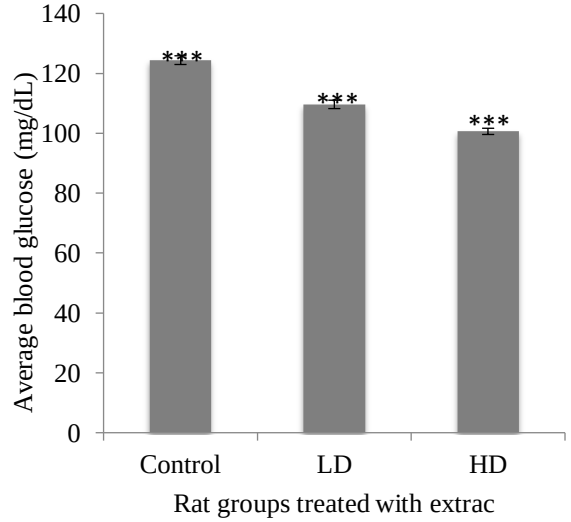


Figure 10. Impact of *P. tuber-regium* extract on ABG, n = 5 ± SE, *** = < 0.0005.

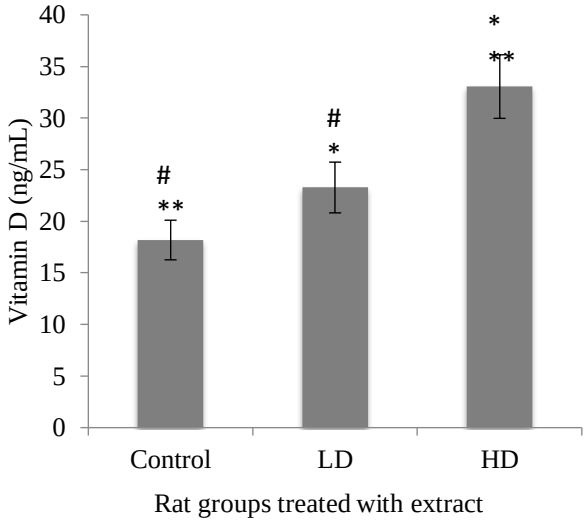


Figure 11. Impact of *P. tuber-regium* extract on vitamin D, n = 5 ± SE, # = No significant differences between control and low dose, * = p < 0.02, ** p = < 0.0025.

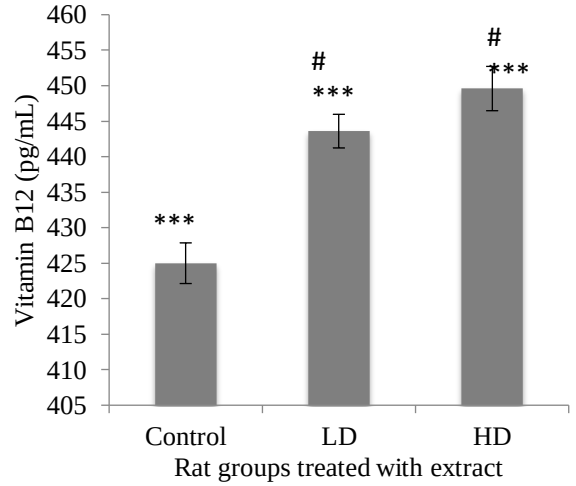


Figure 12. Impact of *P. tuber-regium* extract on vitB12, n = 5 ± SE, #=No significant differences between low dose and high dose, *** = < 0.0005.

group compared to low dose group. It has been reported that food with rich vitamin B12 supplement is associated with maintenance of immune response of body [59]. It was also found significant ($p < 0.001$) decrease in cytotoxicity mediated by natural killer cell [59]. Shawabkeh and Jamal [60] reported that in diabetic patients concentration of vitamin B12 is lower compared to healthy persons. No previous study has been reported on impact of mushroom extract on vitamin B12 and vitamin D level of rats but in present study, *P. tuber-regium* extract significantly ($p < 0.05$) elevate vitamin B12 concentration both in high and low dose group (Figure 12) which were associated with glycemic parameters of rats and must be enhanced immunity of body.

