

Full Length Research Paper

Phytochemical composition of *Pleurotus tuber regium* and effect of its dietary incorporation on body /organ weights and serum triacylglycerols in albino mice

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The phytochemical, mineral and nutrient compositions of *Pleurotus tuber regium* as well as the effect of its dietary incorporation on body organ weight and total triacylglycerols in albino mice (*Mus musculus*) was studied. Phytochemical composition analysis yielded (g/100g) saponins (2.19 ± 0.19), alkaloid (0.32 ± 0.07), flavonoids (2.84 ± 0.24) and oligosaccharides (14.14 ± 0.53). Proximate analysis gave moisture (12.87 ± 0.51), dry matter (87.13 ± 0.51), ash (2.97 ± 0.09), ether extract (1.06 ± 0.21), crude protein (222.10 ± 0.66), crude fibre (10.86 ± 0.58), carbohydrates (63.03 ± 1.12) and a calorific value of 349.98 ± 3.98 . Mineral composition analysis yielded g/100g calcium (0.80 ± 0.00), magnesium (0.36 ± 0.06), potassium (0.40 ± 0.03), sodium (0.20 ± 0.05) and mg/100g copper (0.83 ± 0.00), zinc (1.35 ± 0.00), iron (5.02 ± 0.00), manganese (0.06 ± 0.00) and selenium ($\leq 0.0025 \pm 0.00$). Dietary incorporation of *P. tuber regium* at 5 and 10% levels resulted in a non-significant dose dependent reduction in total triacyl glycerol concentration. There was also no significant change in percentage relative weight of vital organs such as liver, Intestines, kidney and pancreas.

Key words: *Pleurotus tuber regium*, phytochemicals, minerals, nutrients, lipids.

INTRODUCTION

Mushrooms grow naturally in Nigeria during the early and late rainy seasons (Gbolagade *et al.*, 2006). They are usually found in forests, grasslands, damp rotten logs etc. *Pleurotus tuber regium* (tiger milk mushrooms or sclerotia producing mushroom) is a tropical edible mushroom that produces the edible sclerotium or underground tuber as well as mushroom. *Pleurotus* are cultured on a wide variety of agroforestry products for the production of feed, enzymes, and medicinal products (Andrew *et al.*, 2007). Several medicinal properties have been reported in extracts of *Pleurotus* species. They include antitumour

properties attributable to their polysaccharides (Zhang *et al.*, 2004), antigenotoxic, bioantimutagenic activities (Fillipie and Umek, 2002), antiinflammatory activity, antilipidaemic, antihypertensive, and antihyperglycaemic activities (Hu *et al.*, 2006) antibacterial and antifungal activities (Ngai *et al.*, 2006). There is also research evidence that extracts from medicinal mushrooms can function as immunomodulators (Jong *et al.*, 1991).

The present study was designed to investigate the nutrient, phytochemical and mineral composition of *Pleurotus tuber regium* cultured from sclerotia obtained from a local market in the suburbs of Umudike, Abia State Nigeria and the effect of its dietary incorporation on blood lipid profile and percentage relative weights of vital organs in albino mice.

This is a preliminary study aimed at investigating the

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level of dietary incorporation of *P. tuber regium* in diet that will reproduce the nutraceutical benefits that have been reported in studies using extracts from *P. tuber regium*.

MATERIALS AND METHODS

Cultivation of *P. tuber regium*

The sclerotia of *Pleurotus tuber regium* was purchased from Ndoro village market in Abia State, Nigeria. They were taxonomically identified by Dr. I. A. Okwujiako of the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The sclerotia were washed several times with distilled water and then placed in a perforated transparent bucket padded with damp cotton wool to maintain the needed humidity for carpophore formation. It was then kept in a humid chamber in the mushroom culture laboratory of the College of Natural and Applied Sciences, Michael Okpara University of Agriculture, Umudike, with periodic introduction of moisture into the cotton wool to maintain the required humidity. After 21 days, mature fruit bodies were harvested and dried under a blowing fan in the laboratory. They were then chopped into small pieces and milled using a Binatone dry blender. A portion was removed for chemical analyses while the rest was used for feeding studies.

