

Screening of gastric antiulcer potential of chitosan extracted from white button mushroom wastes in wistar rats

Akila. RM^{*1} and Priya. N²

¹*Department of Pharmaceutical Biotechnology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore-641 044, Tamilnadu, India.*

²*Nehru College of Pharmacy, Thrissur, Kerala, India.*

ABSTRACT

*Besides their traditional food usage, mushrooms can be used as a source of many pharmacologically active compounds, especially polysaccharides. Wastes left over in the edible mushroom farms such as *Agaricus bisporus* (white button mushroom) can also become free and alternative source of chitin-chitosan materials, beside the traditional industrial source shellfish waste materials. Chitosan, a natural polysaccharide is an attractive material for multiple industrial applications most of them in nutritional, pharmacological research, biomedical and cosmetic fields. In the present work, chitosan was isolated from *Agaricus bisporus*(white button mushroom) wastes, characterized and screened for its antiulcer potential in aspirin induced and pylorus ligation ulcer models in wistar rats. The isolated chitosan at a dose of 200mg/kg showed significant ($P<0.01$) reduction in gastric volume, total acidity, pH and ulcer index when compared with control.*

Keywords: *Agaricus bisporus*, Chitosan, Pylorus ligation, Aspirin induced ulcer model, Ulcer index.

INTRODUCTION

Agaricus bisporus is the most consumed mushroom. Waste accumulated during mushroom production and harvest consist mainly stalks and mushroom of irregular dimensions and shapes. Waste disposal create environmental problems for producers due to the large volume and volatile degradation products. However, *Agaricus bisporus* is rich in chitinous polysaccharides, Chitin and Chitosan [1]. These polysaccharides have got more applications in pharmaceutical, medical, agriculture, textile, food and paper industries [2, 3, 4, 5]. Chitosan is widely used as a dressing to promote rapid healing of external cuts or burns [6, 7, 8, 9]. Since similar agents used for healing skin ulcers have also been reported to be effective in preventing gastric ulcers, chitosan was also expected to exhibit some protection [10, 11].

Current industrial production of chitosan from chitin of crustacean shells [12] is not a ecofriendly process as the CaCO_3 enriched crustacean shells releases CO_2 and pollutes the environment. Moreover this method has long extraction procedures, seasonal supply of the crustacean shells and product variability [13, 14, 15]. The above disadvantages prompted the research into alternative source such as fungal mycelia where chitin and chitosan are present as cell wall components. Fungal chitin and chitosan potentially differ from those isolated from crustaceans and have more functional properties and so enhanced bioactivity. However research is needed to evaluate the most economical way of obtaining chitinous polysaccharides from fungal sources. So, mushroom wastes (macrofungi) were selected as the fungal source for the present work because they can be obtained all the year round[16] and its antiulcer potential was evaluated.

MATERIALS AND METHODS

Mushroom wastes and storage condition

White button mushroom, *Agaricus bisporus* of irregular shape and dimensions considered as wastes were donated from Neelamalai mushroom farm, Ooty, Tamilnadu, India. Whole fruit bodies were harvested in the closed cap stage with a cap diameter 30±5 mm and transported to the laboratory within one hour after harvest and stalks were separated from the caps, packed in paper bags, air dried, ground to a powder and stored in a dessicator at room temperature for further analyses and extraction.

Chitosan isolation

About 5g of air dried stalk powder was suspended with 1MNaOH solution (1:30w/v) and autoclaved at 121°C for 15min. The alkali insoluble matter was collected by filtration, washed with distilled water, 95% ethanol, acetone and dried at 60°C. The residue was further extracted with 10% acetic acid solution at 60°C for 6h. The separation of acid soluble chitosan from acid insoluble chitin was done by filtration by no.1 Whatman filter paper. The chitosan was precipitated from the extract by adjusting the pH to 10 with 4MNaOH. The chitosan was finally washed with distilled water, 95% ethanol, acetone and dried at 60°C [17, 18, 19].

Chitosan characterization

The IR spectra of the KBr discs containing chitosan and a commercial chitosan (Cochin Fisheries, Cochin, Kerala, India) were measured from 4000cm⁻¹ to 400cm⁻¹ with a JASCO FT-IR spectrophotometer.

Experimental Animals

Female swiss albino mice (40-80g) were used for acute toxicity study and Female wistar rats (150-170g) were used for studying antiulcer activity. Pregnant animals were excluded. The animals were maintained under standard laboratory conditions in animal house approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) under 12h light/dark cycle and controlled temperature(24±2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the lab environment for atleast one week before the commencement of the experiment. The experiment protocol was approved by the Institutional Ethical Committee.

