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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/12611
DOI URL: http://dx.doi.org/10.21474/IJAR01/12611

RESEARCH ARTICLE

PHARMACOLOGICAL IMPACT OF EDIBLE MACROFUNGI *D. SPATHULARIA* AND *S. COMMUNE* ON HEMATOLOGICAL PROFILE OF ALBINO RAT

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Manuscript Info

Manuscript History
Received: 20 January 2021
Final Accepted: 24 February 2021
Published: March 2021

Key words:-
Hematological Profile, Edible Macrofungi, Pharmacological Impact, Extract

Abstract

Hematological investigations had been seen as helpful instruments in diagnosing the condition of wellbeing of an individual, the impact of food consumed can be assessed utilizing these apprehensions. In addition, the distinctive blood cell parameters are likewise helpful in the appraisal of immunological status of the individual. The present work was aimed to assess the impact of aqueous extract of edible macrofungi*Dacryopinaxspathularia* and *Schizophyllum commune* on hematological parameters of albino rats. The two experimental macrofungi have been reported to possess several pharmacological properties like antioxidant, anti-diabetic, antibacterial, hepatoprotective, nephroprotective efficacies, and these are also used as traditional dietary and medicinal sources especially by the local people of North-east India. But the impact of these two edible macrofungi on the haematological parameters is still not reported. The results of the present work revealed that on administration of *D. spathularia* extract (500 mg/kg BW), slight but significant increase in total WBC count, platelets, eosinophils and monocytes was observed. There was no significant change found in rest of the hematological parameters. On the other hand, the administration of *S. commune* extract (500 mg/kg BW) had resulted into slight but significant increase in total WBC count and eosinophils, and rest all hematological parameters had not changed significantly. Thus, the two edible macrofungi under study does not have any adverse effects on haematological parameters and hence they are safe and can be used as dietary and nutraceutical purpose.

Introduction:-

Hematology is a branch of biology concerned with the study of blood, the organs involved in blood production, and blood-related diseases. Hematological tests provide criteria that are used in the diagnosis and control of a variety of diseases (Merck Manual, 2012, Togunet al., 2007). Moreover, the hematological parameters are very useful tools for the assessment of the physiological status of an individual (Khan and Zafar, 2005). Variations in the different hematological parameters are commonly used in the assessment of physiological, pathological or nutritional status of the body and to determine the stresses that may develop in response to certain drugs, dietary components, environmental factors or pathological agents (Afolabi et al., 2010, Olafedehanet al., 2010)

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A large number of edible macrofungi or mushrooms are broadly utilized as dietary and even therapeutic sources across India and furthermore over the world, subsequently the examination on the effect of consumable mushrooms on the hematological parameters is of significant importance. Hematological investigations had been seen as helpful instruments in diagnosing condition of wellbeing (Cheesbrough, 1991), as the impact of food devoured can be checked utilizing these boundaries. In addition, the distinctive blood cell parameters are likewise helpful in the appraisal of immunological status of the individual (Baker and Silverton, 1985). Several works have been done to assess the impact of edible mushrooms on mammalian model. Oyetao and Oyetao (2007) have studied the impact of edible mushroom PleurotusSajor-Caju on hematological parameters of rats. Akperaet al. (2012) have studied the impact of Ganoderma applanatum extract on laboratory rats. Kenneth et al. (2020) have studied the impact of TermitomycesTitanicuson hematological parameters of albino rats. In any case, countless eatable mushrooms have not been evaluated for their effect on hematological parameters till date.

Dacryopinaxspathularia and Schizophyllum commune are included under group Basidiomycota of Kingdom Fungi. Both the macrofungi are used as dietary sources and also use as traditional medicines by the local people especially the tribes of North-Eastern states of India. The two macrofungi viz. D. spathularia and S. commune have been reported to possess antibacterial, anti-inflammatory, antioxidant, hepatoprotective, nephroprotective and anti-hyperlipidemic activities in our previous research works (Kumar et al., 2018, Kumar et al., 2019, kumar et al., 2020). Therefore it had become very much significant to assess the impact of these two macrofungi on the haematological parameters, so that the use of these two macrofungi could be validated as a significant nutraceutical and medicinal source.