Impact of extract on haematological parameters of rat

P. tuber-regium extract enhanced haematopoiesis in rats. Total RBC, haemoglobin concentration and total WBC count of high dose group (RBC = 7.05 ± 0.43 × 10^6/µL, Hb = 14.75 ± 0.24 g/dL, WBC = 8.35 ± 0.31 ×10^3/µL) significantly

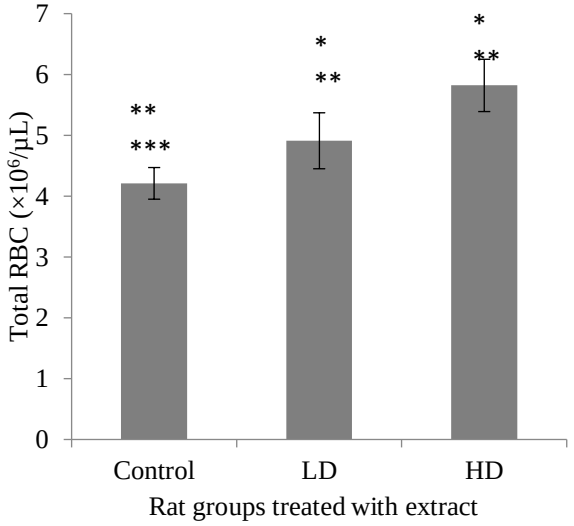


Figure 13. Impact of *P. tuber-regium* extract on total RBC (×10^6/µL), n = 5 ± SE, * = p < 0.05, ** = < 0.005, *** = <.00005.

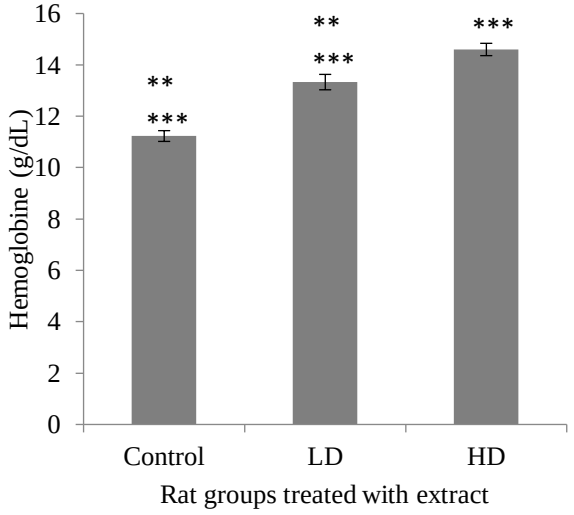


Figure 14. Impact of *P. tuber-regium* extract on Hb, n = 5 ± SE, ** = p < 0.02, *** = p <0.0005.

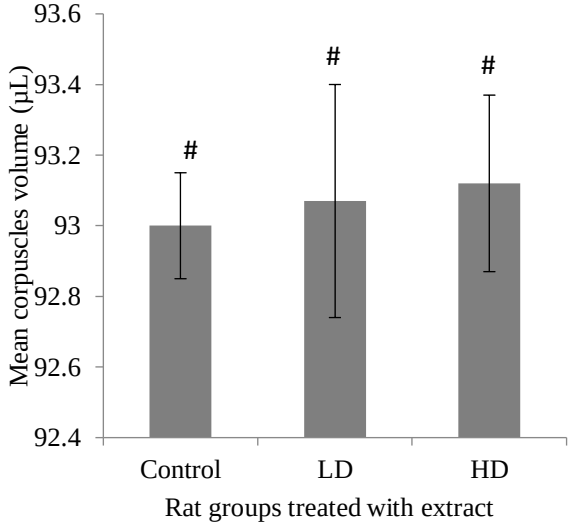


Figure 15. Impact of *P. tuber-regium* extract on mean corpuscles volume (MCV), n = 5 ± SE, # = Non significant differences among the groups of rats.

