



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 polysaccharides 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 merillii* 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, Polysaccharides 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 Bouni'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 & Haimotoxilin 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 show such effect. Consumption of polysaccharide had affected the structure of spleen.



Fig. 3: Physical effects observed on mice



Fig. 4: Change in stool color



Fig. 5: Normal Kidney

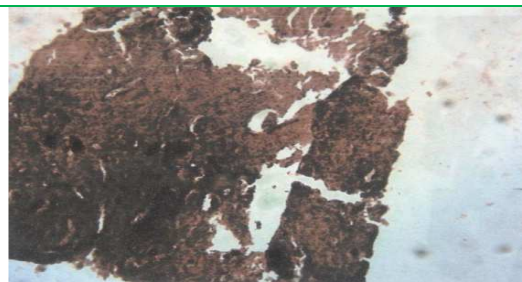


Fig. 6: Kidney with 20% dose of polysaccharides



Fig. 7: Kidney with 40% dose of Polysaccharides

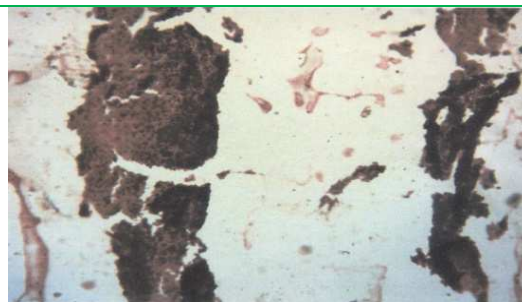


Fig. 8: Kidney with 80% dose of Polysaccharides

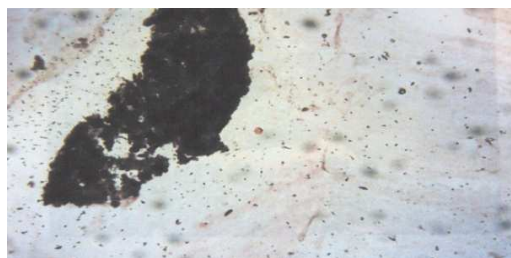


Fig. 9: Kidney with 100% dose of polysaccharides

1.0 Effect of Extracted polysaccharides on kidney



Fig. 10: Normal liver



Fig. 11: Liver with 40% dose of polysaccharides



Fig. 12: Liver with 60 % dose of Polysaccharides

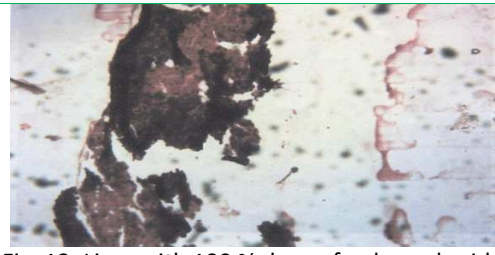


Fig. 13: Liver with 100 % dose of polysaccharide

2.0 Effect of Extracted polysaccharides on liver

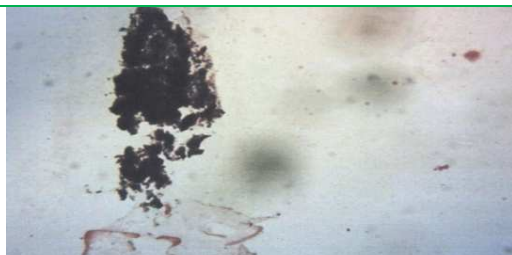


Fig. 14: Spleen with 20% dose of polysaccharides

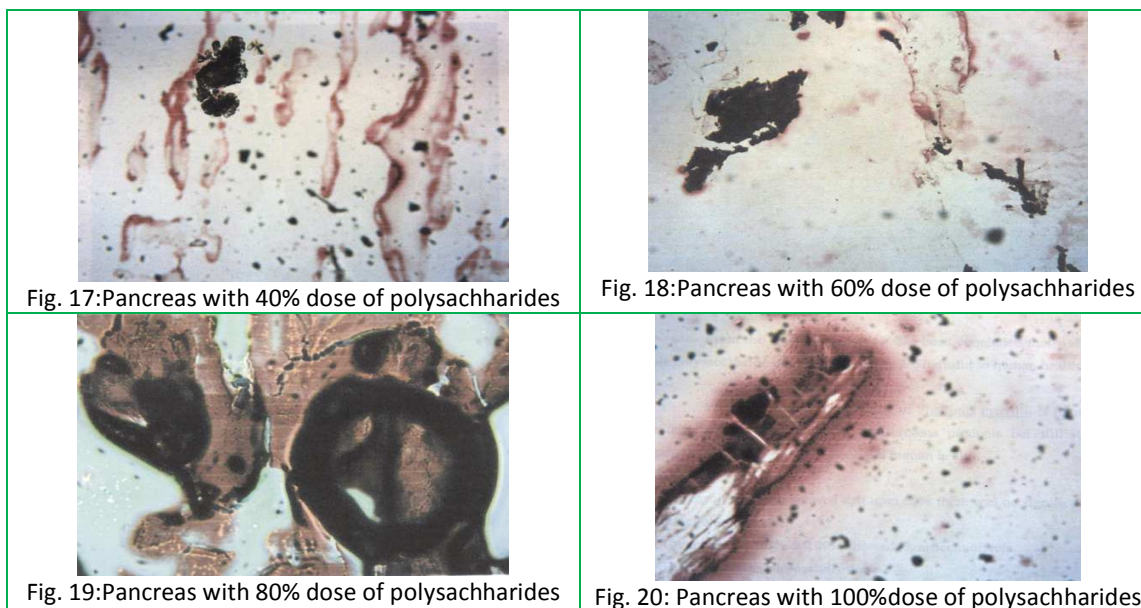


Fig. 15. Spleen with 40% dose of polysaccharides



Fig. 15: Spleen with 60 % dose of polysaccharides

3.0 Effect of Extracted polysaccharides on spleen



4.0 Effect of Extracted polysaccharides on Pancrease

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:

- 1) Ada M.A., H.K. Wong & B Jiang(2005): Fibrinolytic enzymes in Asian Traditional fermented foods. *Food Res. Int.* 38:243-250.
- 2) Adams A.K. & E.O. Wermuth(1999): Antioxidant vitamins and preservation of coronary heart diseases. *Am.Fam. Physician* 60:895-905.
- 3) Bae J.S., K.H.Jang, H.Yim & H.K.Jin(2005): Polysaccharides isolated from *Phellinus gilvus* inhibit melanoma growth in mice. *Cancer Lett.* 218:43-52