Material and Methods:-

Collection of macrofungi and preparation of extract:

Entire work has been carried in the Department of Zoology, Ranchi University, Ranchi. The fresh fruiting bodies of the two experimental macrofungii.e.Dacryopinaxspathularia and Schizophyllum commune were collected from Assam and identified in Department of Botany, Guwahati University.

The collected samples were washed with deionised water repeatedly and then disinfected with HgCl2. The samples were again washed repeatedly to remove the traces of HgCl2. Now, the samples were completely dried under shade for about 10 days. After complete drying, the samples were powdered using electrical griender. The fine powder of the two samples was then subjected to solvent extraction using Sox-let and water as solvent. The extraction procedure was continued for 72 hrs. The extracts were then packed into air-tight containers for further experimental use.

Animals and Acute toxicity studies:

Wistar albino rats (Rattus norvegicus) were used for the present research work with prior approval of ethical committee of Ranchi University. The animals were first acclimatized and maintained at room temperature 25±5ºC, with 12 hrs. light and dark cycle. The rats were fed with commercial pellet diet and water ad libitum. The acute toxicity studies were done following the OECD guidelines.

Experimental design:

Three groups of 6 rats each were formed. Group 1 served as control, Group 2 received 500 mg/kg BW of D. spathularia extract and Group 3 received 500 mg/kg BW of S. commune extract through oral feeding. The experiment was carried out for 14 days. At the end of experimental period of time, all the rats were kept starved overnight and then their blood was collected by retro-orbital bleeding without sacrificing the animal. The blood sample was used for assessment of different hematological parameters as follows:

Haemoglobin determination:

The hemoglobin content in blood was determined by Cyanmethaemoglobin (Haemoglobin-cyanide) method or HiCN method (Zwart et al. 1995). In this method, the hemoglobin is converted into cyanmethemoglobin by adding potassium ferricyanide and potassium cyanide. 5 ml of Drabkin's solution (it contains potassium ferricyanide, potassium cyanide and dihydrogen phosphate as components) is taken in a test tube and 20uL of whole blood was added to it. The test tube was allowed to stand for 5 minutes and then the mixture was poured in a cuvette. Now the absorbance of the mixture was measured at 540 nm. The absorbance was compared with that of the standard solution i.e.hemiglobinocyanide solution using the following formula:

$$Hemoglobin\left(\frac{gm}{dl}\right) = \left[\frac{ABSt}{ABSstd}\right] \times CONCstd \times \left[\frac{dilutionfactor}{100}\right]$$

Where,
ABSt = absorbance of test sample
ABSstd= absorbance of standard
CONCstd= concentration of standard

PCV determination:
The PCV (Packed Cell Volume) is the ratio of the volume occupied by Red Blood Corpuscles to that of the whole blood. It was determined by using Microhaematocrit method. In this method, the whole blood sample was treated with EDTA and then poured into two plain capillary tubes. The dry ends of the capillary tubes were sealed by way of setting into sealing clay at 90º angle. The capillary tubes were positioned in microhaematocrit centrifuge with the sealed ends located in the direction of periphery. The centrifuge was properly balanced and then centrifugation was done for five minutes. The PCV was then decided by means of using microhaematocrit analyzing device.

