



Journal of Biological Sciences

ISSN 1727-3048

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

The Medical Effects of Edible Mushroom Extract on Aflatoxin B₁

¹Q.A. Nogaim, ²H.A.S. Amra and ²S.A. Nada

¹IBB University, Yemen

²National Research Center, Cairo, Egypt

Abstract: Mushroom considered as an important food it has healthy and economic benefits. This study was aimed at investigating the medical effects of edible mushroom extracts on aflatoxin B₁ for the kidney, liver and SOD enzymes. Thirty-six male Sprague-Dawley rats weighing 120-150 g were divided into six groups (6 rats/group) and housed in stainless steel cages housed in a temperature-controlled (23±1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. Fine powder of mushroom was suspended in gum acacia (2.5% v/v in distilled water) into two dose levels (100 mg and 200 mg/1 mL/kg body weight). The 1st group kept as a control group; the 2nd and 3rd groups were given 100 mg and 200 mg kg⁻¹, mushroom, respectively, the 4th group treated orally with aflatoxin B₁ 2.5 mg kg⁻¹, in corn oil, the 5th and 6th groups administered 100 mg and 200 mg kg⁻¹ mushroom, respectively in concomitant with AFB₁. The experimental period was 15 days of daily treatment after which blood samples were collected from the retro-orbital vein of each animal, under light anesthesia by diethyl ether. Serum obtained from each animal was used to estimate the activities of Aspartate amino-transferase, Alanine amino-transferase, Superoxide dismutase, Creatinine and Uric acid. The results indicated that a low dosage of mushroom 100 mg with aflatoxin B₁ was the best treatment to decrease the toxicity in animals significantly (p<0.5) and these clearly demonstrated that, mushroom was a good functional food because it has many medicinal compounds.

Key words: Medical benefits, mushroom extract, aflatoxicoses

INTRODUCTION

Fungi are well known for their medicinal properties. From ancient times oriental traditional medicine has stated that medicine and food have the same origin. Amongst the many species used in medicine, the most common are *Claviceps purpurea*, *Cordyceps sinensis*, *Ganoderma lucidum*, *Laricifomes officinalis*, *Lentinula edodes* and *Trametes versicolor* (Molitoris, 2002). Many edible mushroom including *F. velutipes* and *L. edodes* are used in Japan and China to develop not only food materials but also medicines. Three kinds of carcinostatic polysaccharide drugs such as immuno-potentiators (BRMs, biological response modifiers) have been developed in Japan. Beta-D-glucose, hetero polysaccharides, glycoprotein, steroids, terpenoides and nucleotides were isolated from fruiting bodies or mycelium of many *Mushroom* spp.

Beta-glucan is a polysaccharide (i.e., many sugars, a chain of glucose molecules) that is found in such foods as oats, barley, mushrooms and yeasts. It is extremely difficult to extract and purify. This nutrients benefits anyone who wants to be healthier, live longer, deal with the stress of modern society, be less allergenic, speed up healing and resist the dangerous microbes, bacteria and

viruses that seem to be everywhere. As you saw in the contents, the major reason to take beta glucan is to enhance your immune system. It has been found to help regulate blood sugar levels especially in cases of diabetes. There are various other benefits such as protection from ionizing radiation. Sarangi *et al.* (2006) purified proteoglycan fractions from *P. ostreatus* mycelia that could be used as immuno-modulators and anticancer agents. The polysaccharides represent the major constituent that determines the rigidity and morphological properties of the fungal cell wall and depending on the culture conditions they can also be excreted to the culture medium. Among the polysaccharides produced by *Pleurotus* spp., (β-1,3 glucans) play an important role as Biological Response Modifiers (BRMs), which stimulate the immune system of the host and exert an extensive range of immuno-pharmacological activities, in particular an antitumor effect and the inhibition of metastasis, as well as the stimulation of hematopoiesis (Wang *et al.*, 2005).

