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In vitro and in vivo evaluation on fishes of anti-inflammatory potential of *Agaricus bisporus*

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ABSTRACT

Agaricus bisporus has been studied for many activities except for its anti-inflammatory potential completely both by in vitro and in vivo experiments. In the present study it was evaluated for the same using egg albumin for in vitro study and fish as the model for in vivo evaluation and found to have remarkable anti-inflammatory activity on both experiments. As expected with any natural drug the activity was better at higher doses.

Keywords: Agaricus bisporus, Oreochromis niloticus (Tilapia fish), anti-inflammatory

INTRODUCTION

Agaricus bisporus is a most widely cultivated edible mushroom and represents more than 40% of world's consumption of mushrooms. It is believed to have high biological activity, low toxicity and has significant folklore and ethno-pharmacological use. Although we often think fungi as disease causing organisms, it has other properties also useful to humanity. For example, fungi produce antibiotics like Penicillin and Cephalosporin. It is also useful in the production of immune suppressant drug Cyclosporine and as a precursor for steroid hormones. Ergot is another best example for fungi as a drug [1]. Hence it has been evaluated for many activities like Antioxidative, Antiaging, Hepatoprotective, Antimicrobial, Cytotoxic, Antimelanogenesis and Anti-Inflammatory Activity [2-8] In this work the most popular fungi Agaricus bisporus was taken up for studying its anti-inflammatory potential. Initially in vitro method established its potential and it was further confirmed by in vivo evaluation using fish as a model. Recently researchers through out the world started using fish for pharmacological evaluations as it offer a study one step ahead of in vitro studies since it is carried out on living organism or animal.

MATERIALS AND METHODS

The edible fresh white button mushrooms were purchased from a super market in Thuraipakkam, Chennai and authenticated by the taxonomist of Department of Botany, D.B.Jain College, Chennai. The mushrooms were cut into very small pieces with knife as it is impossible to powder them. It was dried under shadow and subjected to extraction with Petroleum ether [60^{o}-80^{o}c]. The defatted

mushrooms were then dried to remove the traces of Petroleum ether and extracted with ethyl alcohol 60% using a soxhlet apparatus [9, 10]. The extract was then concentrated under vacuum and evaporated to dryness. The yield was 10.7%w/w. This extract was evaluated for its anti-inflammatory potential.

PHARMACOLOGICAL EVALUATION

In vitro anti-inflammatory effect of alcoholic extract was tested by egg albumin assay and the in vivo activity was tested on fish.

In-vitro anti-inflammatory activity

The experiment was carried out with minor modification of the method published in the reference [11] shown below. The standard drug and extract were dissolved in Dimethyl sulfoxide (DMSO). The standard and test sample containing different concentrations (100, 200, 300, 400, 500, 1000 µg/ml) was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 37°C in incubator for 15 min. 3 ml of phosphate buffer (0.2 M, PH 7.4) was added and denaturation was induced by keeping the reaction mixture at 70°C in water bath for 15 min. After cooling, the turbidity was measured at 660 nm. Percentage of Inhibition of denaturation was calculated from control where no drug was added. Diclofenac was used as standard drug. The percentage inhibition of denaturation was calculated by using following formula:-

% of Inhibition = 100 x (At - Ac) / At.

Where, At = O.D. of test solution.

Ac = O.D. of control

The results are shown in table I, II & III,. A graph was also drawn from the readings and given below [Fig 3]

In vivo anti-inflammatory activity with fish as an experimental model organism

In our experiment, we have chosen Oreochromis niloticus (Tilapia fish) [Fig.1] as it is widely used in research studies and also easily available.

Oreochromis niloticus

Fig 1

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Cichliformes
Family: Cichlidae
Genus: Oreochromis
Species: niloticus.

Oreochromis niloticus, commonly known as the Nile tilapia, is a popular food fish that has been farmed in ponds for thousands of years. Despite its name, the Nile tilapia is not only present in the River Nile, it is native to Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Egypt, Gambia, Ghana, Guinea, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo and Uganda. Today, we can find established Nile tilapia populations on all continents except Antarctica.The genus Oreochromis is a part of the cichlid family (Cichlidae) [12]

Conservation status:

Oreochromis niloticus has not been evaluated for the IUCN Red List ofThreatened Species. [13]

Habitat

Nile tilapia is an adaptable fish that can do well in wide range of habitats. It has been found in all sorts of waters, from rivers and lakes to sewage canals and irrigation channels. Despite being considered a freshwater fish it will readily adapt to brackish conditions. Its extended temperature range is 8-42 °C (47-108 °F), but it is typically found in environments where the water temperature stays in the 13.5-33 °C (56-91 °F) interval.