RBC and WBC count:
The RBC and WBC counting was done by using Haemocytometer (Neubauer) counting method. For total RBC count the blood sample was treated with RBC diluting fluid (Hayem's solution, containing 0.25 gm Mercuric chloride, 2.5 gm Sodium sulphate, 0.5 gm Sodium chloride, 100 ml distilled water, pH=5.9±0.1), whereas for total WBC count the blood sample was treated with WBC diluting fluid (2ml Glacial acetic acid, 1 gm Gentian violet 1%w/v, 97 ml distilled water, pH=2.2±0.2). For RBC count the dilution was 1:200 and for WBC count the dilution was 1:20. The dilution was carried out by using the pipette provided in the haemocytometer kit. Following dilution the blood sample was loaded in the loading area of the counting chamber of haemocytometer. Precautions were taken to ensure that there was no air bubble and over filling. The counting chamber was left undisturbed for 3 to 5 minutes so that the blood cells will get settled down. The RBC counting was done under microscope (low power, 10X) in the centre room of the chamber and WBC counting was done under microscope (high power, 40X) in the 4 large squares of the counting chamber.

For WBC counting the blood is diluted 20 times with acid solution, which removes the RBC by way of haemolysis and additionally gentian violet slightly stains the nuclei of WBCs, therefore counting of WBC becomes easy. For RBC count the blood is diluted 200 times with RBC diluting fluid, that's isotonic with blood hence haemolysis does not take place.

$$TLC = no.ofcells \times dilutionfactor \times \frac{deptfactor}{areacount}$$

Where, dilution factor = 200, depth factor = 10, area count = 4

$$TEC = no.ofcells \times dilutionfactor \times depthfactor \times \frac{totalruledarea}{areacount}$$

Where, dilution factor=20, depyh factor = 10, total ruled area = 25, area count = 5

Platelet count:
Haemocytometer was used for platelets count. 50 ul EDTA treated blood mixed with 950 ul dilution solution (11.45 gm Ammonium oxalate, 1 gm Sorensen's phosphate buffer, 0.1 gm Thimerosal, Distilled water 1L) to achieve the dilution up to 1:20. The mixture was then allowed to stay undisturbed to get the complete lyses of erythrocytes. Now, the suspension was again mixed and loaded into the counting chamber of haemocytometer, which in turn, is positioned in a wet Petri dish for about 15 minutes, in order to settle the platelets without the drying of the counting chamber. Now the counting chamber was observed under microscope with appropriate objective and the platelets were counted in the small chambers.

$$platelets(ul) = \frac{totalno.ofplateletscounted \times dilution}{no.ofcountedsquares \times volumeaboveonesmallsquare}$$

Where,

Dilution= 200, volume above one small square= 0.00025 ul

RBC indices:
RBC indices are calculated parameters. These parameters reflect the characteristics of the RBCs. The RBC indices measure the size, shape, and physical characteristics of the RBCs.

MCV (Mean Corpuscular Volue):
It refers to the average RBC size, expressed in units of Femtolitres (1fL=10-35 L).

MCH (Mean Corpuscular Hemoglobin):
It refers to the amount of hemoglobin per RBC or the average weight of RBCs in the sample. It s expressed in the units of Picogram (1pg=10-12gm).

MCHC (Mean Corpuscular Hemoglobin Concentration):
It refers to the average concentration of hemoglobin in the RBCs contained in a sample. It is expressed in gm/dl.

$$MCV = \frac{Hematocrit\ (\%) \times 10}{RBC\ (mio/ul) or (\times 10^{12}\ /L)}$$

$$MCH = \frac{haemoglobin(g/dl) \times 10}{RBC\ (mio/ul) or (\times 10^{12}\ /L)}$$

$$MCHC = \frac{hemoglobin(g/dl) \times 100}{hematocrit\ (\%)}$$

Differential WBC count:
A blood smear is prepared on a slide from the fresh blood sample and the slide was allowed to dry. Now methanolic fixative is applied on the blood smear to stabilize the cellular components. Then the slide was dipped in a buffered solution of Xanthene dye, which stains the cytoplasmic granules into a bright orange colour. The slide is then dipped repeatedly in a buffered solution of thiazine dyes consisting of methylene blue and Azure A. The nucleoli and cytoplasm is stained due to basophilic methylene blue component.