Coletto (2005) reported that, the antimicrobial properties of the mycelia and culture filtrates of 20 *Basidiomycete* strains were evaluated against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Agrobacterium tumefaciens* and *Candida albicans*. The

in vitro antagonistic interaction between 15 strains of Basidiomycetes and 3 strains of pathogenic fungi (*Mucor racemosus*, *Botrytis cinerea* and *Penicillium digitatum*) was also investigated. *Lopharia spadicea*, *Phlebiopsis gigantea*, *Agaricus macrosporus* and *Hebeloma subsaponaceum* showed significant antibacterial activities which are thought to be recorded for the first time. *Omphalotus olearius* exhibited significant activity against the pathogenic fungi but *Mutinus caninus* and *M. elegans* were the most effective, almost completely inhibiting the development of the 3 fungi. The intake of fungi has also been shown to be effective in cancer prevention. Ikekawa (2001) has reviewed studies of the anti-tumour activity of fungi undertaken at the National Cancer Research Institute of Japan since 1966. Several anti-tumour polysaccharides have been isolated, the most important of which is lentinan, a β -1,3-glucan isolated from *Lentinula edodes* (shiitake) which is used clinically in Japan. Lentinan may enhance various immune system functions, such as increasing production of T-helper cells and macrophages (Sadler, 2003). Antioxidant activity of Wild Edible Fungi (WEF) has also been recorded. In a study by Lakshmi *et al.* (2004) the four fungi examined, *Phellinus rimosus*, *Pleurotus florida*, *Pleurotus sajor-caju* and *Ganoderma lucidum*, were reported to show antioxidant activity. Cellular damage caused by reactive oxygen species has been implicated in several diseases and hence the antioxidant properties of fungi may play an important role in human health.

MATERIALS AND METHODS

Experimental animals: Ten weeks-old sexually male Sprague-Dawley rats weighing 120-150 g (purchased from Animal House Colony, Giza, Egypt) were maintained on standard lab diet (Protein : 16.04% ; Fat: 3.63% ; Fiber: 4.10% and metabolic energy : 0.012 MJ) and water *ad libitum* at the Animal House Lab., NRC. After an acclimation period of 1 week, animals were divided into sex groups (6 rats/group) and housed in stainless steel cages housed in a temperature-controlled ($23\pm 1^\circ\text{C}$) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination.

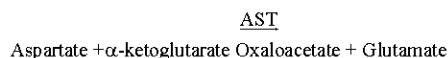
All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of NRC, Cairo, Egypt. Fine powder of mushroom was suspended in gum acacia (2.5 % v/v in distilled water) into two dose levels (100 and 200 mg/1 mL kg^{-1} body weight) according to (Nada *et al.*, 2008). Rats were randomly divided to sex equal groups (6 rats each). The 1st group kept as a control; the 2nd and 3rd groups were given

100 mg and 200 mg kg^{-1} , PO mushroom, respectively the 4th group treated orally with aflatoxin B1 2.5 mg kg^{-1} , in corn oil Abdel-Wahhab *et al.* (1999), the 5th and 6th groups administered 100 mg and 200 mg kg^{-1} mushroom, respectively in concomitant with AFB1. The experimental period was 15 days of daily treatment.

Collection of blood samples: At the end of the experimental period, blood samples were collected from the retro-orbital vein from each animal, under light anesthesia by diethyl ether. Blood was allowed to coagulate and then centrifuged at 1500 rpm for 15 min. The serum was used to estimate the activities of AST, ALT, SOD, Creatinine and Uric acid.

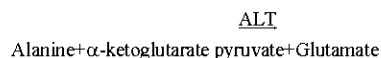
Determination of aspartate Amino-transferase (AST) activity: Colorimetric method determination of serum AST activity was carried out using a test reagent kit according to the method of Reitman and Frankel (1957).

Principle: The method depends on the reaction between aspartate and α -ketoglutarate in the presence of AST to form oxaloacetate, which in turn reacts with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone, which can be estimated colorimetrically at 505 nm. The reaction is illustrated as follows:



Determination of alanine Amino-transferase (ALT) activity: Colorimetric determination of serum ALT activity was carried out using a test reagent kit according to the method of Reitman and Frankel (1957).

Principle: The method depends on the reaction between alanine and α -ketoglutarate in the presence of ALT to form pyruvate and glutamate. The pyruvate formed was measured in its derivative form as 2,4-dinitrophenylhydrazone colorimetrically at 505 nm. The reaction is illustrated as follows:



Determination of Superoxide Dismutase (SOD): Colorimetric method, was done where the activity of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress, according to that described by Nishikimi *et al.* (1972).

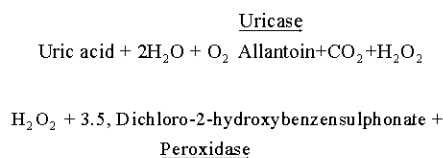
Principle: It depends on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.

Determination of creatinine: Creatinine was determined by colorimetrically method according to Bartels *et al.* (1972).

Principle: Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the Creatinine concentration.

Determination of uric acid: Uric acid was determined by using Enzymatically colorimetric method according to Barham and Trinder (1972).

Principle: The reactions are illustrated as follows:



4-Amino antipyrine $\text{H}_2\text{O} + \text{HCL} + \text{Colored quinoneimine}$.

Statistical analysis: Obtained data were expressed as Mean \pm Standard error of the mean. Data were analyzed by one-way Analysis of Variance (ANOVA), statistical analysis were performed with SPSS, version 17.0 for windows (SPSS Inc., Chicago, IL, USA, 2008). The significance of the differences among treatment group was determined by Waller-Duncan K-ratio, Waller and Duncan, (1969). All statements of significance were based on probability $p = 0.05$.

RESULTS AND DISCUSSION

Mushroom have been used as food and food-flavoring materials in soups and sauces for centuries due to their unique and subtle flavor. Further, mushroom have recently become attractive as functional food. It was considered a source of biologically and physiologically active substances. Mushroom, was reported to possess anti-mutagenic, anti-tumor and immuno-modulating activities (Kawagishi *et al.*, 1988; Shon and Kyung-Soo, 2001). It is used for the prevention of cancer and / or as an adjuvant with cancer chemotherapy drugs after the removal of a malignant tumor, Ishihara (1999). Moreover, these mushroom are used to combat physical and emotional stress, improving life quality of diabetics, osteoporosis and gastric ulcer and they act as excellent antioxidant (Tsai *et al.*, 2006). Nevertheless, scientific studies about their biological properties are still insufficient (Guterrez *et al.*, 2004).

Recent advances in chemical technology have allowed the isolation and purification of some compounds in mushrooms, especially polysaccharides which possess strong immuno-modulation and anti-cancer activities. They are used as biological response modifiers. The polysaccharides isolated from mushroom fruiting bodies are either water soluble or/and insoluble glucans and hetero-polysaccharides with different main and side-chains. There is a great interest on these molecules because they can act as biological response modifiers (Wasser, 2002).

1-Liver function: Data in Table 1, show the effect of mushroom and aflatoxin B1 on the ALT and AST enzymes. Results indicated that either mushroom or aflatoxin had positive effect ($p = 0.05$) on ALT and AST in treated groups. As compared with control group mushroom increased ($P = 0.05$) ALT and AST enzymes by (32.85-67.17) and (16-40.22%), respectively. However, aflatoxin B1 increased them by (314.67 and 99.56%), respectively. Increasing the level of mushroom from 100 to 200 mg kg^{-1} body weight resulted in a significant ($p = 0.05$) increase in ALT and AST enzymes as compared with control group. The combination between aflatoxin B1 and mushroom resulted in a significant ($p = 0.05$) reduction in ALT (4.40-33.27) and AST (6.34-30.06%) enzymes compared to aflatoxin B1 group. Mushroom at 100 mg kg^{-1} level was more effective ($p = 0.05$) in reducing ALT and AST enzymes compared to mushroom at 200 mg kg^{-1} level. The enzyme activity of serum AST and ALT are shown in (Table 1) sensitive in detecting early hepatocytes cell injury.

2-Super Oxide Dismutase enzyme (SOD): This enzyme plays an important role in protection of tissues against oxidative stress by the reaction with superoxide anion (O_2^-) and convert it to hydrogen peroxide (H_2O_2).

Data in Table 2 show that the level of SOD was significantly increased in the group (3) which treated with 200 mg kg^{-1} mushroom, when compared with group (2) which treated with 100 mg kg^{-1} mushroom. Meanwhile, the lower dose of mushroom (100 mg kg^{-1}) had no effect on SOD level and it non-significantly changed than normal control value. On the other side, in aflatoxicosis groups, SOD showed severe depletion in animal treated with AFB1 more than any treated groups. Surprisingly, SOD activity was increased significantly in the groups of rats treated with AFB1 and the lower dose (100 mg kg^{-1} mushroom) than that higher dose (200 mg kg^{-1} mushroom); the recorded % of increase were 704.78 and 582.04% in AFB1+100 mg mushroom and AFB1+200 mg mushroom, respectively, relative to AFB1 values. These figures did not reach to the normal groups value.