Size and appearance

The body of the Nile tilapia is decorated with regular vertical stripes that continue throughout the depth of the caudal fin. The dorsal fin margin is black or grey. The largest scientifically measured *O. niloticus* specimen was 60 cm (nearly 24 inches) long.

In-vivo Anti-inflammatory activity:- Methodology

Experimental design

The experiment was performed in 500L FRP tanks and the fishes were equally and randomly divided into four groups (untreated control, carrageenan alone induced, standard diclofenac sodium induced and treatment) and each group was maintained in triplicate set containing 10 numbers of fishes, following a completely randomized design (CRD). The fish were anesthetized with clove oil (Merck, Germany) @ 4-5 ppm prior to the injection and the treatment group were injected intra-peritoneal (i/p) @ 50 l/fish with 0.5% of I-Carrageenan (Sigma-Aldrich, India) dissolved in 100 ml of sterile saline solution (0.85%) and the control group were kept in tanks without injection. The experiment was conducted for 24 hours and the sampling for histopathological parameters was carried out. For each sampling, fishes were selected randomly from each tank and analyzed for various parameters.

Fig 2

Histopathology

The collected tissue sample (liver, and intestine) were fixed in 10% neutral buffered formalin (NBF) for histopathology and processed byroutine paraffin embedding technique. Five microns thick section was cut [Fig 2] using microtome (Leica RM 2125 RT, Germany) and stained with haematoxylin and eosin. Pathological changes manifested in the tissue sections were observed and recorded using a light

binocular microscope (Olympus CX-31, Japan) and given below.

RESULTS AND DISCUSSION

The total extract obtained with alcohol 60% was subjected to pharmacological evaluation for in-vitro and in-vivo anti inflammatory activities. The alcoholic extract was first evaluated for anti inflammatory activity by in-vitro egg albumin assay.

It was found that the extract has less anti-inflammatory activity than the standard drug Diclofenac sodium. But, in higher doses it showed good improvement in activity. Hence it can be safely concluded that the Agaricus bisporus extract has notable anti-inflammatory activity than standard at high concentrations. [Tables I, II & III] & [Fig 3]

Table I

Anti Inflammatory activity

Egg Albumin Assay

Standard: Diclofenac sodium

CONCENTRATION	ABSORBANCE			S-C/S*100			% INHIBITION		IC50
	1	2	3	1	2	3	MEAN	S.D	443.5
100	0.152	0.156	0.155	14.474	16.026	15.484	15.328	0.788	
200	0.173	0.17	0.175	24.277	22.941	25.143	24.120	1.109	
300	0.194	0.191	0.199	32.474	31.414	34.171	32.686	1.391	
400	0.236	0.247	0.256	44.492	46.964	48.828	46.761	2.175	
500	0.315	0.321	0.363	58.413	59.190	63.912	60.505	2.976	
1000	0.471	0.419	0.497	72.187	68.735	73.642	71.521	2.520	
CONTROL	**0.131**	**0.129**	**0.133**	**0.131**					

Table II: SAMPLE: AGARICUS BISPORUS EXTRACT

CONCENTRATION	ABORBANCE			S-C/S*100			% INHIBITION		IC50
	1	2	3	1	2	3	MEAN	S.D	639.2
100	0.132	0.133	0.135	0.758	1.504	2.222	1.495	0.732	
200	0.134	0.137	0.136	2.239	4.380	3.676	3.432	1.091	
300	0.148	0.147	0.147	11.486	10.884	10.884	11.085	0.348	
400	0.185	0.179	0.181	29.189	26.816	27.624	27.876	1.207	
500	0.256	0.236	0.245	48.828	44.492	46.531	46.617	2.170	
1000	0.547	0.456	0.535	76.051	71.272	75.514	74.279	2.618	

Table III

Concentration	Absorbance		% Inhibition	
	Sample	Standard	Sample	Standard
100 µg	0.133	0.154	1.495	15.3277
200 µg	0.135	0.172	3.432	24.1205
300 µg	0.147	0.194	11.085	32.6862
400 µg	0.181	0.246	27.876	46.7611
500 µg	0.245	0.333	46.617	60.5049
1000 µg	0.512	0.462	74.279	71.5213
Control	0.131	0.131	-	-