- 4) Bhone R.R. Lambrood P.Y., Vaidya J.G. (2001): Anticarcinogenic activity of two samples of Phansomba – *Phellinus merrillii* (Murr.) Ryv. and *P. fastuosus* (Lev.) Ryv. on siHa lines. *International journal of Medicinal Mushrooms* 3,119.
- 5) Heng – Yuan Chang, Yu – Ling Ho, Ming – Jyh sheu, Yaw-Huei Lin, Mu – Chuan Tseng, Sheng – Hua Wu, Guan – Jhong Huang and Yuan –Shiun Chang (2007): Antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts. *Botanical studies.* 48: 407 –417.
- 6) Heng Yuan Chang et al (2007): Analgesic & Anti-inflammatory activities of *Phellinus merrillii* 12:76-82.
- 7) Kim G.Y., Y.H.Oh & Y.M. Park(2003): Acidic polysaccharides isolated from *Phellinus linteus* induce nitric oxide mediated tumoricidal activity of macrophages through protein tyrosine kinase & protein kinase C. *Biochem. Biophys. Res. Commun.* 309:399-407.
- 8) Li G, D.H.Kim, T.D. Kim, B.J. Park, H.D.Park, J.I. Park, M.K.Na, H.C. Kim, N.D. Hong, K.Lim, B.D.Hwang & W.H. Yoon(2004): Protein bound polysaccharide from *Phellinus linteus* induces G2/M phase arrest & apoptosis in SW480 human colon cancer cells. *Cancer Lett.* 216:175-181.
- 9) Lin S.B., C.H. Li., S.S. Lee & L.S. Kan(2003): Triterpene enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells

- via supporting protein kinase C, activating mitogen activated protein kinases & G-2 phase cell cycle arrest. *Life sci.* 72:2381-2390.
- 10) Wasser S.P. & A.L. Weis (1999): Medicinal properties of substance occurring in higher basidiomycetes mushroom. *Int.J.Med. Mushrooms* 1:31-62.
 - 11) Yuan chang, Shyh-Shyun-Huang, Ying – chih Lin and Yueh – Hsiung kuo (2011): α Glucosidase & Aldose reductase I activity from the fruting body of *Phellinus merillii*. *Journal of Agricultural & Food chem.* 59(10), 5702-5706.
 - 12) Hiralal Sonawane, Shekhar Bhosle, Gauri Bapat, Sandhya Garad, Vikram Ghole (2013): Bioactive metabolites & Compound from medicinal mushrooms. *International Journal of Scientific & Engineering research* 4(6) 304-315.
 - 13) Chang S.T. and Buswell J.A. (1999): *Ganoderma lucidum* P. Karst (Aphyllophoromycetideae)- A mushrooming medicinal mushrooms. *International Journal of Medicinal Mushrooms* 1:139-146.