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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of *Pleurotus eous* Supplemented Diets on the Albumin, Globulin and Total Proteins in experimental Animal (Male albino Wistar rats)

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ABSTRACT

India blessed with various climatical condition which seems to be quite suitable for large-scale production of Oyster mushroom P.eous. Hence studies were taken up with an objective to find new technologies to test the Albumin, Globulin and Total Proteins of P.eous .With regard to the studies on medicinal properties of P.eous, The level of albumin, globulin and total protein showed steady increase in the rats co-administrated with various levels of P.eous the level of RBC, WBC, hemoglobin and haematocrit showed a significant increase in rats co-administered with different concentration and durations of observation. Among the three levels of Pleurotus eous diet tested, the 10 per cent level recorded the maximum increase level when compared to 2.5 and 5 per cent level in the both serum and liver. The level of albumin, globulin and total protein are analysed by feeding rats with 10 per cent of Pleurotus eous diet (Group D) over a period of 90th day observed a gradual increase when compared with control (Group A) and therefore the study revealed the beneficial effects of P.eous on the increased level of albumin, globulin and total protein content.

Key words : *Pleurotus eous, Hemoglobin, Haematocrit, Albumin, Globulin, Wistar rats .*

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INTRODUCTION

Mushrooms having magnificent medicinal, delicacy as well as nutritive values are interestingly used as human food from the time everlasting. Moreover, they contain proteins and nutritive fibers, as well as other vitamins necessary for the normal functioning of the human body. Mushrooms are widely consumed as an edible and medicinal resource. Mushrooms are exquisite food with an extraordinary nutritional profile and many scientifically proven medicinal properties. Medicinal value and very high productivity level per unit area rightly have been identified as an excellent food source to fight against malnutrition in developing countries. Many mushrooms have been used in the human diet because of their desirable taste and aroma. Mushrooms provide a rich addition to the diet in the form of proteins, carbohydrates, valuable salts, vitamins and minerals besides being low in fat and calories. 100 to 200 g (per day) of mushrooms are required to maintain nutritional balance in a normal human being [8]. Cultivation of the oyster mushroom, Pleurotus spp., has increased greatly throughout the world during the last few decades. In Tamil Nadu the farmers have developed preference to Pleurotus spp. because it can be cultivated in plains not only during the cooler months of the year but also during summer. There are about 38 species described under the genus Pleurotus from different parts of the world. The species like *P. ostreatus, P. flabellatus, P. citrinopileatus, P. eous, P. sapidus, P. sajor-caju, P. platypus and P.eous* are widely cultivated in India.. *Pleurotus* is an efficient lignin- degrading mushroom and can grow well on different types of lignocellulolosic materials. New technologies and production techniques are being constantly developed as the number of required controllable environment parameters increases. Oyster mushrooms are food-stuff with a high therapeutically and nutritive value [4].

Mushrooms provide a wide variety of physiologically active components: *Pleurotus sajor-caju* inhibits hypertensive effects through its active ingredients, which affect the renin-angiotensin system, *Tricholoma*

magnivel are produces asorelaxation because of its lectin content, *P. ostreatus* possesses antitumor or activity and hypoglycemic effects on experimentally induced diabetic rat, *Lentinus edodes* and *Grifola frondosa* have antihypertensive effects in spontaneously hypertensive rats and *Agaricus bisporus* decreases low -density lipoprotein-cholesterol (LDL-C) in serum by increasing the expression of LDL receptor at mRNA level and LDL receptor activity. Hence, the objective of the present study was to generate awareness of the beneficial effects of edible mushrooms, particularly oyster mushrooms, on increasing the level of albumin, globulin and total protein content.

MATERIAL AND METHODS

Source and maintenance of culture

The culture of *Pleurotus eous* was obtained from Tamil Nadu Agricultural University, Madurai. The fungus was also cultured from fresh sporophore by tissue culture method. The mushroom cultures were maintained in PDA slants and used for the present investigation.