The slide was then rinsed with distilled water and allowed to dry. The dried slide was placed under the microscope and scanned to find a good distribution of cells. A drop of Cedar wood oil was located at the slide and the cells were examined at higher magnification using the oil emersion objective. Two hundred WBCs were counted and then the percentage of each type of WBC was determined.

Results:-

Table 1:- Impact of D. spathularia extract and S. commune extract on haematological parameters.

Parameters	Control	*D. spathularia*(500mg/kg BW)	*S. commune* (500mg/kg BW)
Total WBC	6.82±1.46	8.46±1.02a	9.16±0.92a
Total RBC	4.36±0.47	4.82±0.59	4.68±0.54
Platelets	349.21±5.39	368.45±4.86a	356.38±5.92
HB (g/dL)	10.86±1.04	11.24±1.02	12.04±1.16
Haematocrit (PCV%)	26.79±1.33	26.18±1.89	27.89±1.63

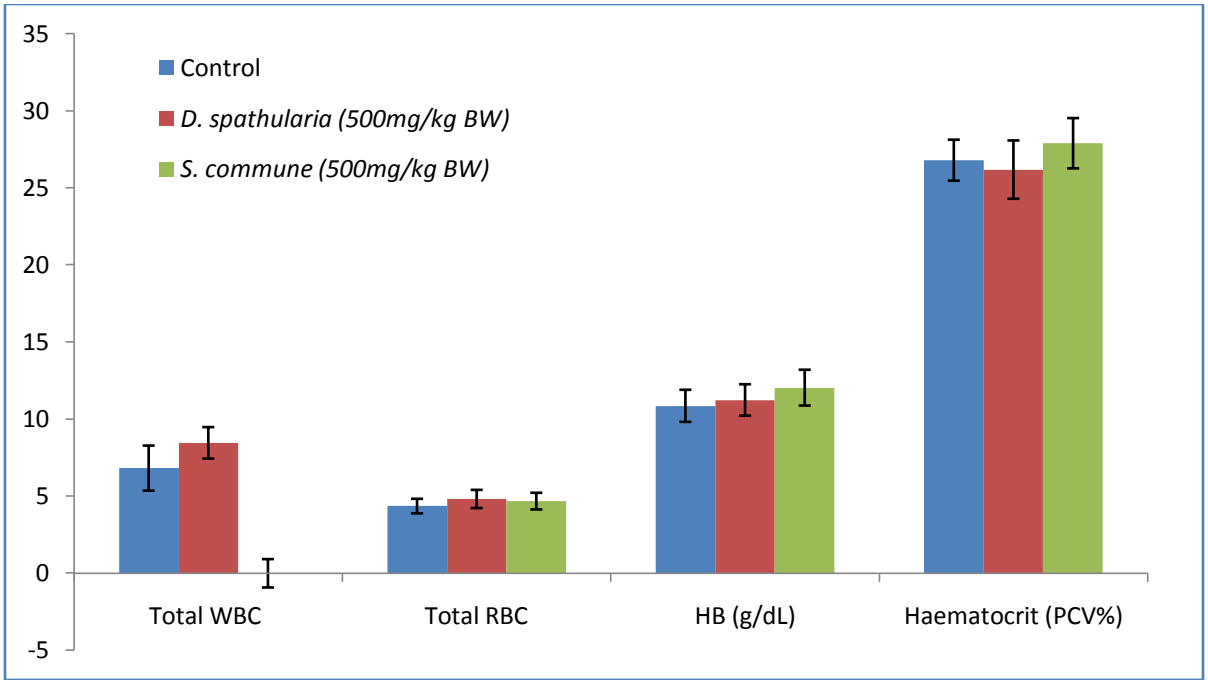
Table 2:- Impact of ofD. spathularia extract and S. commune extract on RBC indices