Feed preparation

The milled *P. tuber regium* was mixed with standard Guinea growers mash to obtain a 5 and 10% dietary incorporation of the mushroom and extruded through a 5 ml syringe whose end had been cut to obtain the mixture in pelletized form.

Experimental animals

Weanling albino rats, aged 5 to 6 weeks were purchased from the animal breeding unit of the College of Veterinary Medicine of the University of Nigeria, Nsukka, Enugu State, Nigeria. The mice were housed in well ventilated stainless steel mice cages under humid tropical conditions and exposed to 12 h light/dark cycles. After three days of equilibration on the standard growers feed, the animals were separated into 3 groups. Group 1 which served as control received the control diet while groups 2 and 3 received the 5 and 10% *P. tuber regium* incorporated diet. Feed and water were supplied *ad libitum*.

At the end of the one week feeding studies, the animals were sacrificed by dazing them with a cervical blow. Blood was drawn by cardiac puncture into labelled fluoride bottles. Vital organs were excised, promptly dabbed on a filter paper to remove blood stains and weighed.

Proximate composition analyses

Crude protein contents were determined using the microKjedahl method as described by AOAC (1990). Determination of total ash, moisture and total lipid contents were carried out as described by AOAC (1990). Crude fibre analysis was done as described by Yeshajagu *et al.* (1996). Carbohydrates were estimated by difference (AOAC, 1990).

Phytochemical analyses

Total alkaloids, saponins and flavonoids were extracted and yield

determined by gravimetric method (AOAC, 1990). Oligosaccharide contents were determined by anthrone spectrophotometric method as described by Ojiako and Akubugwo (1997).

Mineral composition analyses

Total calcium (Ca^{2+}) and magnesium (Mg^{2+}) contents were determined by EDTA versanate complexometric titration method as described by Harbourne (1973). Sodium (Na^+) and potassium (K^+) ion contents were determined by flame photometry as described by Onwuka (2005). Other metal contents (Zn, Fe, Cu, Mn and Se) were determined by the atomic absorption spectrophotometric technique as described by Onwuka (2005).

Biochemical analyses

Determination of plasma lipid concentration: The blood collected from sacrificed animals was centrifuged at 4,000 rpm for 5 min to allow for separation of the plasma. Plasma triacylglycerols concentrations were determined using commercial kits purchased from Biosystems Reagents and Instruments Ltd. Total plasma triacylglycerols were measured using the method of Bucolo *et al.* (1973) as modified by Fossati *et al.* (1982).

Determination of percentage relative organ weights: This was calculated by multiplying the ratio of each organ weight by the body weight on the day of sacrifice and multiplying by a factor of 100.

RESULTS AND DISCUSSION

P. tuber regium fruit body has been shown to be highly nutritive (Adejumo and Awosanya, 2004; Akindahunsi and Oyetayo, 2006). The present study (Table 1) indicates a much higher protein yield (22.10 ± 0.66 dry wt) in the fruit body than that reported by Akindahunsi and Oyetayo, 2006. This protein content compares well with that of several leguminous seeds and implies that fruit bodies of *P. tuber regium* could serve as a suitable meat substitute. Protein evaluation has shown that mushroom proteins have a higher quality than green leafy vegetables (Chan, 1981). Our carbohydrate yield of 63.03 ± 1.12 g% is also higher than 56.2 g/100g reported by Akindahunsi and Oyetayo (2006). A considerable fraction of this carbohydrate is contributed by oligosaccharides as shown in Table 3. Several health benefits have been attributed to mushroom oligosaccharides including immunomodulatory effects which have been shown to be beneficial to individuals living with HIV (Jong *et al.*, 1991).

In the present study, ether extract (1.06 ± 0.21 g%) and ash (2.96 ± 0.09 g%) contents agree with findings of other workers (Akindahunsi and Oyetayo, 2006) that *P. tuber regium* has a low fat and ash content. The present finding of a high crude fibre content (10.86 ± 0.58 g%) indicates that the incorporation of *P. tuber regium* in diet could aid bowel movement as well as reduce the incidence of colon cancers in its users. Epidemiological studies have found an association between high fibre diets and a lower incidence of cardiovascular diseases and large bowel cancers (Honda *et al.*, 1999).