Anti Inflammatory activity

Egg Albumin Assay

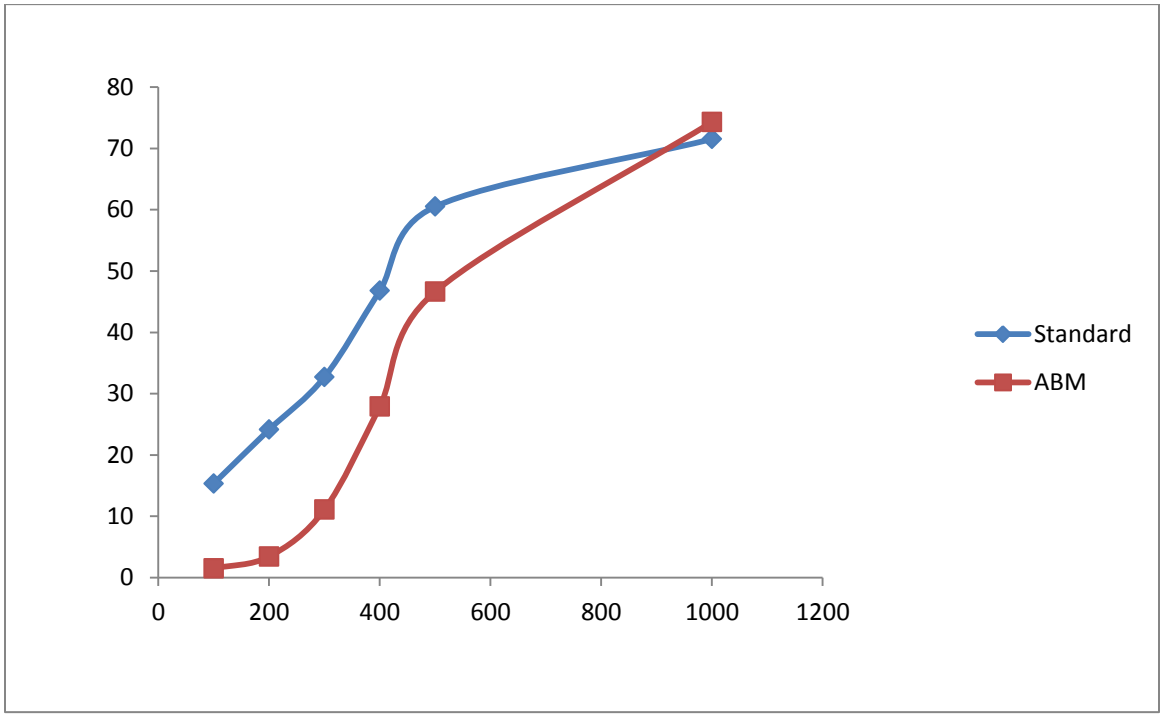
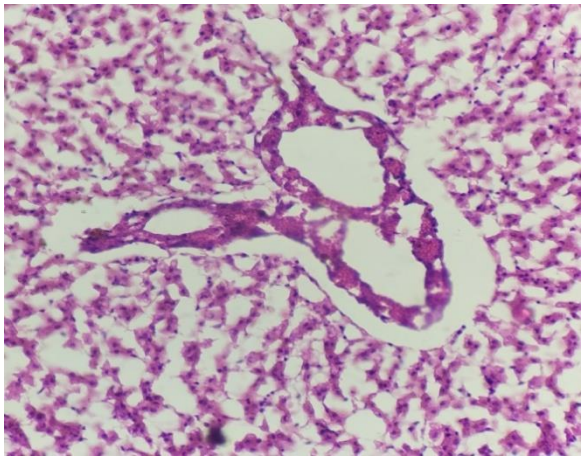


Fig 3

Then, the same alcoholic extract was subjected to evaluation of in vivo anti-inflammatory activity in Tilapia fish. Inflammation was induced in the fishes using carrageenan. From the histopathological observations [14] of liver [Fig.4 to 7] and intestine [Fig 8 to 11] of the fishes, it can be concluded that the extract has remarkable anti-inflammatory activity.

Histopathological observations



Liver Fig 4

(a) Untreated Control group - showing intact hepatocytes and central portal triad appearing normal.

