Plant Archives Vol. 19 No. 1, 2019 pp. 535-538 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PHARMACOLOGICAL EFFECT OF *LENTINUS EDODES* ON THE SERUM ENZYME PROFILE OF MALE ALBINO WISTAR RATS

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Abstract

Edible Mushrooms have been highly regarded as possessing enormous nutritive and medicinal values. *Lentinus edodes* is revered in Asian medicine for its health-promoting effects. Pharmacological functions of polysaccharides isolated from *L. edodes* known to inhibit the growth of various transplantable tumors in experimental animals and increased the survival rate. The present study was aimed to find out the pharmacological effect of *L. edodes* on the serum enzyme profile of male albino wistar rats. The result revealed that on 30th day the Aspertate Amino Transferase (AST) value of Group D which was fed with *L.edodes* (10 %) and Group E (cholesterol feed) recorded the AST value of 65.92 units / L on same day. In general, a reduction in the level of Alanine amino transferase (ALT) was observed in Group D (31.32 units/ L) and Group H (39.25units / L) when compared with their respective control. The rats fed with cholesterol diet recorded significantly the maximum values of Alkaline phosphatase at all observations.

Key words: *Lentinus edodes*, male albino wistar rats and serum enzyme

Introduction

Mushrooms have been highly regarded as possessing enormous nutritive and medicinal values. *Lentinus edodes* is revered in Asian medicine for its health-promoting effects. Numerous bio-components present in *L. edodes* aid in its pharmacological potency against hypertension, hyperlipidemia and cardiovascular complications, depressed immunity, hepatic disorders and cancer. In addition, its antioxidative, anti-fungal and anti-microbial aspects have been duly attributed to its bio-functional components (Bisen *et al.*, 2010). With such unmatched potentials the commercial interest in the shiitake mushroom has increased in recent years, mainly because of its high value on the international market and an increase in dried mushrooms imports by some countries. This has occurred, not only because of its excellent aroma, flavor and nutritional profile, but also because of its medicinal properties **(**Rigoberto *et al.,* 2011). Shiitake is globally a well known cultivated species, but yet to find a place in Indian markets. Hence, in view of the potentials of shiitake to find a place in Indian markets the present research had been designed to evaluate the serum chemistry of *L. edodes* supplemented diet on male albino wistar rats.

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Materials and methods

Medicinal properties of *Lentinus edodes*

The medicinal properties of *L. edodes* were evaluated by testing the serum chemistry of *L. edodes* supplemented diet on male albino wistar rats. Accordingly, serum enzyme assay *viz.,* Aspertate Amino Transferase (AST), Alanine Amino Transferase (ALT) and Alkaline phosphatase (ALP) were investigated with standard procedures.

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 *L. edodes* was powdered and mixed thoroughly with the lab stock diet with the help of a little amount of hot water, and made into pellet form and air dried. Then it was stored in an air tight container at room temp.

Cholesterol feed: Feed rich in cholesterol, *viz.*, groundnut oil and egg yolk were mixed with normal feed and used.

Cholesterol plus mushroom feed : Hundred g of cholesterol feed was powdered, then 2.5, 5 and 10 g of

L. edodes was powdered and mixed thoroughly with the help of little amount of hot water, and made in to pellet form and then air dried. Then it was stored in an air tight container at room temp.

Animals and diets

Male wistar rats weighing 100 g and five weeks old were used for the study. The rats were individually housed in wire mesh cages and kept in an isolated room at a controlled temp. of 28±2°C and ambient relative humidity of 50-60% on a 12-hour light: dark cycle (lights on from 0600 to 1800 h) and an air changes of 10 to 12 per hour. Animals were acclimated to the facility and given free access to water and the powdered laboratory stock diet. The animals belonging to experimental groups were given five per centage powdered *L. edodes* mixed with laboratory stock diet. For animals belonging to cholesterol group, oils, egg yolk and ground nut were mixed with normal feed to increase the serum cholesterol level for experimental purpose. Necessary ethical clearance was obtained from Institutional Animal Ethical Committee of the Rajah Muthiah Medical College, Annamalai University to perform experimental studies on male wistar rats. The animals were reared with standard management practices and clinical as well as other parameters were recorded at 30 days, 60 days and 90 days duration.