Isolation and Maintenance of Pure Cultures

A well-developed sporophore was selected and cut into two equal halves. A small piece of mushroom tissue was removed with a sterile forceps exactly form the center of junction of pileus and stipe from the half cut mushroom. The tissue bit was then sterilized with 70 per cent ethanol for 30 sec, washed thrice serially in sterile distilled water, air dried near a flame to remove excess moisture and placed aseptically into Petri dishes containing PDA or MA medium. The dishes were incubated at room temperature (25 ±2º C) for seven d. Pure cultures were transferred to PDA or MA slants and maintained at 25 ±2º C for further studies.

Spawn Production

Pure cultures maintained in PDA medium were used for the preparation of sorghum grain spawn. Cleaned grains were thoroughly washed and soaked in water for 30 min and half cooked in an open vessel for 20 min. After draining the excess water, the grains were mixed with calcium carbonate at the rate of 20 g per kg of grains (dry weight), filled in autoclavable polypropylene bags (25 x 10 cm size) and sterilized at 1.42 kg cm^{-2} pressure in an autoclave for 1.5 h. After cooling, the bags were aseptically inoculated with the pure cultures of the respective mushroom fungus, incubated at room temperature (25 ±2^{0}C) for 15 d and used for spawning the substrate.

Bed preparation

Polypropylene bags of 60 × 30 cm size (100 G thickness) were used as containers for the preparation of cylindrical mushroom beds with layer spawning or thorough spawning. For each bag 500 g of substrate (dry weight basis), respectively were used. Fifteen d old sorghum grain spawn was used to seed the substrate at the rate of 2 per cent (dry weight basis). The beds after spawning were provided with 4 - 8 holes of one cm dia. depending upon the size of the bed for air circulation.

Evaluation of medicinal values of *P.eous*

Experimental Animal : **Male albino Wistar rats**
Age : **5 week old**
Duration of Experiment : **Three months**

The rats were individually housed in wire mesh cages and kept in an isolated room at a controlled temperature (22-25^{o}C) and ambient relative humidity of 50-60% on a 12 hour light: dark cycle and air changes of 10-12 times per hour. Necessary ethical clearance was obtained from Institutional Animal Ethical Committee of Rajah Muthiah Medical College, Annamalai University to perform experimental studies on male wistar rats.

Preparation of rat feed

Normal feed : lab stock feed in pelleted form.

Normal plus mushroom feed: 100 g of lab stock feed in pelleted form was powdered. Then 2.5, 5, and 10 g of *P. eous* was powdered and mixed thoroughly with the lab stock diet with the help of a little amount of hot water, the moisture was made into a pelleted form and then air dried. Then it was stored in an air tight container at room temperature.

Cholesterol feed: feed rich in cholesterol, *viz*, groundnut, coconut scrapping, egg yolk, etc., were fed.

Cholesterol plus mushroom feed : 100 g of cholesterol feed was powdered, then 2.5, 5, and 10 g of *P. eous* was powdered and mixed thoroughly with the help of little amount of hot water, and made in to a pelleted form and then air dried. Then it was stored in an air tight container at room temperature.

Treatment details:

Experimental Design:

The experimental rats were grouped as

Group A Rats fed with Normal Feed

Group B Rats fed with Normal Feed + 2.5% *P.eous*
Group C Rats fed with Normal Feed + 5% *P.eous*
Group D Rats fed with Normal Feed + 10 % *P.eous*
Group E Rats fed with Cholesterol feed
Group F Rats fed with Cholesterol feed + 2.5% *P.eous*
Group G Rats fed with Cholesterol feed + 5% *P.eous*
Group H Rats fed with Cholesterol feed + 10 % *P.eous*

Clinical Symptoms and Body Weight:

Both the controls as well as the experimental groups of rats were weighed at weekly intervals. The animals were observed daily for clinical symptoms if any and recorded.

