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Research on Acute Toxicity and the Effect of Methanol Extract *Pleurotus Eous* Mushroom in Rats: An *In Vivo* Study

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ABSTRACT

Objective: The present study was aimed to investigate the toxic effects of acute toxicity administration of methanol extract of *Pleurotus Eous* in wistar rats weighing (150-200g) as per OECD guidelines.

Materials & Methods: In acute toxicity the effects of single oral dose (100, 500, 1000 mg/kg) of *Pleurotus Eous* methanol extract have been administered. Group 1 was used as control. Mortality rate, organ weight and hematological parameters were studied.

Results: In acute toxicity study, a single dose administration of *Pleurotus Eous* upto 1000 mg/kg did not induced any mortality. LD50 of the *Pleurotus Eous* has been estimated higher than 1000 mg/kg. No significant difference were found in relative organ weight and the outcomes of the current study revealed that no significant changes in the hematological parameters between the control and the methanol extract of *Pleurotus Eous* administered groups in acute toxicity studies.

Conclusion: The oral administration of *Pleurotus Eous* did not produce any toxic effect in albino rats. Acute toxicity studied in animals is usually required for any pharmaceutical purpose. It was observed as no rats were dead at maximum dosage which indicates large therapeutic index.

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Introduction

Mushrooms have a long traditional record of use for their promotion of healthy lifestyles [1]. Edible mushrooms have been widely accepted as human food for years, and have been acknowledged for organoleptic properties as well as some medicinal attributes [2]. Mushrooms are now getting significant importance, due to their nutritional and medicinal value and today their cultivation is being done in about 100 countries. However, the awareness of mushrooms as a healthy diet and as an importance source of biological active substance with medicinal value has only recently emerged [3]. Study documented that mushroom contains soluble β -glucan which has glucose reducing properties and boost pancreatic β cell in chemically induced diabetic rats over the block of α -glucosidase and improve insulin secretion [4].

Pleurotus species grows on various types of lignocellulosic un-composted agro wastes and produce Oyster mushroom. Oyster mushroom contains very low amount of carbohydrate, sugars and no or very few amount of cholesterol [5]. *Pleurotus* species have given a comprehensive account of nutritional with some medicinal aspects [6].

Guanide, a compound related to the bi-guanide class of oral anti-diabetic drugs was isolated from *Pleurotus* species [7]. Mushrooms contain certain compounds which help proper functioning of the liver [8]. Pancreas and other endocrine glands, promotes formation of Insulin and related hormones [9,10]. *Pleurotus* species contain excellent source of essential amino acids and several medicinal properties and antioxidants [11,12].

Toxicological assessment is the initial step in validating the medicinal potential of mushrooms [13]. Hence, the present study is to investigate the acute toxicity of methanol extract of *Pleurotus eous* with reference to hematological parameters in albino Wister rats.

Materials and Methods
Mushroom Extract

The fresh fruiting bodies were shade dried and powdered in an electrical grinder. Dried powder weighed carefully and used for methanol extract preparation through soxhlet apparatus at respective temperature.

Acute Toxicity Studies

Male Albino Wistar rats weighing (150-200g) were obtained from Kerala Agricultural University. The animals were maintained in

a cage with free water *ad libitum* and food. They were fed with standard pellet diet from AVM feeds, Coimbatore. All experimental procedure was in compliance with the Animal Ethical Committee, with an approval number 659/PO/Re/S/02/CPCSEA. Animals were accustomed to laboratory condition for a week before start.

Experimental Set Up

Table 1: Experimental Set Up in Acute Toxicity Study

Groups	Treatment
Group I	Control rat
Group II	100mg/kgbw
Group III	500mg/kgbw
Group IV	1000mg/kgbw

Single Dose Formulation

As stated by the instructions of OECD guidelines 423 with little conversion, the rats were divided into four groups. Administration of different doses of test substance to various groups, which has 6 animals each. The first group of 3 rats were control which was given only food and water *ad libitum* from the second group onwards receives different doses of the test substances 100, 500 and 1000mg/kg of body weight. All treatments were given orally. The animals were monitor for signs of mortality and toxicity during practicum. At the withdrawal of the study, the animals were sacrificed and the blood was drawn through cardiac puncture.

Hematological Parameters

Blood samples were collected to analyze hematology parameters into heparinized tubes. The hematological parameters scrutinized, which included total hemoglobin (Hb) packed cell volume (PCV) total WBC count, polymorphs, lymphocytes, monocytes, eosinophil, total RBC count, MCV, MCH, MCHC, RDW, Platelet count, MPV.

Statistical Analysis

The results were expressed as the mean \pm standard deviation (SD). Differences between groups were analyzed by one-way analysis of variance (ANOVA) using SPSS Software package for windows version 16.0. Post-hoc study was performed for inter-group comparison using the least significant difference (LSD) test. $P < 0.05$ values are considered statistically significant.

Results and Discussion

Acute toxicity study was carried out using OECD guidelines. However, the effect of the *Pleurotus eous* mushroom on hyperglycemia condition in diabetes was not documented in the previous studies according to our knowledge. Hence, the present investigation was carried out to find out the antihyperglycemic action of Oyster mushroom *Pleurotus eous*. Rats weighing 150-200g were selected and fasted for 12 hrs prior to experiments and allowed an excess *ad libitum*. The study was conducted by administration of 100, 500, 1000 mg/kg body weight of methanol extract of *Pleurotus eous* mushroom to rats in the respective groups and the control group received no extract. The LD 50 of *Pleurotus eous* of methanol extract in rats were determined to be greater than 1000mg/kg body weight. In the acute toxicity study no mortality was recorded and presented in the table 2. It was observed that no rats were dead at the maximum dosage of 1000 mg/kg body weight, which indicated large therapeutic index.

