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Effects of graded doses of ethanol extract of *Pleurotus ostreatus* (Oyster mushroom) on the sperm quality and haemo-biochemical parameters of the male Wistar rats

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ABSTRACT: The effects of graded doses of ethanol extract of *Pleurotus ostreatus* (EEPO) on the sperm quality and haemo-biochemical parameters of the male Wistar rats were studied. Twenty-five adult male rats were randomly assigned into 5 groups A-E, (n=5). Groups A, B, C, and D were treated with 200, 400, 600 and 800 mg/kg of EEPO respectively while group E received 0.2 ml Propylene glycol serving as the control. Animals were treated for 14 days after which samples were collected. Sperm motility, liveability and sperm count of EEPO-treated rats in groups A, B, C and D showed a dose-dependent increase compared to the control. The treatment significantly ($p<0.05$) increased the PCV, Hb, RBC and platelets counts of rats across the treated groups A-D. There were no significant changes ($p>0.05$) in other haematological parameters except for a marked reduction in the WBC count of group B. The mean ALB and TP increased across the treated groups when compared to the control). It was concluded that ethanol extract of *P. ostreatus* produced a dose-dependent increase in sperm motility, liveability, sperm count, PCV, Hb, RBC, TP and ALB, thereby enhancing the fertility of the rats, especially at higher doses. Hence, the plant should be considered as feed supplements for the male breeding stock.

Keywords: Fertility, *Pleurotus ostreatus,* rats, semen.

INTRODUCTION

Pleurotus ostreatus is a tropical sclerotial mushroom that produces a sclerotium or an underground tuber as well as a fruiting body (sporocarp). Both the sclerotium and the sporocarp are edible (Jin *et al*., 2003). They are documented as being rich in proteins, minerals, vitamins while they are low in lipids (Kalac, 2009). Mushrooms contain a huge diversity of biomolecules with nutritional (Lindequist et al., 2005) and/or medicinal properties (Borchers *et al*., 2004; Lindequist *et al*., 2005; Kalac, 2009).

Studies have shown that mushrooms contain phytochemicals and other compounds which are strong antioxidants (Fang *et al.,* 2002; Liu, 2004). Phenolic compounds, alkaloids, saponins, flavonoids, tannins, steroids, triterpenes, coumarins and cyanogenic glycosides have been detected in mushrooms analyzed in Sudan and in Nigeria (Egwim *et al.,* 2011, Adebayo *et al.,* 2012; Ehssan and Saadabi, 2012). The compounds seem to mop the free radicals generated in the normal natural metabolism of aerobic cells, mostly in the form of Reactive Oxygen Species (ROS). These include superoxide (O2-) and hydroxyl (OH-) radicals among several others.

Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides (Barja, 2004). However, non-enzymatic molecules like ascorbic acid and carotenoids are reported to be present in Mushrooms which act as antioxidants (Fang *et al*., 2002; Isabel *et al.,* 2004).

Several medicinal properties have been reported in extracts of *Pleurotus* species. They include antioxidant, anti-tumour, anti-genotoxic, anti-mutagenic, anti-inflamatory, anti-lipidaemic, anti-hypertensive, anti-hyperglycaemic, anti-bacterial and anti-fungal activities (Hu e*t al.,* 2006; Ngai and Ng, 2006). However, the

potential effects of *Pleurotus ostreatus* on fertility of male animals are not well elucidated. Therefore, the current study investigated the effects of graded doses of the ethanol extract of *Pleurotus ostreatus* on the fertility potentials of the male Wistar strain albino rats.

MATERIALS AND METHODS

Plant collection and phytochemical screening

Cultivated macrofungi (*Pleurotus oestratus*) were collected fresh from Forest Research Institute of Nigeria, Ibadan, Oyo State and were identified at the herbarium of the Department of Botany, University of Ibadan.