Table 1: Effect of mushroom (100 and 200 mg kg⁻¹, OS) and/or aflatoxin B1 on the activities of serum ALT and AST enzymes in rats

Parameters	Groups with aflatoxinB1				Normal groups	
	Control	Mush-1 ⁴	Mush-2 ⁵	AFB1 ⁶	AFB1+Mush-1	AFB1+Mush-2
ALT ¹	A3	B	C	D	E	D
IU mL ⁻¹	22.83±1.08	30.33±0.99	38.17±1.45	94.67±1.98	63.17±1.74	90.50±3.48
% of effect	0	32.85	67.17	0	-33.27	-4.4
AST ²	A	B	C	D	C	E
IU mL ⁻¹	75.00±2.08	87.00±2.93	105.17±2.24	149.67±5.43	104.67±1.89	140.17±3.22
% of effect	0	16	40.22	0	-30.06	-6.34

ALT¹: Alanine aminotransferase enzyme.-AST²: Aspartate aminotransferase enzyme. ³Means in the same row with different letters are significantly different at (p≤0.05). ⁴Mush-1: 100 mg mushroom kg⁻¹ body weight. ⁵Mush-2: 200 mg mushroom kg⁻¹ body weight. ⁶AFB1 was added at a rate of 2.5 mg kg⁻¹ body weight. IU mL⁻¹: International Unit of Enzyme/milliliter, Means±SE, n = 6

Table 2: Effect of mushroom (100 and 200 mg kg⁻¹, OS) and/or aflatoxin B1 on the activity of serum SOD enzyme in rats

Parameters	Groups with aflatoxinB1			Normal groups		
	Control	Mush-1 ²	Mush-2 ³	AFB1 ⁴	AFB1+Mush-1	AFB1+Mush-2
SOD	A1	A	B	C	D	E
IU mL ⁻¹	226.50±1.89	226.71±1.77	251.00±4.62	22.17±1.96	178.42±4.62	151.21±2.43
% of effect	0	0.09	10.81	0	704.78	582.04

¹Means in the same row with different letters are significantly different at (p≤0.05). ²Mush-1: 100 mg mushroom /kg body weight. ³Mush-2: 200 mg mushroom kg⁻¹ body weight. ⁴AFB1 was added at a rate of 2.5 mg kg⁻¹ body weight-IU mL⁻¹: International Unit of Enzyme/milliliter, Means±SE, n = 6

Table 3: Effect of mushroom (100 and 200 mg kg⁻¹, OS) and/or aflatoxin B1 on Creatinine and Uric acid in rat serum

Parameters	Groups with aflatoxinB1			Normal groups		
	Control	Mush-1 ²	Mush-2 ³	AFB1 ⁴	AFB1+Mush-1	AFB1+Mush-2
Creatinine	A1	B	C	D	C	D
mg dL ⁻¹	0.27±0.01	0.42±0.02	0.52±0.01	0.76±0.01	0.55±0.02	0.73±0.01
% of effect	0	55.55	92.59	0	-27.63	-3.94
Uric acid	A	B	C	D	E	
mg dL ⁻¹	0.67±0.019	0.89±0.022	1.22±0.015	1.44±0.017	1.01±0.015	1.38±0.031
% of effect	0	32.83	82.08	0	-29.86	-4.16 ¹

Means in the same row with different letters are significantly different at (p≤0.05). ²Mush-1: 100 mg mushroom kg⁻¹ body weight. ³Mush-2: 200 mg mushroom kg⁻¹ body weight. ⁴AFB1 was added at a rate of 2.5 mg kg⁻¹ body weight. mg dL⁻¹: Milligram/deciliter, Means±SE, n = 6

3-Kidney function: Data in Table 3, show the effect of mushroom and aflatoxin B1 on the Creatinine and Uric acid. The results indicated that either mushroom or aflatoxin had positive effect (p = 0.05) on Creatinine and Uric acid of rats. As compared with control group mushroom increased (p = 0.05) Creatinine and Uric acid (55.55-92.59) and (32.83-82.08%), respectively. However, aflatoxin B1 increased them by (181.48 and 114.92%), respectively. Increasing the level of mushroom from 100 to 200 mg kg⁻¹ body weight resulted in a significant (p = 0.05) increase in Creatinine and Uric acid as compared with control group. The combination between aflatoxin B1 and mushroom resulted in a significant (p = 0.05) reduction in Creatinine (3.94-27.63) and Uric acid (4.16-29.86%) compared to aflatoxin B1 group. Mushroom at 100 mg kg⁻¹ level was more effective (p = 0.05) in reducing Creatinine and Uric acid compared to mushroom at 200 mg kg⁻¹ level. Serum Creatinine and Uric acid are good indicators for kidney injury.