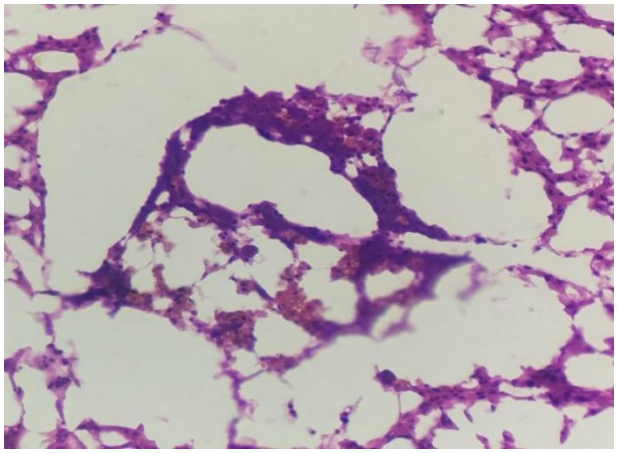


Fig. 5

b) Carrageenan alone induced group – showing loss of nucleus and strong inflammation in hepatocytes.

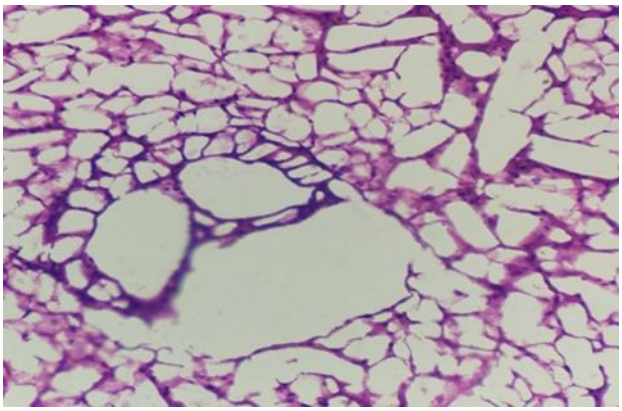


Fig. 6

(b) Standard Diclofenac sodium treated group – showing normal hapatocyte with no such inflammation.

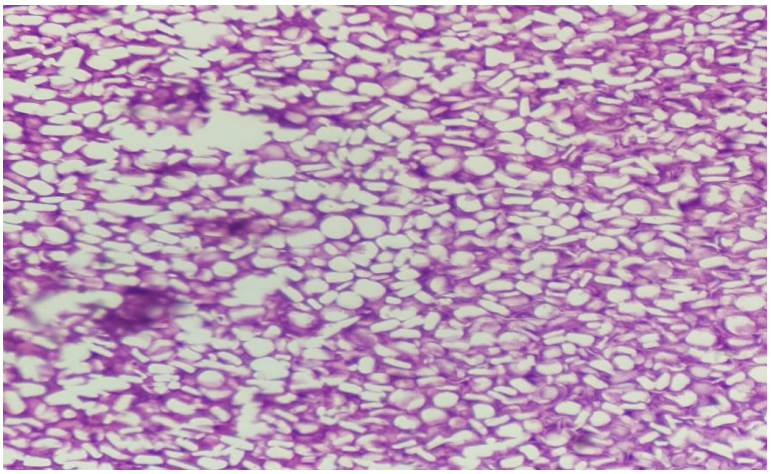
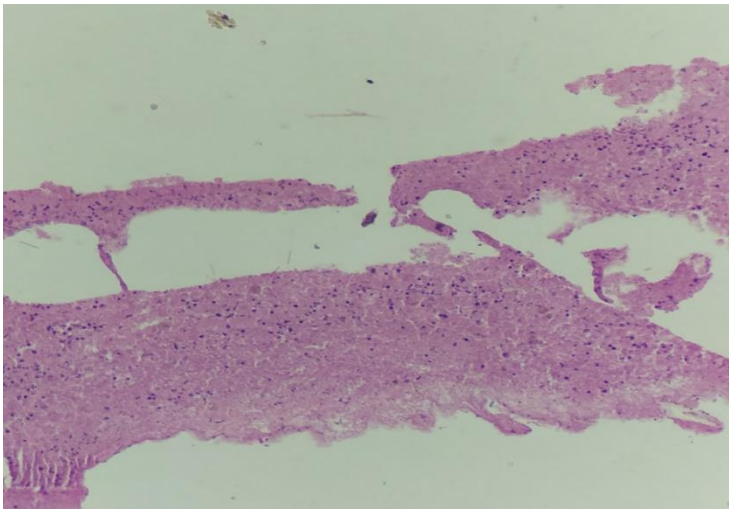


Fig.7

(c) ABM Extract treated group –Hepatocytes returning to normal architecture, but still mild hepatosteatosis.



Intestine Fig.8

(a) Untreated Control group - showing normal intestinal architecture.

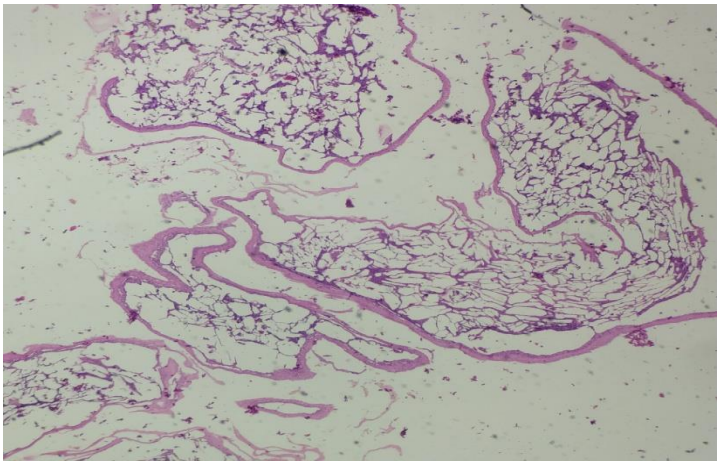


Fig.9

(b) Carrageenan alone induced group – showing inflammation in intestinal epithelial cells.

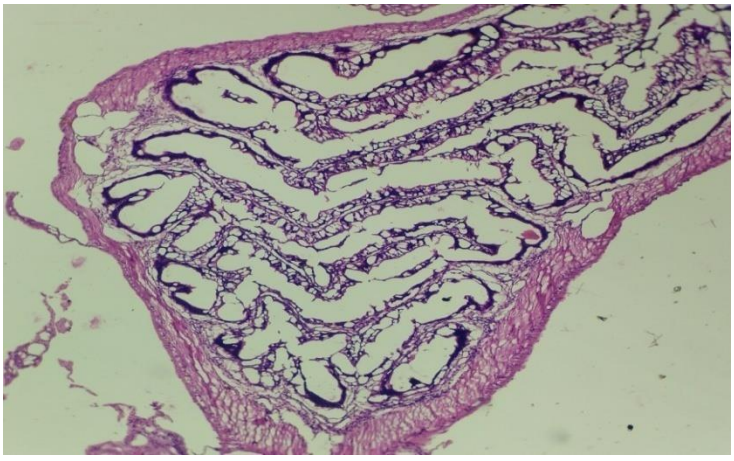


Fig.10

(C) Standard Diclofenac sodium treated group – showing normal intestinal epithelial cells with no such inflammation.

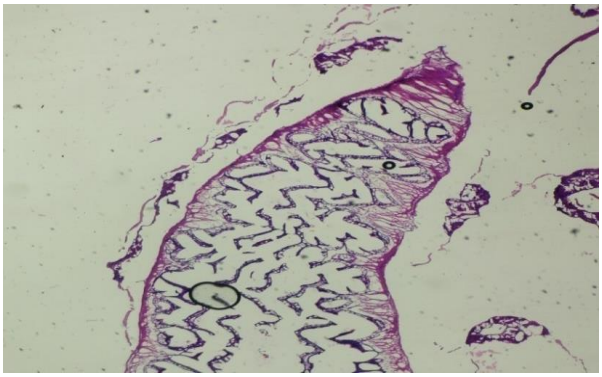


Fig 11

d] ABM Extract treated group - Intestinal epithelial cells returning to normal architecture degeneration.
[It is the description of Fig 11. So it should be printed just below the figure 11, as in Fig 9, 10 etc]

There is further scope in this project that the constituent or constituents responsible for this remarkable anti-inflammatory activity of alcoholic extract of Agaricus bisporus mushroom can be isolated in pure form and their identification including structure can be elucidated, after which they can be used for confirming above, as well as other pharmacological activities.

CONCLUSION

Hydro alcoholic extract of Agaricus bisporus has remarkable anti-inflammatory activity in both in vitro and in vivo pharmacological evaluations. Fish as a model for in vivo studies offer alternative source in pharmacological evaluations where getting higher animals for such studies are highly restricted. Compound(s) responsible this activity can be isolated and confirmed for anti-inflammatory and other activities like analgesic activity which are planned and under execution in our laboratory.

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