The extracts of *Pleurotus ostreatus.* were chemically tested, using standard methods (Harbone, 1984; Evans, 2002) for the presence of carbohydrates, proteins and amino acids, alkaloids, anthraquinones, cardiac glycosides, tannins, flavonoids, saponins, phenols and steroids (Sofowora, 1993).

Experimental animals

Twenty-five adult male albino rats (Wistar strain) kept in a standard cage in the animal house of the Faculty of Veterinary Medicine, University of Ibadan were utilized. All the rats were kept under controlled conditions of temperature (25 ± 2.0°C), relative humidity (50 ± 15%) and normal photoperiod (12 hour light and 12 hour dark). The animals were stabilised for two weeks before being fed standard commercial pellet diet and water was provided *ad libitum*.

Research approval

This study was approved by the Animal Care and Use Research Ethics Committee of the University of Ibadan (UI-CUREC/App/2015/068).

Solvent extraction

The macro fungi materials were air dried at 45°*C* in a hot air drier and ground to a powder using a grinder. The extract were prepared by maceration by shaking for 2 hours in 70% ethanol solvent to dry weight ratio according to the method described by Eloff (1998).

Ten grams (10 g) of grounded mushroom were weighed in the beaker (155.18 g), 100 mls of solvents (ethanol, were added to achieve a 10:1 solvent to dry weight of sample ratio. There was constant shaking of the mixture every 15 minutes for 2 hours before filtering. The process was repeated twice to make a total of 6 hours. The filtrate was then kept in a hot air drier (45°*C*) until the extract yield was produced.

Experimental design

The twenty-five experimental rats were assigned into five groups (A-E), each group having 5 rats (n=5). They were administered with oral dose of ethanol extract of *Pleurotus ostreatus* constituted with propylene glycol at dosage rate of 200, 400, 600, 800 mg/kg bodyweight respectively for 2 weeks. Animals in groups A, B, C and D received 200, 400, 600 and 800 mg/kg of ethanol extract of *Pleurotus ostreatus* respectively while group E received 0.2 ml propylene glycol serving as the control. Samples were collected from all the animals at 2 weeks (14 days) post-treatment.

Sample collection

Blood collection and biochemical assay

The rats were anaesthetized using diethylether in a desiccator and blood samples were collected via venous puncture (infraorbital vein) into sterile heparinized sample tubes for haemogram and also into plain sample tubes without anticoagulant for serum chemistry. The samples were analysed as described by Reece (1997).

Semen collection and analysis

After blood collection, mid caudoventral abdominal incision was made on the rats with sterilized scissors, permitting instant access to the testes once pushed upward from the scrotum. The right and left epididymides were trimmed off the body of the testes and semen sample was collected from the tail of the epididymis through an incision made with a scalpel blade (Oyeyemi and Fayomi, 2011). The semen samples were evaluated for sperm motility, liveability, count and morphology as described by Zemjanis (1977).

Data analysis

The data generated were expressed in mean ± SEM (standard error of mean) and analyzed using One-Way Analysis of Variance (ANOVA). SPSS Version 20 for Windows (SPSS Inc, 2006) and Microsoft Excel Professional Plus (Microsoft Corporation, 2010) were used to carry out all procedures. The values for $p<0.05$ were considered to be statistically significant.

RESULTS

Phytochemical screening of ethanol leaf extract of *Pleurotus ostreatus* revealed the presence of compounds such as saponins, anthraquinones, alkaloids and terpenoids (Table 1).

Table 1. Qualitative phytochemical screening of ethanol extracts of *Pleurotus ostreatus* (Oyster Mushroom).

No.	Constituents	Qualitative tests	Inference
1.	Tannins	Ferric chloride	+ve
2	Saponins	Frothing	+ve
3	Cardiac glycosides	Keller-killanins	-ve
		Ammonia/H_2SO_4	-ve
4	Flavonoids	Aluminium solution	-ve
		Ethyl acetate/Ammonia	-ve
5	Anthraquinones	Chloroform/Ammonia	-+ve
		Dragendoff's	+ve
6	Alkaloids	Mayer's	+ve
		Wagner	+ve
7	Terpenoids	Chloroform/H_2O_2	+ve

+ve = present, -ve = absent.