Parameters	Control	*D. spathularia*(500mg/kg BW)	*S. commune*(500mg/kg BW)
MCV (fL)	59.24±2.68	62.38±3.04	63.18±2.59
MCH (pg)	28.15±1.79	26.64±1.37	27.14±1.46
MCHC (g/dL)	46.72±3.16	44.95±2.81	42.96±3.12

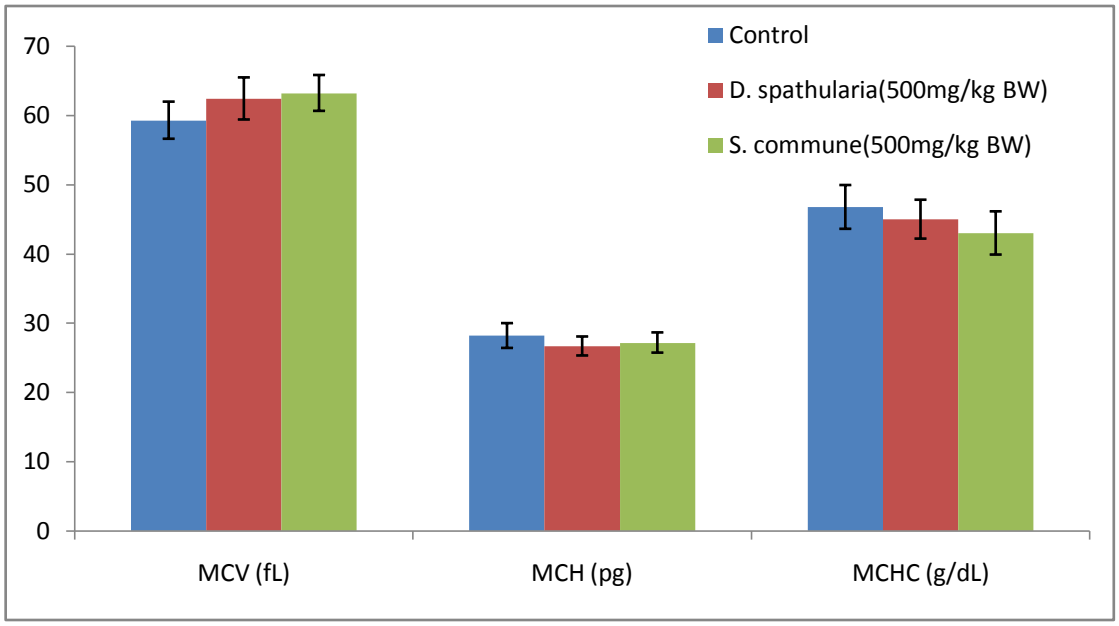
Table 3:- Impact of ofD. spathularia extract and S. commune extract on differential WBC count.

Parameters	Control	D. spathularia(500mg/kg BW)	S. commune(500mg/kg BW)