Serum Chemistry

Serum enzyme assay was done by using ERBA CHEM semi auto analyzer. The values were taken on 30,60 and 90th day of experiment. The serum concentrations of total cholesterol, HDL cholesterol, free cholesterol, triacylglycerols and phospholipids were measured enzymatically with kits (Cholesterol C-Test, HDL Cholesterol-Test, Free Cholesterol C-Test, Triglyceride G-Test and Phospholipid B-Test, respectively, Wako). The difference between total cholesterol and HDL cholesterol or free cholesterol was assumed to be VLDL + LDL cholesterol or esterified cholesterol, respectively.

Estimation of serum total protein and albumin

Serum total protein and albumin were estimated by Reinhold [13] method. Protein form a purple coloured complex with cupric ions in alkaline solution. The reaction takes its name from the simple compound biuret which reacts in the same way. The intensity of the purple colour is proportional to the amount of protein present in the sample. Values are expressed as g/dl.

Estimation of globulin

Serum globulin conc. was calculated using the following formula after the estimation of total protein and albumin.

Globulin = Total protein – Albumin

Estimation of Tissue Protein

Tissue protein was determined by the method of Lowry *et al.* [14]. Protein react with the folin –ciocalteau reagent to give a colour complex. The colour so formed is due to the reaction of the alkaline copper with the protein as in the biuret test and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour formed was proportional to the conc. of protein in the sample. The protein levels were expressed as mg/g tissue.

RESULTS

Determination of Albumin (g/l)

Level of albumin showed a steady increase in rats fed with normal feed or cholesterol feed along with various levels of mushroom. Also, the level of albumin showed a positive correlation with level of mushroom diet and duration of supplementation. The maximum albumin level of 4.06 g/l was recorded in Group D (normal feed + 10 per cent *P.eous)* on the 90th day of observation. The albumin level steadily increase in the duration of administering cholesterol feed plus mushroom diet in Group F (2.34, 2.89 and 2.99 g/l on 30th, 60th and 90th days of observation, respectively), Group G (2.43, 3.09 and 3.18 g/l on 30th, 60th and 90th days of observation, respectively) and Group H (2.52, 3.29 and 3.38 on 30th, 60th and 90th days of observation, respectively) when compared with Group E (2.45, 2.49 and 2.50 on the 30th, 60th and 90th days) of treatment.

Table.1.Effect of *P.eous* on the albumin (g/l) levels of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	1.97	2.10	2.49
Group B (2.5% P.eous)	2.36	2.59	2.88
Group C (5% P.eous)	2.56	2.79	3.47
Group D (10% P.eous)	2.76	2.99	4.06
Group E (Cholesterol feed)	2.45	2.49	2. 50
Group F (Cholesterol feed + 2.5% P.eous)	2.34	2.89	2.99
Group G (Cholesterol feed + 5% P.eous)	2.43	3.09	3.18
Group H (Cholesterol feed + 10% P.eous)	2.52	3.29	3.38
SE	0.03	0.01	0.02
CD (P=0.05)	0.01	0.04	0.06

Determination of Globulin (g/l)

The result present in revealed that co-administration of mushroom (*P.eous*) diet alone with normal diet increased the level of globulin when compared to their respective control. Accumulation of globulin was found to be the maximum (3.30 g/l) in Group D at 90th day observation. The globulin level is reduced when the animals were fed with cholesterol rich feed (Group E) for duration of 30, 60 and 90 days (2.29, 2.26 and 2.14, respectively). Administration of 10 per cent mushroom (*P.eous*) diet alone with the cholesterol feed (Group H 2.67, 2.62 and 2.54) showed a reduction in the values of globulin on 30th, 60th and 90th day observation.