Table 2. Semen characteristics of male albino rats in different groups at 2 weeks post-treatment.

Parameters	A (200 mg/kg)	B (400 mg/kg)	C (600mg/kg)	D (800 mg/kg)	E (Control)
Sperm motility (%)	95.50±6.29^b	96.00±5.48^b	98.00±2.45^b	99.00±4.00^b	90.33±1.67^a
Sperm liveability (%)	95.00±3.08^a	95.20±2.40^a	95.20±2.40^a	96.20±0.73^a	95.00±1.00^a
Sperm count (x10^6 spermatozoa/ml)	142.75±6.33^b	145.20±5.15^b	155.80±3.06^b	160±6.45^b	137.33±5.61^a

^{a,b,c}Mean(±SEM) with same superscript are not significantly different at 0.05 level along the rows.

The mean percentage sperm motility, liveability and sperm count of rats in groups A, B, C and D increased significantly ($p<0.05$) with increasing doses of ethanol extract of *Pleurotus ostreats* across the groups compared to the control (Table 2).

The treatment significantly increased ($p<0.05$) the PCV, Hb, RBC and platelets counts compared to the control. Other blood parameters were not significantly affected by the treatment. The PCV increased in groups A to D compared to the control, and the group B and C values were significantly ($p<0.05$) higher than the control. The mean Hb concentration increased ($p>0.05$) across the group A to D compared to the control. This changes was significant ($p<0.05$) for group B rats. Changes observed in the mean RBC followed a similar pattern. WBC decreased across groups A to D compared to control. The mean WBC decrease in group B was significant ($p<0.05$) compared to the control (Table 3).

There was significant increase ($p<0.05$) in the Albumin (ALB) and Total Protein (TP) of groups A to D treated with ethanol extract of *P. ostreatus (EEPO)* compared to the control (group E). The mean TP for group B (treated 400 mg/kg EEPO) and group D (treated 800 mg/kg EEPO) were significantly ($p<0.05$) higher than the control (Table 4).

DISCUSSION

In the present study, the phytochemical screening revealed the presence of compounds such as saponins, anthraquinones, alkaloids and terpenoids. Hiremath and Rao (1990) observed that these compounds exhibited pro-fertility activity of ethanol extract *of Pleurotus ostreatus* (EEPO). The presence of one or more of these compounds may also be the cause of the dose-dependent increase in the sperm motility, liveability and count observed in the current study. This implies that the EEPO possesses a spermatogenic property and thus would enhance the fertility of the male rats. The finding is similar to Ghaly *et al*. (2011) in which dried sporocarp of *Pleurotus ostreatus* enhanced the fertility of diabetic rats.

The significant increase in PCV, Hb, and RBC observed in the rats across the groups following the administration of EEPO implies that the EEPO has a potent haematinic property and could be useful in the management of anaemic animals.

The increase in the haematological indices observed in this study is consistent with the observations made when rats were fed with the diet preparation of the sporocarp of *P. ostratus* for 28 days (Majesty *et al*., 2019). The current study has also shown that new blood cells would have started appearing in the circulation after 14 days of treatment of the male rats with EEPO and the increase would become significant at a dose of 400 mg/kg

The observed significant increase in the mean ALB and TP across the experimental groups especially group D treated with 800 mg/kg EEPO implies that that the extract is protein-rich and this may suggest the extract possesses an immune-modulatory property.

Table 3. Haematological profile of male albino rats in different groups at 2 weeks post-treatment.