Table 1. Proximate composition of *P. tuber regium* fruit body.

Nutrient	Dry matter	Moisture	Ash	Ether extract	Crude protein	Crude fibre	CHO	Calorific value
Content g/100g	12.87 ± 0.51	87.13 ± 0.51	2.97 ± 0.09	1.06 ± 0.21	22.10 ± 0.66	10.86 ± 0.58	63.03 ± 1.12	349.98 ± 3.69

*Results are means of duplicate analysis and deviations from the mean.

Table 2. Mineral composition of *P. tuber regium* fruit body.

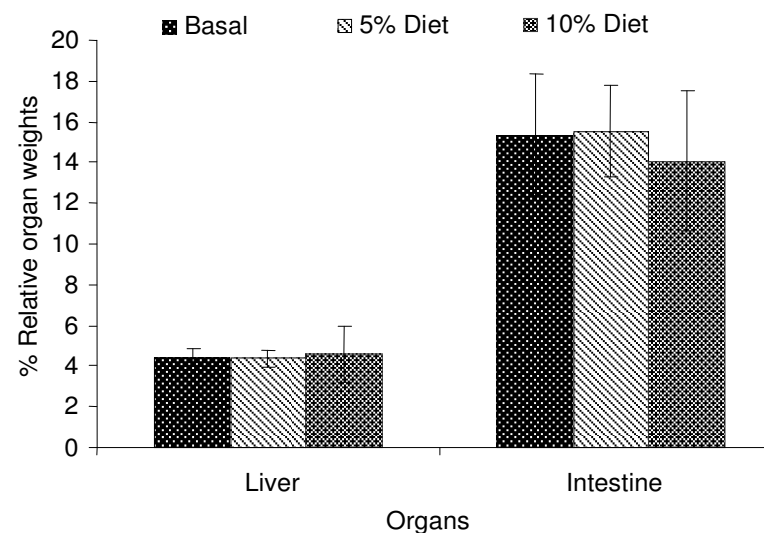
Mineral	Ca (g/100 g)	Mg (g/100g)	K (g/100 g)	Na (g/100 g)	Cu (mg/100 g)	Zn (mg/100g)	Fe (mg/100 g)	Mn (mg/100 g)	Se (mg/100 g)
content	0.80 ± 0.00	0.36 ± 0.06	0.20 ± 0.03	0.83 ± 0.00	0.83 ± 0.00	1.35 ± 0.00	5.02 ± 0.00	0.06 ± 0.00	≤ 0.0025 ± 0.00

*Results are means of duplicate analysis and deviations from the mean.

Table 3. Phytochemical analyses of *P. tuber regium* fruit body.

Phytochemical	Saponins	Alkaloids	Flavonoids	Oligosaccharides
Content (g%)	2.19 ± 0.19	0.32 ± 0.07	2.84 ± 0.24	14.46 ± 0.53