From the previous data it can be conclude that, a dosage of 100 mg kg⁻¹ mushroom powdered with aflatoxin gave a very important and significant effect in blood parameters which were examined in this study. These

result are in agreement with those in worldwide which mentioned that mushroom had very important healthy effects for human and reported by Ikekawa (2001), Molitoris (2002), Lakshmi *et al.* (2004), Boa (2004), Coletto (2005), Nakajima *et al.* (2007) and Kim *et al.* (2007).

Angeli *et al.* (2006) found that, mushrooms have been used for many years for the preparation of infusions and as foods. Because of their biochemical components, such as carbohydrates, proteins, lipids and vitamins, they have been considered as functional foods. On the basis of several investigations, it has also been proposed that this mushroom has anti-tumor, anti-mutagenic and anti-clastogenic properties. Among the polysaccharides found in this mushroom, special attention has been paid to β-glucans (BGs). These polysaccharides have a central linear structure of D-glucose molecules, linked in the β-(1→3) position, containing one or more glucose side-chains [β-(1→6) linkage]. Beta-glucans (BGs) are polysaccharides that are found in the cell walls of organisms such as bacteria, fungi and some cereals. The biological effects of BGs include stimulation of the immune system as well as bactericidal and antiviral activity.

Other study by Kim *et al.* (2005) mentioned that, beta-glucans were prepared from *Agaricus blazei* Murrill by repeated extraction with hot water. The average molecular weights of beta-glucans were 30-50 kDa by gel filtration chromatography and Oligosaccharides, derived from hydrolyzing beta-glucans. Though beta-glucans and Oligosaccharides both showed anti-hyperglycemic, anti-hypertriglyceridemic, anti-hypercholesterolemic and anti-arteriosclerotic activity indicating overall anti-diabetic activity in diabetic rats, Oligosaccharides had about twice the activity of beta-glucans with respect to anti-diabetic activity.

The objective of their study was to investigate the genotoxic and antigenotoxic effects of beta-glucans extracted from the mushroom *Agaricus brasiliensis* (*Agaricus blazei* Murrill ss. Heinemann). The mutagenic activity of beta-glucans was tested in single-cell gel electrophoresis assays with human peripheral lymphocytes. Finally, fungi can also have a role as functional foods, known as (nutraceuticals) which are those materials that are beneficial but not absolutely vital, for the human body. Modern diets are based on relatively few domesticated plants, which in terms of biochemical composition tend to be blander than their wild counterparts and some theories suggest a return to more primitive eating habits, much closer to those of man's hunter-gatherer ancestors (Tudge, 2001).

CONCLUSION

Biological assessment on rats experiment indicated that, a low dosage of mushroom 100 mg with aflatoxin B1 was the best treatment to decrease the toxicity in animals for 15 days and these clearly demonstrated that, mushroom was a good functional food because it has many medicinal compounds but mushroom as a row food not drugs or pure medicines.