Drugs and Chemicals

Aspirin was obtained from German Remedies Ltd., Mumbai, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Acute toxicity study

A safe oral dose of the extract was determined by acute oral toxic class method of organization of Economic Co-operation and Development (OECD) as per 425 guidelines. Swiss albino mice were kept overnight fasting and food was withheld for 3-4 h prior to isolated chitosan administration. A total of five animals were used which received a single oral dose (2000mg/kg b.w.) of isolated chitosan. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 24h) and daily thereafter for a period of 14 days [20].

Antiulcer activity –Aspirin induced and Pylorus ligation ulcer model

The anti ulcer activity was evaluated in aspirin induced and pylorus ligated rats. The animals were divided into four groups of six animals each. They were fasted for 24 h prior to the experiment and care was taken to avoid caprophagy. The animals were treated according to the experimental design given in Table 1. All the rats were treated with aspirin (200mg /kg) after 30 minutes of isolated chitosan treatment. The animals were subjected to fasting for 18 hours after three days of drug treatment. Pyloric ligation was made after fasting. The rats were sacrificed four hours later by cervical dislocation and the esophagi were clamped, the stomach was exposed carefully, opened along the greater curvature, the luminal contents were removed, [21] the total volume of gastric secretion and total acidity were estimated by titration method [22]. The ulcer index was calculated[23]. The lesions were counted with the aid of hand lens (10x) and each gives a severity rating as follows.

Ulcer score	Descriptive observation
0	Normal coloured stomach
0.5	Red colouration
1	Spot ulcers
1.5	Haemorrhagic streak
2.0	Ulcers
3.0	Perforation

Mean ulcer score for each animal was expressed as ulcer index. Ulcer index (UI) was then calculated from the above scoring as follows:-

$$UI = UN + US + UP \times 10^{-1}$$

where UN is the average of number of ulcers per animal, Us is the mean severity of ulcer score and Up is the percentage of animals with ulcer incidence. The percentage of ulcer protection was determined as follows:

$$\% \text{ Ulcer Protection} = \frac{\text{Control mean ulcer index} - \text{test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

The values were expressed as MEAN \pm SEM and found out by using one way ANOVA followed by Newman kevel's multiple range test. The probability value $p < 0.01$ was considered significant.

Histopathological evaluation

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/or anti-ulcerogenic activity of isolated mushroom chitosan. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5- μ m thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes [24].

RESULT AND DISCUSSION

As a first step in the alternate and most economical production of commercial crustacean chitosan, chitosan was isolated from *Agaricus bisporus* mushroom wastes. The isolated mushroom chitosan gave similar IR spectrum to that of commercial crustacean chitosan as shown in Figure 1 and Figure 2. The mushroom chitosan was then screened for gastric antiulcer effect. The acute toxicity was carried out and no mortality was found even at 2000 mg/kg. So, 200mg/kg, p.o. was selected as the dose for pharmacological activity studies. The histopathologic studies showed that administration of 200mg/kg Aspirin suspension consistently caused hemorrhagic lesions in the mucosa of the glandular stomach, indicating true ulcer formation as stated in the histological findings (Figure 3 - Figure 6). In aspirin induced and pyloric ligation ulcer model, oral administration of mushroom chitosan at a dose of 200mg/kg showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. It was showing protection index of 65% and prevented gastric ulcerogenesis significantly $P < 0.01$ in comparison to control whereas Omeprazole as reference standard drug showed 78% (Results are tabulated in Table 1). The antiulcer effect of isolated chitosan may be due to its neutralization effect on H^+ ions and pepsin in the gastric juices and exert its protective effect by coating the ulcerated area [10].

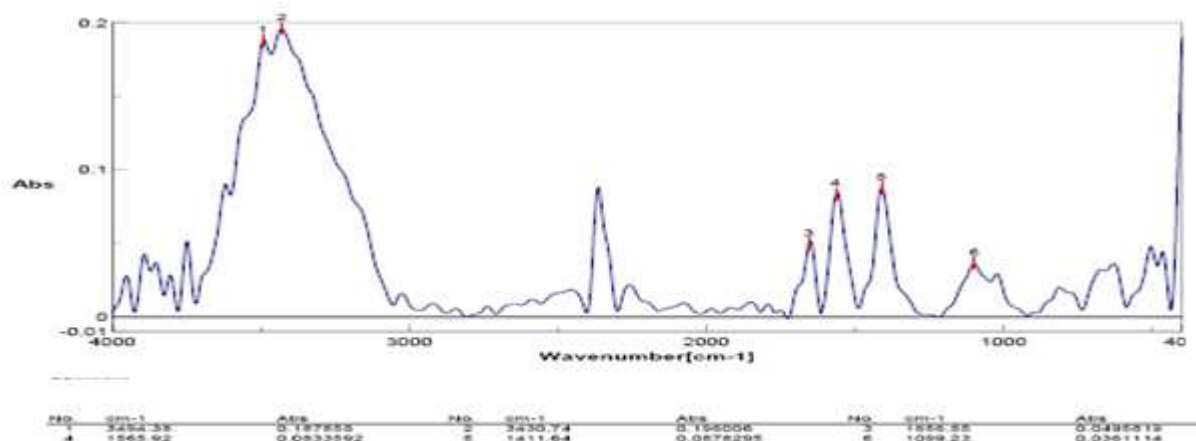


Figure 1: IR Spectrum of Commercial Chitosan

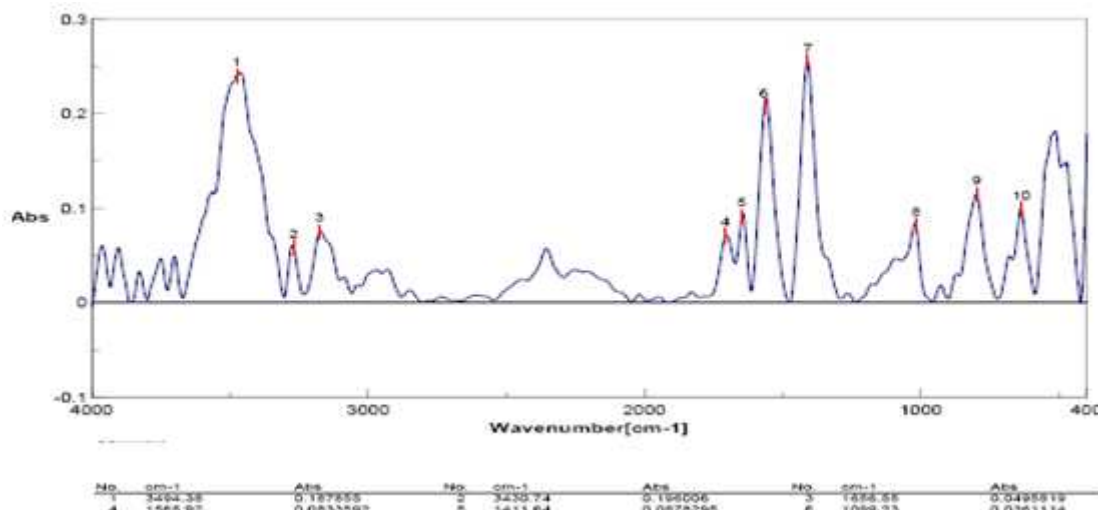


Figure 2: IR Spectrum of mushroom chitosan from mushroom *Agaricus bisporus* wastes

Table 1: Antilucer activity of isolated chitosan from mushroom *Agaricus bisporus* wastes

Group	Treatment	Dose mg/kg	Total volume of gastric secretion (ml/100gm)	Total acidity (meq/L/100g)	pH	Ulcer score	% Protection
I	Normal Control	1ml of 1%CMC	4.2± 0.10	445.26±22.47	1.8±0.20	0.3±0.01	0.000
II	Ulcer Control	200mg/kg ASA	5.4± 0.20 ^a	496.60±0.18 ^a	1.2±0.18 ^a	2.3±0.06 ^a	0.000
III	Standard Control	2mg/kg Omeprazole	2.1 ± 0.10	336.92±1.10 ^b	4.4±0.23 ^b	0.5±0.08 ^b	78.26
IV	Treatment Control	200mg/kg of isolated chitosan	2.1± 0.25 ^b	390.9±35.10 ^b	3.3±0.11 ^b	0.8±0.15 ^b	65.21

Values are expressed as Mean±SEM
 (ANOVA followed by Newman kevel's multiple range test)
 a - P<0.01 when compared to Normal control group
 b - P<0.01 when compared to ulcer control group

Histopathological evaluation:

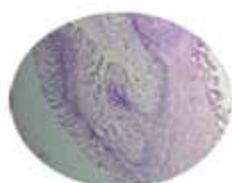


Figure 3: Normal control

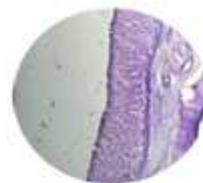


Figure 4: Ulcer control



Figure 5: Positive control



Figure 6: Treatment control

CONCLUSION

Based on the results, it may be concluded that chitosan isolated from *Agaricus bisporus* mushroom waste was more economical and provided preliminary data that mushroom chitosan showed significant gastric antilucer activity in animal models.