Medicinal mushrooms have been used for thousands of years in cultures around the world. Now modern scientists have identified some attracting healing properties of mushrooms. Currently there are more than hundred species of mushrooms studied across the

world today for their prospective health benefits. Acute toxicity studies in animals are usually required for any pharmaceutical purpose. The chosen mushrooms was subjected to toxicological studies by giving methanol extract of the mushrooms to the rats for the safety confirmation of future clinical investigations.

Table 2: Mortality Rate of Rat in Acute Toxicity Study

Dose sample mg/kg	Number of animals survived/utilized	% Mortality
100	6/6	0
500	6/6	0
1000	6/6	0

Earlier reports have shown that if the median lethal dose of a test substances is three times more than the minimum effective dose, the substance is recognized as positive aspirant for future studies [14]. The effect of *Pleurotus eous* methanol extract at different doses that is weight of the vital organs were monitored and documented which is represented in the Table 3.

No remarkable differences were noticed in the relative organ weight. Generally toxic nature of the drug leads to abnormalities in body weight [15]. However the doses of 100, 500, 1000 mg/kg of *Pleurotus eous* methanol extract showed increased in bodyweight. Furthermore, the increase in the body weight was not remarkably dissimilar from that of control.

Organ weight is also an essential indicator of physiological and pathological status in animals. The relative organ weight was necessary to diagnose whether the organ is injured or not. However, the results observed that there was no infection in the vital organ among the control and treated animals. Hence, it can be recommended that *Pleurotus eous* methanol extract is practically non-toxic.

Table 3: Relative Organ Weight of Rats Treated With Different Doses

Organs	Control mg/kg	100 mg/kg	500 mg/kg	1000 mg/kg
Heart	0.47 \pm 0.04	0.48 \pm 0.09	0.50 \pm 0.05	0.51 \pm 0.01
Liver	3.13 \pm 0.14	3.53 \pm 0.19	4.01 \pm 0.12	4.23 \pm 0.22
Spleen	0.63 \pm 0.09	0.60 \pm 0.01	0.68 \pm 0.23	0.71 \pm 0.16
Pancreas	0.58 \pm 0.18	0.63 \pm 0.34	0.64 \pm 0.66	0.67 \pm 0.34
Kidney	0.78 \pm 0.05	0.83 \pm 0.19	0.87 \pm 0.09	0.93 \pm 0.18

Hematological Analysis

The study was carried out at different concentration of methanol extract of *Pleurotus eous* (represent in Table-4) on some hematological parameters such as Red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet and differential count such as neutrophils, lymphocytes, monocytes, eosinophil's were estimated in blood samples. Hematological analysis is important to diagnosis and monitor hemolytic diseases and also a basic tools to check the health or test for certain conditions, illness and abnormalities. Hematological parameters are useful for predicting the pathological and toxicological effect of potentially toxic substances in humans and animals [16,17]. The outcomes of the current study revealed that no significant changes in the hematological parameters between the control and the methanol extract of *Pleurotus eous* administered groups in acute toxicity studies.

Table 4: Hematological Profile Treated With Different Doses of Rat in Acute Toxicity Study

Parameters	Control	100mg/kg	500mg/kg	1000mg/kg
Complete blood cell count				
Hemoglobin g/dl	14.2±0.23	13.5±0.54	12.2±1.09	13.2±1.86
Packed cell volume %	44.3±1.72	40.8±2.33	37.7±1.10	40.6±2.21
WBC (10 ³ /mm ³)	7.35±0.52	6.53±0.88	7.87±1.15	8.24±0.67
RBC (10 ⁶ /mm ³)	12.18±2.31	10.67±3.01	10.88±2.85	09.18±3.67
MCV fL)	79.50±0.87	78.19±1.04	72.59±1.43	76.21±2.03
MCH (pg)	26.02±1.45	29.21±1.98	25.10±2.05	26.24±2.45
MCHC (g/dL)	36.35±2.76	39.49±1.83	37.39±2.40	33.53±3.12
Platelet (10 ³ /mm ³)	274.33±09.43	256.81±08.22	230.72±05.98	201.33±07.43
Differential Count				
Neutrophils %	45.09±3.32	41.06±2.58	39.10±3.15	36.09±2.13
Lymphocytes %	88.13±2.43	91.19±3.03	88.27±2.89	90.08±2.12
Monocytes %	03.11±3.56	02.21±4.50	02.19±2.50	0322±3.89
Eosinophil %	04.98±1.38	04.86±2.45	03.45±3.01	05.34±3.58

Results Are Expressed as Mean ± S.D (n=6). Values are Statistically Significant at the Level of P<0.05.

Conclusion

The acute toxicity study of the methanol extract of *Pleurotus eous* did not produce adverse effects of the rats of the treated doses. LD50 of the methanol extract of *Pleurotus eous* was greater than 1000 mg/kg. Hematology parameters were with the reference range of rats. The result obtained from this study revealed that the *Pleurotus Eous* mushroom will implement new application in the pharmaceutical industries in the upcoming years.

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