Experimental Design:

The experimental rats were grouped as

Group A Rats fed with Normal Feed

Group B Rats fed with Normal Feed + 2.5% *L.edodes*

Group C Rats fed with Normal Feed + 5% *L.edodes*

Group D Rats fed with Normal Feed + 10 % *L.edodes*

Group E Rats fed with Cholesterol feed

Group F Rats fed with Cholesterol feed + 2.5% *L.edodes*

Group G Rats fed with Cholesterol feed + 5% *L.edodes*

Group H Rats fed with Cholesterol feed + 10 % *L.edodes*

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.

Assay of Aspertate Amino Transferase AST (Units/L)

Serum Aspertate Amino Transferase (AST) was assayed by using the diagnostic kit based on the method of Reitman and Frankel (1957). AST catalyses the transfer of amino group from L-aspertate to α-ketoglutarate with the formation of oxaloacetate and glutamate. The amount of oxaloacetate was measured by converting it into pyruvate by treating with aniline

Table 1: Effect of *L.edodes* on the serum enzyme profile (AST) (units/L) of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	59.75_{b}	60.27_{a}	62.09_{b}
Group B (2.5% *L.edodes*)	59.50_{b}	60.21_{a}	62.00_{b}
Group C (5% *L.edodes*)	59.21_{a}	60.11_{a}	61.82_{a}
Group D (10% *L.edodes*)	59.00_{a}	60.02_{a}	61.52_{a}
Group E (Cholesterol feed)	65.92_{d}	70.42_{b}	73.45_{c}
Group F (Cholesterol feed + 2.5% *L.edodes)*	65.25_{c}	70.38_{b}	73.32_{c}
Group G (Cholesterol feed + 5% *L.edodes)*	65.10_{c}	70.20_{b}	73.12_{c}
Group H (Cholesterol feed + 10% *L.edodes)*	65.00_{c}	70.10_{c}	73.05_{c}

Table 2: Effect of *L.edodes* on the serum enzyme profile (ALT) (units/L) of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	32.21_{b}	35.31_{a}	41.92_{b}
Group B (2.5% *L.edodes*)	32.01_{b}	35.27_{a}	41.85_{b}
Group C (5% *L.edodes*)	31.96_{b}	35.13_{a}	41.70_{b}
Group D (10% *L.edodes*)	31.32_{a}	34.98_{a}	41.20_{a}
Group E (Cholesterol feed)	39.78_{c}	42.82_{b}	45.74_{d}
Group F (Cholesterol feed + 2.5% *L.edodes)*	39.72_{c}	42.78_{b}	45.71_{d}
Group G (Cholesterol feed + 5% *L.edodes)*	39.61_{c}	42.62_{b}	45.62_{c}
Group H (Cholesterol feed + 10% *L.edodes)*	39.25_{c}	42.41_{b}	45.42_{c}

Table 3: Effect of *L.edodes* on the serum enzyme profile (ALP) (units/L) of male albino wistar rats

Groups	30 days	60 days	90 days
Group A (Normal Feed)	118.82_{a}	120.72_{a}	127.81_{a}
Group B (2.5% *L.edodes*)	118.74_{a}	120.67_{a}	127.78_{a}
Group C (5% *L.edodes*)	118.61_{a}	120.58_{a}	127.65_{a}
Group D (10% *L.edodes*)	118.32_{a}	120.40_{a}	127.51_{a}
Group E (Cholesterol feed)	129.62_{b}	135.78_{b}	138.54_{b}
Group F (Cholesterol feed + 2.5% *L.edodes)*	129.51_{b}	135.61_{b}	138.48_{b}
Group G (Cholesterol feed + 5% *L.edodes)*	129.42_{b}	135.42_{b}	138.31_{b}
Group H (Cholesterol feed + 10% *L.edodes)*	129.12_{b}	135.10_{b}	138.10_{b}

citrate and then reacting the pyruvate with 2, 4-dinitrophenyhydrazine to form 2, 4 dinitrophenylhydrazone derivative which brown coloured in alkaline medium. The absorbance of this hydrazone derivative is correlated to AST activity. The result were expressed as IU/L of serum.

