Effects of Water Soluble Polysaccharide of *Phellinus Merrillii* on Laboratory Rats (Albino Mice)

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Abstract:

*Phellinus merrillii* is a non edible, very common mushroom found on a jack fruit tree mostly used in western part of Maharashtra. It is used by tribal people to cure sour mouth; excessive salivation etc. Variety of experimental systems is used to screen toxicity including animals. In the present study rats were used for the experiments. The water soluble polysaccharide (WSP) complex has been isolated by chilled alcohol extraction method from *Phellinus merrillii* and purified to study its effect on Albino mice. Different doses (20%, 40%, 60%, 80% & 100%) of solutions mixed with wheat flour and given to each mouse each day for 8 days. And their physical and histological effects were studied. Maximum hair loss and sleeping mode is observed when the dose of the extracted polysachharides is increased on mice. Stool color of the mice also changes with increasing concentration of polysaccharides. Harmful effects of extracted polysaccharides are observed on kidney, liver, spleen and pancreases.

Keywords: polysaccharide, mushroom, physical effects, histological effects

1.0 Introduction:

*Phellinus merrillii*: A Mushroom:

Mushrooms are macrofungi widely consumed as food throughout the world. The major civilization which includes mushrooms in its regular diet is the Chinese and Japanese (Chang S.T. *et al* 1999, Sonawane H. *et al* 2013). Mushroom is a fleshy, spore bearing structure of the fungus produce on soil or underneath. Some mushrooms are used as a food because they are rich in essential nutrients such as proteins, minerals, carbohydrates and vitamins. While few mushrooms like are widely used as medicines.

The anti-oxidants present in dietary mushrooms are of great importance because it helps human body to reduce oxidative damage (Adams & Wermuth 1999). The ethanol extracts of *Phellinus merrillii* showed the strong α glucosidase and aldose reductase activities (Yuan chang *et al* 2011). Now they are used as important foods and as a source of physiologically beneficial components (Wasser & Weis 1999). Mushrooms have various importance’s because they helps to increase heart health, lowers the risk of cancer, increases immune function, helps to reduce inflammation, combat allergies, also helps to balance blood sugar levels & also supports bodies detoxification mechanism ( Ada *et al* 2005 ). Mushrooms accumulate a variety of secondary metabolites such as phenolic compounds, polypeptides, terpens, steroids etc. In Taiwan, several different species of Phellinus used for anticancer, antioxidant & hepatoprotective effect. *Phellinus linteus* shows anti tumor activity in several studies (Lin *et al* 2003; Kim *et al* 2003; Li *et al* 2004; Bae *et al* 2005, Sasaki T. *et al* 1971). *Phellinus merrillii* is a non edible very common mushroom found on a jack fruit tree mostly used in western part of Maharashtra. It is used by tribal people to cure sour mouth, excessive salivation etc, however information about dose range is not available.In this study, Polysachharides was Extracted & then dissolved in distilled water and different doses were given to mice to observe the toxic effect of these polysaccharides with the objectives to study toxicity screening.
1.1 Objectives of the study:
Objectives include study related to the effect of polysaccharide sample on physical effects of rat. It includes movement, weight loss, hair loss, stool color etc. It also includes study in respect to the effect of polysaccharide sample on histology of different organ includes kidney, liver, pancreas, spleen etc.

2.0 Materials and Methods:
1. Sample collection:
The samples were collected from the University of Pune campus. The fresh fruiting body of Phellinus merrillii grown on Gliricidia sepium plant was collected. Identification of mushroom was done using a key of Larsen and Cobb pauli on microscopic and macroscopic characters. These fruiting bodies were dried in the oven at 38-45°C for 24 to 36 hours and then powdered using mixer grinder. The powdered mushroom were boiled in distilled water and kept overnight.

2. Extraction of Polysaccharide:
Mushroom powder was boiled in distilled water and filtered. Drop wise precipitation of filtrate was done with chilled alcohol (Filtrate to alcohol ratio 1:4). The precipitated mixture was then kept in refrigerator for separation of two phases i.e. alcohol and polysaccharide, it was followed by centrifugation to remove alcohol. Polysaccharide pellets were isolated and re-suspended in distilled water and boiled till water gets vaporized and crystalline form of powder obtained.

3. Maintenance of animals:
Healthy swiss albino mice were kept in laboratory cages for a period of 15 days and feed properly prior to beginning the experiment so as to acclimatize them to laboratory conditions.

Experimental Setup: Six mice were randomly kept in 6 different compartments. Extract were reconstituted to obtain approximate stock solution and were given to each mice. Different doses (5%, 10%, 40%, 60%, 80% & 100%) of solutions mixed with wheat flour and given to each mouse each day for 8 days. After that mice were dissected. Kidney, liver, pancreas & spleen were rinsed in water and quickly fixed in Bouin’s solution. Then dissected organs were transfer into different grades alcohol such as 10%, 30%, 50%, 70%, 90% & 100% for one hour. After that paraffin section 10 microns cut on rotary microtome at room temperature were processed by double staining using alcoholic Eosin & Haimotoxylin section were dehydrated in graded alcohol series and mounted in DPX

3.0 Result and Discussion:
1. Physical effects:
Mice (M0 control) – Showed normal motility and normal sleeping mode as comparative to experimental.
Mice – (20 %) M1- Showed less hair loss, normal motility % frequent in sleeping mode.
Mice – (40%) M2- Showed hair loss greater than 20%, motility is frequent; sometimes they were in sleeping mode.
Mice – (60%) M3- As comparative to 40% mice, these mice showed significant hair loss, motility & sleeping mode.
Mice – (80%) M4- Showed great hair loss and was always in sleeping mode.
Mice – (100%) M5- Showed largest hair loss as compare to other experimental mice.

Fig. 1: Phellinus merrillii
Fig. 2: Extraction of Polysaccharides
2. Histological effects:
Polysaccharide consumption affected various properties of kidney, spleen, pancreas & liver, stool color was observed, structure of collecting tubules and nephron has shrunken. Hepatic cords, sinusoids and lobule got damaged with internal lining of liver. Pancreas had less damaged because of polysaccharide consumption and acini structure of pancreas did not showed such effect. Consumption of polysaccharide had affected the structure of spleen.

1.0 Effect of Extracted polysaccharides on kidney
2.0 Effect of Extracted polysaccharides on liver

3.0 Effect of Extracted polysaccharides on spleen
4.0 Conclusion:
From the experiment carried out it is concluded that the water soluble Polysaccharides from *Phellinus merrillii* has various harmful effects on albino mice. Higher doses i.e. doses above 80% showed significant effect. Polysaccharide consumption affects Physical state as well as causes degradation and disintegration of kidney, liver, spleen and pancreases. Similarly it can cause harmful effects on the humans and other organisms if taken in greater quantity. Hence use of *Phellinus merrillii* is avoided.

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References:


