Indian J.Sci.Res. 18(2): 115-118, 2018

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

IN VITRO CYTOTOXICITY ASSAY OF SELECTED WILD EDIBLE MUSHROOMS AGAINST DALTON'S LYMPHOMA ASCITES (DLA) CELLS

N.K. SHAHINA[a1] AND K. MADHUSUDHANAN[b]

[ab]Department of Botany & Research Centre, St. Albert's College, Ernakulam, India

ABSTRACT

Mushrooms are used by people from ancient times due to its pleasing flavour, palatability and medicinal value. The bioactive components and pharmacological activities of many mushrooms were proven by various workers. Edible mushrooms extracts are natural, less expensive and in general have minimal side effects. The ethnomedicinal studies about wild edible mushrooms among tribes in Kerala suggests that many wild edible mushrooms are used by different tribal communities for medicinal/health boosting purposes. The present investigation evaluates the in vitro cytotoxicity assay of four wild edible mushroom crude extracts in four different concentrations (100, 50, 20, and 10 µg) by trypan blue dye exclusion assay on Dalton's Lymphoma Ascites (DLA) cells. The inhibition percentages of extract in 100 µg were found to be 99.33±0.53, 99.16±0.76, 55.5±0.5 and 89.96±0.05 for *Termitomyces microcarpus*, *Phlebopus portentosus*, *Lentinus bambusinus* and *Coprinellus micaceus*respectively. Even though *Termitomyces microcarpus*and *Phlebopus portentous* showed almost similar results in 100 µg, the inhibitory percentage in 10 µg was different (56.17±0.17, 12.16±0.25) for both of them.

KEYWORDS: In Vitro Cytotoxicity Assay, Wild Edible Mushrooms, Dalton's Lymphoma Ascites (DLA), Tribes

By invitro cytotoxicity tests we can predict human toxicity to certain compounds/chemicals (Clemedson and Ekwall; 1999; Scheers et al., 2001). Cytotoxic compounds often compromise cell membrane integrity, so the most common ways to measure cell viability and cytotoxic effects is by assessing cell membrane integrity. Trypan blue is an essential dye with two azo chromophores group (Riss and Moravec; 2004). It penetrates into the dead cells and gives blue colour and gave an exact number of dead and viable cells. Cytotoxic compounds are an area of interest for developing a therapeutic that targets rapidly dividing cancer cells.

Block et al.,(1992) showed that functional foods reduce the risk of cancer in people. When considering a natural plant or organism as source of functional food, scientists normally take in to account its traditional usage. Mushrooms are used by people from ancient times due to it's pleasing flavour, palatability and medicine value (Mizuno; 1995) and considered as functional food. The bioactive components and pharmacological activities of many mushrooms were proven. Unique structural features of mushrooms ie polysacharide-protein, polysacharide peptide complexes, sugar to sugar linkages contributing to cytotoxic effects/antitumor effects (Lakshmi *et al*., 2004, Nitha *et al*., 2006). Many anticancer drugs in market were isolated from mushrooms like *Lentinus edodus*, *Coriolus versicolor* and *Schizophyllum commune*.The ethnomedicinal studies about wild edible mushrooms among tribes in Kerala suggests that many wild edible mushrooms are used by different tribal communities for medicinal/health boosting purposes.

The present study intended to screen the in vitro cytotoxicity of four wild edible mushroom (*Termitomyces microcarpus f elongates, Coprinellus micaceus, Phlebopus portentosus, Lentinus bambusinus)* extracts against DLA Cell line using Trypan blue dye method.

MATERIALS AND METHODS

Four wild edible mushrooms *Termitomyces microcarpus f elongates, Coprinellus micaceus, Phlebopus portentous and Lentinus bambusinus*were collected from forest regions of Wayanad with the help of tribal key knowledge holders. Recorded field notes, the collected mushrooms were dried in 40°C in hot air oven for 4-8 hours. The identification of mushrooms was done by macro and micro characterisation. Extracts were taken in 70% ethanol in soxhlet extractor and crude extract prepared by lyophilisation.

Dalton's lymphoma ascites (DLA) cell lines were maintained as ascites tumors in swiss albino mice. The cells were aspirated, washed thrice in phosphate buffer saline (PBS) and counted using a haemocytometer. The cell suspension of 1million cells/ml was prepared. Added viable suspension to test compounds in various concentration (200,100, 50, 20 &10 µg) made the final volume 1ml using PBS, incubated 3 h, 37°C. Control tubes containing only cell suspension. Each cell suspension mixed with 0.1 ml of 1% trypan blue, the dead cells take up dye(Kuttan et al., 1985). The number of dead cell was counted using a haemocytometer (Shrivastava and Ganesh., 2010).

[1]**Corresponding Author**

Percentage of cytotoxicity = [No.of dead cells/No.of livecells+No.of dead cell] x 100

RESULTS

Description of Wild Edible Mushrooms Collected for Study

1. *Termitomyces microcarpus f elongates* (Berk. &Br.)