Parameters	Group A	Group B	Group C	Group D	Group E
PCV (%)	39.80±0.49[b]	47.4±0.81[a]	41.00±1.7[a]	38.60±2.42[b]	37.67±1.45[b]
Haemoglobin (g/dl)	13.20±0.19[b]	16.62±0.74[a]	13.26±0.64[b]	13.28±0.68[b]	12.50±0.4[b]
RBC (mm^3)	6.55±0.10[b]	7.86±0.21[a]	6.93±0.28[a]	6.63±0.41[b]	6.09±0.26[b]
Lymphocytes (mm^3)	65.40±1.91[a]	66.4±3.08[a]	64.60±3.09[a]	65.80±2.33[a]	65.00±1.53[a]
Monocytes (mm^3)	1.20±0.2[a]	1.8±0.37[a]	1.80±0.20[a]	1.6.00±0.24[a]	2.00±0.00[a]
Neutrophils (mm^3)	32.20±2.01[a]	30.2±2.96[a]	32.40±2.93[a]	32.20±2.85[a]	32.67±1.33[a]
Platelets (ml)	190000±23381.62[c]	147600±17500.29[ab]	172000±34033.81[c]	150400.00±22303.81[ab]	105666.67±3480.1[a]
WBC (mm^3)	6240±650.08[ab]	5050±362.63[b]	5130±620[b]	6990±881.39[ab]	8333.33±1244.43[a]
Eosinophils (mm^3)	1.60±0.51[a]	1.60±0.60[a]	1.20±0.58[a]	2.40±0.40[a]	1.00±0.58[a]

[a,b,c]Mean(±SEM) with same superscript are not significantly different at 0.05 level along the rows.

Table 4. Serum chemistry of male albino rats in different groups at 2 weeks post-treatment.

Parameter	A	B	C	D	E (Control)
A.G Ratio	0.64±0.09	0.7±0.03	0.56±0.02	0.68±0.04	0.5±0
ALB(g/dl)	3±0.33b[c]	3.56±0.12[a]	3.04±0.16[ab]	3.4±0.07[ab]	2.37±0.15[c]
ALP (mmol/l)	119±2.59	113.2±1.39	116.4±3.01	114.8±3.43	111.33±4.18
ALT (mmol/l)	31±1.14	31.4±1.21	31.6±60.37	33.8±0.73	30.67±0.88
AST (mmol/l)	42.4±1.25	43.4±2.01	43.8±1.74	45.4±1.4	41.33±1.33
BUN(mmol/l)	16.84±0.27	16.7±0.35	17.12±0.12	17.04±0.23	16.33±0.64
CHOL(mg/dl)	54.4±5.64	65.2±6.55	58.6±3.85	56.6±3.93	53.67±1.76
CREA(mg/dl)	0.92±0.07	1.14±0.09	1.06±0.09	1.0±0.09	0.77±0.03
GLOB(mg/dl)	4.6±0.19	5.04±0.18	5.06±0.08	4.92±0.07	4.83±0.2
GLU(mg/dl)	143±0.95	142.4±2.04	141.8±2.78	142.6±1.12	144.67±2.85
TP(g/dl)	7.6±0.36bc	8.5±0.07a	8.08±0.2ab	8.32±0.09a	7.2±0.35c

[a,b,c]Mean(±SEM) with same superscript are not significantly different at 0.05 level along the rows.
Keys: ALB = Albumin; ALP = Alkaline phosphatase; ALT = Alanine Amino transferase; AST = Aspartate Amino transferase; BUN = Blood Urea Nitrogen; CHOL = Cholesterol; CREA = Creatinine; GLOB = Globulin; GLU = Glucose; TP = Total Protein.

Conclusion

In conclusion, ethanol extract of *P. ostreatus* produced a dose-dependent increase in sperm motility, liveability, sperm count, PCV, Hb and RBC, TP and ALB and can be said to possess a pro-fertility and haematinic property at higher doses (800 mg/kg). It can therefore be recommended for consumption in the male animals used for breeding purpose.

CONFLICT OF INTEREST

The author declares no conflict of interest in regards to the publication of this research work.

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