Assay of Alanine Amino Transferase ALT

Serum alanine amino transferase (ALT) was assayed by using the diagnostic kit based on the method of Reitman and Frankel (1957). ALT catalyses the transfer of amino group from L-alanine to α- ketoglutarate with the formation of pyruvate and glutamate. The pyruvate so formed is allowed to react with 2,4-dinitrophenyhydrazine to produce 2,4dinitrophenylhydrazone derivative which brown coloured in alkaline medium. The absorbance of this hydrazone derivative is correlated to ALT activity. The results were expressed as IU/L of serum.

Assay of Alkaline phosphatase ALP

Plasma alkaline phosphatase was estimated by using the diagnostic kit based on Kind and king's (1954) method. ALP catalyses disodium phenylphosphate into phenol and disodium-hydrogen phosphate at pH 10.0. Phenol so formed reacts with 4-aminoantipyrine medium in the presence of oxidizing agent potassium ferricyanide to form a red coloured complex whose absorbance is proportion to the enzyme activity was expressed as IU/L serum.

Effect of *L. edodes* on the serum enzyme profiles of male albino wistar rats

No mortality or abnormalities in appearance or behavior were observed in either mixture feed (cholesterol feed + mushroom feed) or mushroom feed during the administration period.

Determination of Asparatate Amino Transferase (AST)

The result revealed that on 30th day the AST value was 59.75 units/L in Group A which was fed with normal rat feed. The same group showed an increasing trend up to day 90 (62 .09 units/L). Group D which was fed with *L. edodes* (10 %) showed an AST value of 59 units/L on day 30 and the same increasing trend was observed till the last day of observation and the value recorded was 61.52 units/L on 90th day. The treatment Group E (cholesterol feed) recorded the AST value of 65.92 units /L on day 30. Group H (cholesterol feed + 10 % *L. edodes*) recorded 65.0 units /L. Thus, it is evident that the value of AST was decreased in Groups D and H (table 1).

Determination of Alanine Amino Transferase (ALT)

In general, a reduction in the level of ALT was observed in Group D (31.32 units/L) and Group H (39.25units/L). The administration of *L. edodes* diet to the male wistar rats showed a non- significant effect on the level of ALT when compared with their respective control. Further, the cholesterol feed showed more of ALT values when compared with the normal diet and mushroom co-administered diet (table 2)

Determination of Alkaline Phosphatase (ALP)

Generally an increase in the duration of observation showed an increase in the values of ALP in all the treatments. Also, the both the treatment Group D and Group H showed no significant effect and recorded statistically at par results with their respective controls in all days of observation (table 3). The rats fed with cholesterol diet recorded significantly the maximum values of ALP at all observations.

The enzyme AST is widespread in tissue and therefore elevation may represent non-specific tissues damage. Clinically, AST is primarily related to liver functioning and is a specific indicator of liver dysfunction. Aside from liver, heart or muscle damage, elevations in AST can indicate a deficiency of certain hormones and vitamin E (Benjamin, 1985).

Alkaline phosphatases (ALP) are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (is enzyme ALP-2). The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. An increase in serum alkaline phosphatase may be due to congestion or obstruction within the liver (Benjamin, 1985).

In general, the serum enzyme profiles are simply the marker of liver or biliary track dysfunction (American liver foundation, 1985). In the present study, co-administration of various levels of *L.edodes* showed slight decrease in the values of serum enzymes profile when compared to their respectively control. Similarly, Oyetayo and Oyetayo (2005) observed decreased level of AST and increased levels of ALT in rats administered with mushroom diet when compared with standard protein diet. Sang Chul Jeong *et al.,* (2010) reported that male sprague – Dawley rats fed with the *Agaricus bisporus* powder for 3 weeks had significantly reduced liver enzyme activites.

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