Pileus 2.05 ±0.62 cm diam., campanulate to planoconvex , umbonate; soft and fleshy, surface creams at the disk, whitish elsewhere, dry, smooth and glabrous; margin straight entire, sometimes wavy. Lamellae free, white then pinkish. Stipe 4.1±1.70 cm x 1.76±0.69 mm, central, cylindric, equal or narrow towards the base, surface white, fibrous, smooth. Veil absent. Pseudorrhiza absent. Context white. spore print light pink. Cheilocystridia and pleurocystidia present.

2. *Coprinellus micaceus (Bull.) Fr.*

Plieus 3.66±1.56 cm high and of similar diameter when open out. At first ovoid covered with white granules the remains of veil and then expanding to bell shaped with split or sometimes rolled back margin . Cap colour ochre brown with a russet central eye and turns grey brown as it ages. Gills are attached, close,moderately broad, white turning purple browned then blackening. Auto- digesting. Stipe 6.35 ±1.31 cm tall and 3.12 ±0.85 mm diameter. White discolouring to brown. Spore print is dark brown. Spores ellipsoidal.

3. *Phlebopus portentosus,* Berk & Br.

Description: Pileus 13.37 ±8.69 cm diam., convex becoming plano convex with a depression.Surface fleshy olive brown, slimy when wet,smooth and glabrous; margin involute. Hymenophore tubulate, lemon yellow 9.75 ±3.8 mm wide, bruised on cutting,pores greenish yellow. Stipe 7.1 ±3.25 cm x 5.37 ±2.5cm, central, cylindric with swollen base, solid; surface olive brown, bruised when cut. Context butter yellow. Spore print olive brown. Spores ovoid, olive brown.

4. *Lentinus bambusinus.* Kumar & Manmohan

Pileus 8 ±6.04 cm diameter at first convex with a depression, infundibuliform at maturity, white with reddish tint at young become dull white to yellowish white, fine squammules on young ones. Margin incurved and smooth when young; later irregularly lobed. Lamellae free, close deeply decurrent,yellowish white, lamellulae present. Stipe 5.57 ±3.24 x 1.24 ±0.98 mm, central, cylindric, solid; surface whitish, become yellow or brown tapering towards the base. Context white. Spore print white 4.78 ±0,71µm. Cheilocystidida 23 ±1.4 µm and gloeocystidia 30 ±11.13µm present.

Cytotoxicity Assay

The percentage toxicity of DLA cells at different concentrations of 4 wild edible mushrooms ranging from10µg/ml to 200µg/ml were shown in table-1. The results showed (Fig-1) maximum inhibitory percentage in 200 µl 99.33±0.53,99.16 ±0.76, 55.5±0.5 and 89.96±0.05 for *Termitomyces microcarpus*, *Phlebopus portentosus, Lentinus bambusinus, Coprinellus micaceus* respectively. The percentage of growth inhibition was increasing with the concentration of test compounds. Even though *Termitomyces microcarpus* and *Phlebopus portentosus* showed almost similar results in higher concentrations the activity of *Phlebopus portentous* was less in lower concentration. Curcumin was used as the reference drug and it produced 100% cytotoxicity at 100 µg/ml , 200 µg/ml and 39.23 ±1.01% for 10µg/ml.

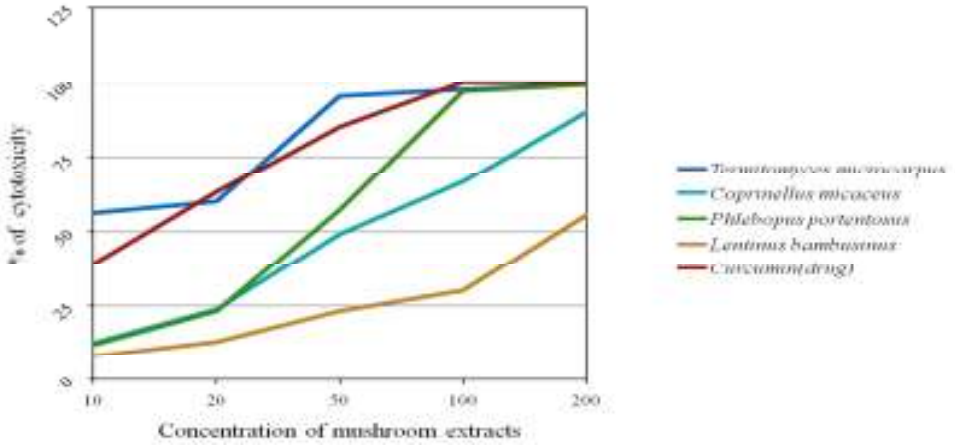

Figure 1: Cytotoxicity of wild edible mushrooms

Table 1: The percentage toxicity of DLA cells at different concentrations of 4 wild edible mushrooms extracts