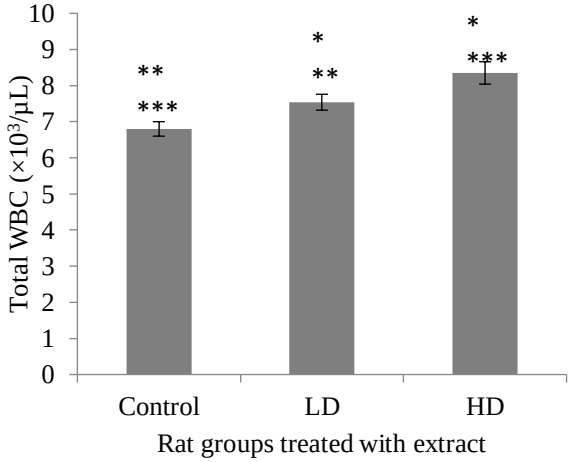


Figure 16. Impact of *P. tuber-regium* extract on total WBC (×10^3/µL), n = 5 ± SE, * = p < 0.05, ** = p < 0.025, *** = p < 0.0025.

(p < 0.05) increased compare to low ((RBC = 5.83 ± 0.46 × 10^6/µL, Hb = 12.73 ± 0.30 g/dL, WBC = 7.54 ± 0.22 × 10^3/µL) and control group (RBC = 4.12 ± 0.02 × 10^6/µL, Hb = 11.66 ± 0.21 g/dL, WBC= 6.80 ± 0.20 ×10^3/µL). However, a non-significant increase in MCV was observed in high dose group (93.12 ± 0.25 µL) and low dose group (93.07 ± 0.33 µL) compare to control group (93.00 ± 0.15 µL).

Hematological parameters are usually associated with health status and are of diagnostic importance in clinical assessment and they are good indicators of the physiological, pathological, nutritional and immunological status of the body [61, 62]. Previously it was reported rats feed with mushroom extract of edible *Plurotus* species (*P. ostreatus* and *P. pulmonarius*) enhance the total RBC and WBC count, hemoglobin concentration and PCV in treatment group compared to non-treatment group [63]. Previously impact of *P. florida* on hematological parameters such as RBC count, Hb concentration and MCV of cadmium toxicity rats fed with *P. florida* supplement diet was studied and reported *P. florida* can prevent cadmium toxicity, maintain these parameters and enhance the level of these parameters [64]. In pre-

sent study *P. tuber-regium* extract significantly ($p < 0.05$) increased RBC count (Figure 13) and Hb concentration (Figure 14) of treated groups which may be due to enhancement of erythropoiesis in myeloid tissues of body, proper absorption of iron from food and their incorporation in hemoglobin molecules during their synthesis [65]. MCV is a measure of the average volume or size of a red blood cell. In the present study non-significant increase in MCV of both the treatment groups (Figure 15) was observed which may be due to increase in vitamin B concentration [66]. White blood cells count and its indices play a vital role in immune function and they are formed from pluripotent myelogenous tissue and are associated with immunity to the body against antigen invasion [65].

It has been reported mushroom belongs to *Plurotus* spp. possess immunomodulatory properties [14]. Previously it has been studied and reported feeding of *P. ostreatus* extracts significantly increase in the number of total white blood cells of Rainbow trout [69]. Similar study has been done to investigate immunomodulatory activity of oyster mushroom *Pleurotus sajor-caju* on mice. The result of this study reveals mice treated with various concentrations of *P. sajor-caju* significantly increased populations of cluster of differentiation molecule for immunophenotyping of cells (CD) molecules specially $CD3^+/CD4^+$, CD8 and WBC cells. It has also been reported *Pleurotus* mushroom contains non cellulosic fibers associated with effectively growth of probiotic and that encourage the growth of beneficial microorganisms in the gastro intestinal tract [68]. It has also been reported that intestinal microbe flora is the endogenous source of vitamin b in human [69, 70]. In the present study *P. tuber-regium* extract significantly ($p < 0.05$) elevated the total WBC count (Figure 16) as well as vitB12 concentration (Figure 12) and provides the linked confirmation about the beneficial immunomodulatory response of extract on the rat.

Conclusion

Mushroom *P. tuber-regium* traditionally has been used as a nutraceutical and medicinal food supplement. The fruiting body of *P. tuber-regium* contains different mycochehemicals, protein, carbohydrate, fibers and low-fat content and high calorific value. Along with-it calorific value *P. tuber-regium* extract shows good antioxidant capacity, decreases the average blood sugar and glycosylated hemoglobin, elevates the vitamin D and B12 level, increases hemoglobin, RBC and WBC count of the body without producing toxicity. Thus *P. tuber-regium* can be consumed as a hypoglycaemic, haematinic agent which also boost the immune system of body and maintain the good health of the diabetic patient.

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