REFERENCES

- Abdel-Wahhab, M.A., S.A. Nada and H.A. Amra, 1999. Effect of alluminosilicates and bentonite on aflatoxin-induced development toxicity in rat. J. Applied Toxicol., 19: 199-204.
- Angeli, J.P.F., L.R. Ribeiro, M.L.C. Gonzaga, S.A. Soares and M.P.S.N. Ricardo *et al.*, 2006. Protective effects of β -glucan extracted from *Agaricus brasiliensis* against chemically induced DNA damage in human lymphocytes. Cell Biol. Toxicol., 22: 285-291.
- Barham, D. and P. Trinder, 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst, 97: 142-145.
- Bartels, H., M. Bohmer and C. Heierli, 1972. Serum creatinine determination without protein precipitation. Clin. Chim. Acta, 37: 193-197.
- Boa, E.R., 2004. Wild Edible Fungi: A Global Overview of Their Use and Importance to People. FAO Publishing Management Services, Rome..
- Coletto, M.A.B., 2005. Antibiotic activity of *Basidiomycetes*. XIV. Antibacterial and antifungal activities of some new recently isolated strains. Allionia, 40: 33-37.
- Gutierrez, Z.R., M.S. Mantovani, A.F. Eira, L.R. Ribeiro and B.Q. Jordao, 2004. Variation of the antimutagenicity effects of water extracts of *Agaricus blazei* Murrill *in vitro*. Toxicol. *In vitro*, 18: 301-309.
- Ikekawa, T., 2001. Beneficial effects of edible and medicinal mushrooms on health care. Int. J. Med. Mushrooms, 3: 291-298.
- Ishihara, T., 1999. Polysaccharide of *Agaricus blazei* Murrill. In: Handbook of Active Substances in Plant Resources, Tamimura, A. (Ed.). 2nd Edn., Science Forum, Tokyo, pp: 348-350.
- Kawagishi, H., R. Katsumi, T. Sazawa, T. Mizuno, T. Hagiwara and T. Nakamura, 1988. Cytotoxic steroids from the mushroom *Agaricus blazei*. Phytochemistry, 27: 2777-2779.
- Kim, S.W., M.G. Kim, J. Kim, H.S. Lee and H.S. Ro, 2007. Detection of the mycovirus OMSV in the edible mushroom, *Pleurotus ostreatus*, using an SPR biosensor chip. J. Virol. Methods, 148: 120-124.
- Kim, Y.W., K.H. Kim, H.J. Choi and D.S. Lee, 2005. Anti-diabetic activity of β -glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus blazei*. Biotechnol. Lett., 27: 483-487.
- Lakshmi, B., J.C. Tilak, S. Adhikari, T.P.A. Devasagayam and K.K. Janardhanan, 2004. Evaluation of antioxidant activity of selected Indian mushrooms. J. Pharma. Biol., 42: 179-185.
- Molitoris, H.P., 2002. Mushrooms in medicine, folklore and religion. Feddes Repertorium, 113: 165-182.
- Nada, S.A., E.A. Omara, H.G. Zahran, G.F. Asaad, D.F. Mansour and H. Mohram, 2008. Hepatoprotective effect of mushroom-insoluble polysaccharides in albino rat. Proceedings of the 7th International Conference of Medical Sciences, December, 23-25, 2008, National Research Center, Cairo, Egypt.
- Nakajima, Y., Y. Sato and T. Konishi, 2007. Antioxidant small phenolic ingredients in *Inonotus obliquus* (persoon) Pilat (Chaga). Chem. Pharm. Bull., 55: 1222-1226.
- Nishikimi, M., N.A. Roa and K. Yogi, 1972. Measurement of superoxide dismutase. Biochem. Biophys. Res. Commun., 46: 849-854.

- Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol.*, 28: 56-63.
- Sadler, M., 2003. Nutritional properties of edible fungi. *Nut. Bull.*, 28: 305-308.
- Sarangi, I., D. Ghosh, S.K. Bhutia, S.K. Mallick and T.K. Maiti, 2006. Anti-tumor and immunomodulating effects of *Pleurotus ostreatus*, mycelia-derived proteoglycans. *Int. Immunopharmacol.*, 6: 1287-1297.
- Shon, Y.H. and N. Kyung-Soo, 2001. Antimutagenicity and induction of anticarcinogenic phase II enzymes by basidiomycetes. *J. Ethnopharmacol.*, 77: 103-109.
- Tsai, S.Y., S.J. Huang and J.L. Mau, 2006. Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. *Food Chem.*, 98: 868-875.
- Tudge, C., 2001. The best medicine. *New Sci.*, 172: 40-43.
- Waller, R.A. and D.B. Duncan, 1969. A bayes rule for the symmetric multiple comparisons problems. *J. Am. Stat. Assoc.*, 64: 1484-1503.
- Wang, J.C., S.H. Hu, Z.C. Liang and C.J. Yeh, 2005. Optimization for the production of water-soluble polysaccharide from *Pleurotus citrinopileatus* in submerged culture and its antitumor effect. *Applied Microbiol. Biotechnol.*, 67: 759-766.
- Wasser, S.P., 2002. Medicinal mushroom as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiol. Biotechnol.*, 60: 258-274.