Drug concentration	% of cell death in DLA cell lines (expressed in standard mean of triplicate)				
µg/ml	*Termitomyces microcarpus*	*Coprinellus micaceus*	*Phlebopus portentosus*	*Lentinus bambusinus*	*Curcumin*
10	56.17±0.17	12.76 ±0.86	12.16 ±0.25	8.23±0.3	39.23 ±0.9
20	58.9 ±0.09	23.96 ±0.55	23.26±0.10	13.03 ±0.15	63.21±0.6
50	95.26±0.64	49.25±0.92	57.19±2.3	23.33±0.17	85.24 ±0.75
100	97.6 ±0.06	66.66±0.5	97.02±0.64	30.06 ±0.4	100.00±0.60
200	99.33 ±0.53	89.96 ±0.05	99.16 ±0.76	55.16 ±0.5	100.00±0.50

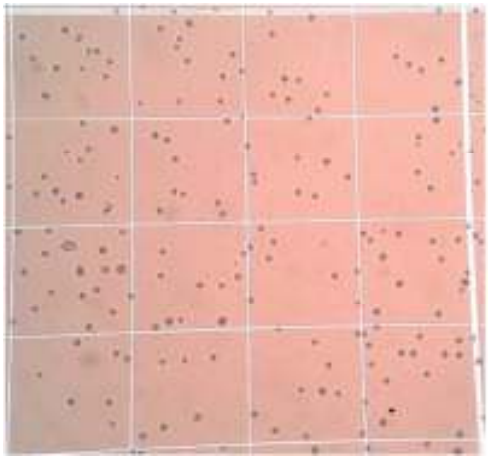

Figure 2: ***Termitomyces microcarpus (100%)***

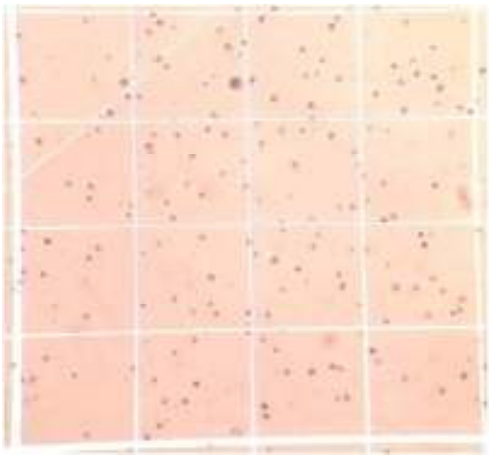

Figure 3: ***Coprinellus micaceous(90%)***

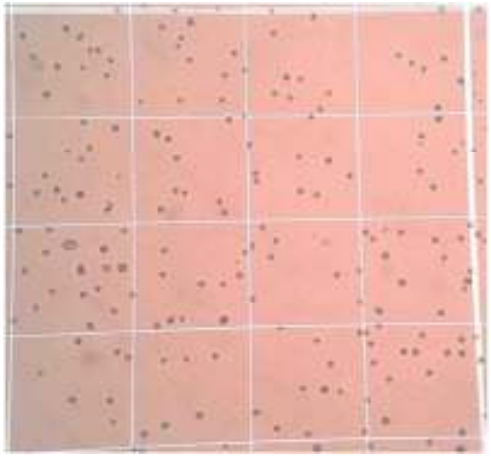

Figure 4: ***Phlebopus portentosus (100%)***

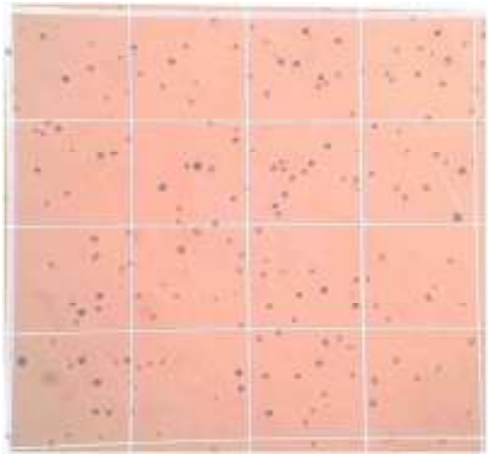

Figure 5: ***Lentinus bambusinus (56%)***

Figure 2 to Figure 5: Haemocytometer vision of cell cytotoxicity of 100µg/ml cell suspensions of wild edible mushroom extracts studiedin DLA cell line by trepan blue method

CONCLUSION

The preliminary cytotoxic screening of wild edible mushroom extracts against DLA cell line showed good results compared to reference drug curcumin and all the four wild edible mushrooms selected for the study were widely consumed by tribal's of Kerala. These results are consistent with earlier studies demonstrating that various basidiomycetes have antitumor activity by direct or indirect mechanisms (Ajith and Janardhanan; 2007). MTT assay to determine effects of the extracts on normal primary cells, on tumor cell lines and on animal's *in vivo* conditions are under progress.

ACKNOWLEDGEMENT

We show our gratitude towards Women Scientist Division(661/2015/KSCSTE) of KSCSTE for funding this project and authorities of 27th Swadeshi science Congress for giving the opportunity to publish this paper. We also acknowledge Tribal Development Department of Kerala and Forestry for giving permission for wild mushroom collection from forest areas near to tribal hamlets. Thanks to Amala Cancer Research Institute for providing facility for this study.

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