Table.2.Effect of *P.eous* on the globulin (g/l) levels of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	1.97	2.07	2.16
Group B (2.5% *P.eous*)	2.34	2.44	2.66
Group C (5% *P.eous*)	2.46	2.94	2.98
Group D (10% *P.eous*)	2.58	3.24	3.30
Group E (Cholesterol feed)	2.29	2.26	2. 14
Group F (Cholesterol feed + 2.5% *P.eous)*	2.49	2.45	2.44
Group G (Cholesterol feed + 5% *P.eous)*	2.55	2.53	2.49
Group H (Cholesterol feed + 10% *P.eous)*	2.67	2.62	2.54
SE	0.01	0.03	0.05
CD (P=0.05)	0.01	0.11	0.15

Determination of serum total protein (g/l)

The serum protein level in all the treatments recorded significant increase over control. The increase in the level of total protein could be positively correlated with the increase in the mushroom level. The serum protein level increased with an increase in the duration of administering normal feed plus mushroom diet in Group B (4.61, 5.59 and 6.18 g/l on 30th, 60th and 90th days of observation, respectively), Group C (5.49, 6.08 and 6.77 g/l on 30th, 60th and 90th days of observation, respectively) and Group D (6.09, 6.68 and 7.73 on 30th, 60th and 90th days of observation, respectively) when compared with Group A (4.39, 4.67 and 4.86 on the 30th, 60th and 90th days) of treatment. The total protein was found to be reducing in rats fed with cholesterol rich feed (5.06, 4.86 and 4.59 g/dl, respectively) when compared with rats fed with normal feed. whereas, serum protein level decrease in the duration of administering cholesterol feed plus mushroom diet in Group F (4.66, 4.71 and 4.92 g/l on 30th, 60th and 90th days of observation, respectively), Group G (5.31, 5.38 and 5.45 g/l on 30th, 60th and 90th days of observation, respectively) and Group H (5.72, 5.79 and 5.86 g/l on 30th, 60th and 90th days of observation, respectively) when compared with Group E (5.06, 4.86 and 4.59 on the 30th, 60th and 90th days) of treatment.

Table.3.Effect of *P.eous* on the serum total protein (g/l) level in the serum of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	4.39	4.67	4.86
Group B (2.5% *P.eous*)	4.61	5.59	6.18
Group C (5% *P.eous*)	5.49	6.08	6.77
Group D (10% *P.eous*)	6.09	6.68	7.37
Group E (Cholesterol feed)	5.06	4.86	4.59
Group F (Cholesterol feed + 2.5% *P.eous)*	4.66	4.71	4.92
Group G (Cholesterol feed + 5% *P.eous)*	5.31	5.38	5.45
Group H (Cholesterol feed + 10% *P.eous)*	5.72	5.79	5.86
SE	0.04	0.06	0.14
CD (P=0.05)	0.12	0.18	0.42

DISCUSSION

Determination of Albumin, Globulin and Total protein

Albumin is one of the two major types of proteins in the blood that promotes the transfer of nutrients and wastes to and from the blood and cell. Albumin is a major protein, which is formed by the liver, and chronic liver disease causes a decrease in the amount of albumin produced. In the present study,

increased levels of albumin was observed in rats fed with mushroom diet which confirmed that the dietary supplementation of *P.eous* did not affect the functioning of liver of rats but also enhanced its functioning. The albumin level and total serum protein level of rats fed with mushroom diet were higher though not significantly different from control rats fed with protein diet. However, the level of serum globulin was higher in rat fed with mushroom diet when compared with control fed with protein diet as reported earlier by Oyetayo and Oyetayo [7]. Higher albumin levels were observed in mice fed with the *P. atroumbonata* [5, 6]. Similarly, higher level of albumin was observed in the present study also. This could be due to the reason that *P.eous* could have had a better amino acid profile as observed by Nwanze and Adamu [5] with the edible mushroom *P. atroumbonata.*

Similarly, the serum globulin level in the treatment with mushroom diet was higher and significantly different from rats fed with protein diet as control, Oyetayo and Oyetayo [7]. An increase in serum globulin is an indication of immunostimulation [1]. A higher serum globulin level observed in the present study in rats fed with various levels of mushroom diet may indicate that immunostimulatory effect is associated with the edible mushroom *P.eous.* In present study, the rats co-administered with *P.eous* diet produced significantly higher total serum protein level than the normal feed and cholesterol diets. This could have been as a result of the breakdown of muscle protein through gluconeogenesis [9-12, 2].

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