Acknowledgement

The authors wish to thank Dr.S.Krishnan, Head, Department of Pharmaceutical Biotechnology, College of Pharmacy, SRIPMS, Sri Ramakrishna Hospital Campus, Coimbatore for providing necessary facilities to carry out this work.

REFERENCES

- [1]W.Tao, Z.F.Svetlana, A.Draughon, E.S.Carl, **2004**, *J. Agric. Food Chem*, 52, 7905–7910.
- [2]K.Kurita, **1998**, *Polym.Degrad.Stability*, 59, 117-120.
- [3]N.V.R.M.Kumar, **2000**, *React.Funct.Polymer*, 46, 1-27.
- [4]Shweta Kalyan, Pramod Kumar Sharma, Vipin Kumar Garg, Nitin Kumar, Jonish Varshney, **2010**, *Der Pharmacia Sinica*, 1, 195-210.
- [5]Prabodh Chander Sharma, Sandeep Jain, Geeta Yadav, Archana Sharma, **2010**, *Der Pharmacia Sinica*, 1, 95-108.
- [6]R.A.Muzzarelli, M.Mattioli-Belmonte, A.Pugnali, G.Biagini, **1999**, *EXS*, 187, 251–264.
- [7]A.K.Azad, N.Sermsintham, S.Chandrkrachang, W.F.Stevens, **2004**, *J Biomed Mater Res B Appl Biomater*, 69, 216–222.
- [8]H.Ueno, H.Yamada, I.Tanaka, N.Kaba, M.Matsuura, M.Okumura, T.Kadosawa, T.Fujinaga, **1999**, *Biomater*, 20, 1407–1414.
- [9]S.Sheik Mansoor, A.M.Hussain, K.Aswin, K.Logaiya, P.N.Sudhan, **2012**, *Der Chemica Sinica*, 3, 683-688.
- [10]S.R.Rosalee, T.M.Michael, In: J.B.Colin, Fereidoon Shahidi(Eds.), (Boca Raton, FL:CRC Press, **2007**), 162-163.
- [11]R.Anandan, P.G.Nair, S.Mathew, **2004**, *J.Pharm Pharmacol*, 56, 265- 269.
- [12]K.Prabhu, E.Natarajan, **2012**, *Advances in Applied Science Research*, 3, 1870-1875.
- [13]N.A.Ashford, D. Hattis, A.E. Murray **1977**, Industrial Prospects for chitin and protein from shellfish wastes. MIT Sea Grant Program. Report no. MITSG 77-3, Index no.77-703-Zle. Massachusetts Institute of Technology, Cambridge.
- [14]C.Crestini, B.Kovac, G.Giovanazzi-Sermanni, **1996**, *Biotechnol. Bioeng*, 50, 207–210.
- [15]N.Nwe, W.F.Stevens, In: H. Tamura (Ed.), (Research Signpost, Trivandrum, Kerala, India, **2008**) 161–176.
- [16]N.Nitar, F.Tetsuya, T.Hiroshi, In: S.K.Kim (Ed.), (CRC Press, New York, **2011**) 6-8.
- [17]P.Pochanavanich, W.Suntornstusuk, **2002**, *Lett.Appl.Microbiol*, 35, 17-21.
- [18]M.Yen, J.Mau, **2007b**, *LWT—Food Science and Technology*, 40, 558–563.
- [19]F.D.Mario, P.Rapaná, U.Tomati, E.Galli, 2008, *International Journal of Biological Macromolecules*, 43, 8–12.
- [20]OECD. 2000, Acute and toxicity-acute and toxic class method guideline 423, adopted 23.03.1996. In: Eleventh addendum to the OECD guidelines for the testing of chemicals. Organization for economic co-operation and development, Paris.
- [21]Madhu.C. Divakar, S.Lakshmi Devi, **2011**, *Der Pharmacia Sinica*, 2, 355-360.
- [22]K.Srinivas, R.V.Celestin Baboo, **2011**, *International Journal of Current Pharmaceutical Research*, 3, 1-2.
- [23]A.K.Gangly, O.P.Bhatnagar, **1973**, *Canad. J. Physiol. Pharmacol*, 51,748-750.
- [24]C.F.Culling, (3rd Ed.) Handbook of histopathological and histochemical techniques, (Butterworth and Co, London, **1974**) 37-38.