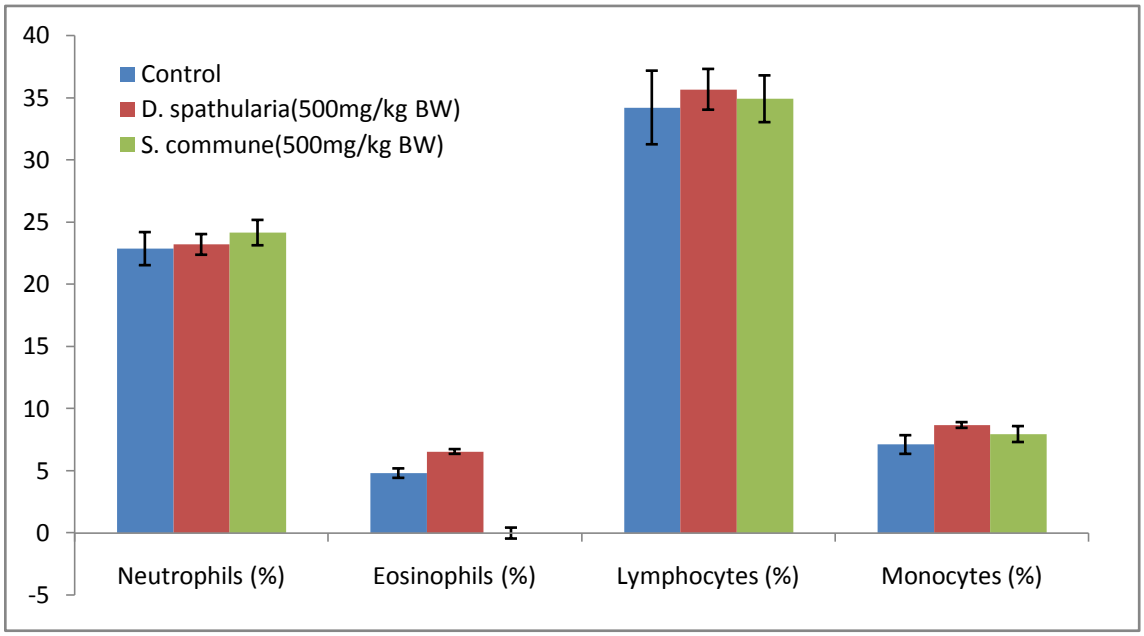
Neutrophils (%)	22.87±1.33	23.21±0.83	24.16±1.02
Basophils (%)	0.81±0.07	0.87±0.09	0.84±0.08
Eosinophils (%)	4.82±0.38	6.56±0.19a	7.08±0.44a
Lymphocytes (%)	34.22±2.96	35.68±1.64	34.92±1.88
Monocytes (%)	7.12±0.75	8.69±0.23a	7.96±0.64



Graph 1:- Impact of D. spathularia extract and S. commune extract on haematological parameters (Total WBC, Total RBC, Hb and Haematocrit)



Graph 2:- Impact of ofD. spathularia extract and S. commune extract on RBC indices.



Graph 3:- Impact of ofD. spathularia extract and S. commune extract on differential WBC count.

Discussion:-

The results of the impact of D. spathularia extract and S. commune extract on haematological parameters of the test animals have been shown in table 1. The results revealed that on administration of D. spathularia extract (500 mg/kg BW), slight but significant increase in total WBC count, platelets, eosinophils and monocytes was observed. There was no significant change found in rest of the hematological parameters. On the other hand, the administration of S. commune extract had resulted into slight but significant increase in total WBC count and eosinophils, and rest all hematological parameters had not significantly changed. Since mushrooms are consumed broadly across the globe, therefore the knowledge of toxicity or pharmacological properties of mushrooms is of great significance. Several previous works have been done till date on the impact of mushroom extracts on hematological profile of mammalian model, since the hematological studies not only reveals the wellbeing status of an individual but also reflects the immunological status of the body. Soji Fakoya (2013) had reported that the edible mushroom Pleurotusostreatus has no adverse effects on hematological profile of albino rats. Madhanrajet al. (2019) have reported the anti-hemolytic properties of many edible Basidiomycetes macrofungi including different species of Oyster mushrooms (Pleurotus sp.).

The two edible mushrooms taken for the present study are used as traditional dietary and medicinal sources especially by indigenous populations of North-eastern states of India. Kumar et al. (2018,2019a,2019b) have reported that these two edible mushrooms possess significant antioxidant, anti-inflammatory, anti-diabetic, hepatoprotective and nephroprotective properties. The results of the present work revealed that the two experimental macrofungi does not have any adverse effect on the hematological or blood cell parameters of the experimental animal. Thus, the two edible macrofungi under study are safe and can be used as dietary and nutraceutical purpose.

Acknowledgement:-

The authors acknowledge the facilities provided by the Zoology department of Jamshedpur Cooperative College and University Department of Zoology, Ranchi University for the lab facilities provided.

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