*Results are means of duplicate analysis and deviations from the mean

**Figure 1.** Percentage mean relative liver and intestinal weights of mice fed on *P. tuber regium* incorporated diets.

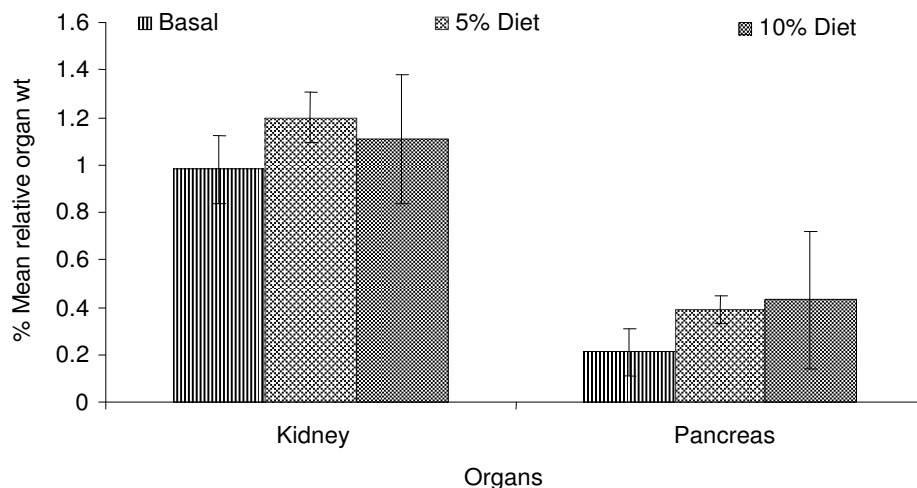


Figure 2. Percentage mean relative kidney and pancreatic weights of mice fed on *Pleurotus tuber regium* incorporated diets.

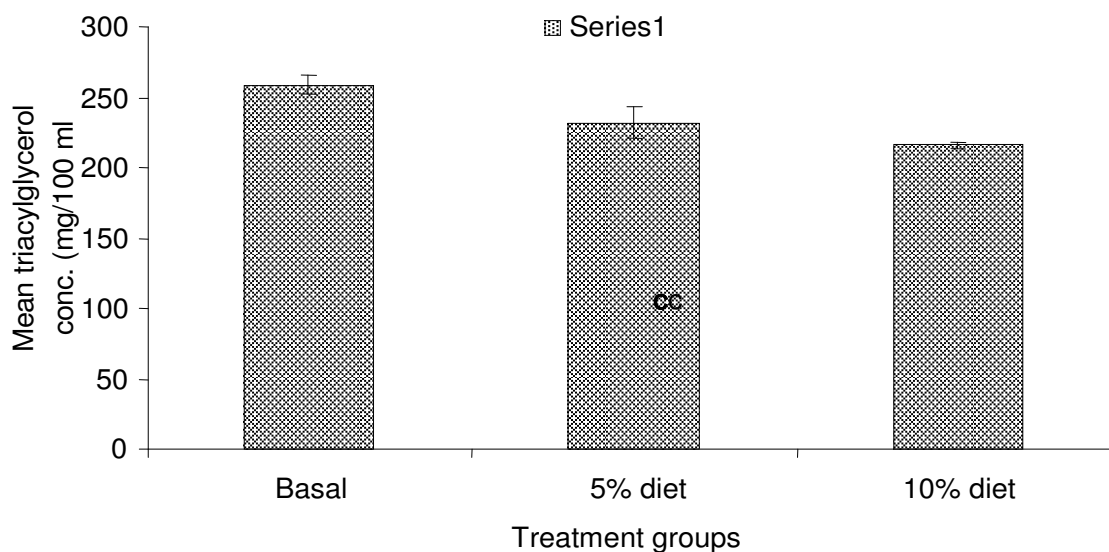


Figure 3. Plasma triacylglycerol concentrations in mice fed incorporated diets.

Mineral composition analyses (Table 2) indicate that the fruit body is rich in iron and zinc. This finding agrees with the findings of Adejumo and Awosanya (2004). The iron content (5.02 mg/100 g) obtained in this study implies that consumption of 200 g of *P. tuber regium* will provide sufficient iron to meet the recommended daily allowance (RDA) for school age children. High zinc content also suggests that increased consumption of edible mushrooms could help reduce the growing incidence of micronutrient deficiency.

Results of phytochemical analyses (Table 3) indicates that the mushrooms are rich in alkaloids, saponins, flavonoids and oligosaccharides. These alkaloids and saponins

which could be toxic at high doses were found to occur in non-toxic doses.

Dietary incorporation of *P. tuber regium* at 5 and 10% levels resulted in a dose dependent increase in pancreatic weight and decrease in intestinal weight but had no significant effect on liver and kidney weights (Figures 1, 2 and 3). The effect on pancreatic weight could be attributable to increased synthesis of pancreatic proteins/enzymes. These findings are indicative that the consumption of *P. tuber regium* at the 5 and 10% level of dietary consumption may have no adverse effects on vital organs. This implies that a higher percentage dietary incorporation may be required to achieve significant hy-

polipidaemic effects.

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