CHEMOPROTECTIVE EFFECT OF *POLYPORUS GRAMMOCEPHALUS* (BERK) EXTRACT ON DIETHYLNITROSOAMINE INDUCED HEPATOCARCINOGENESIS

Delphi Roy, Runa G. Auddy and Sriparna Datta*

Department of Chemical Technology, University of Calcutta,
92, APC Road, Kolkata-700009,
West Bengal, INDIA
E-mail: sriparna_d@yahoo.com

Summary

The present study was designed to investigate the chemoprotective effect of different extracts from an edible Mushroom *Polyporus grammocephalus against* chemically induced hepatocarcinogenesis in rats and evaluation of the glutathione (GSH) level and glutathione-S-transferase (GST) activity. Persistent nodules were developed in rat liver by diethylnitrosoamine (DENA) and its activity was further increased by a carcinogen promoter phenobarbitol (PB). A 20 week study was carried out by supplementing the rats with different extracts of the mushroom *Polyporus grammocephalus* in drinking water at doses of 0.2 ppm and 0.5 ppm for a period of 4 weeks, 8weeks and 12weeks to study the effect on glutathione (GSH) level and glutathione-S- transferase (GST) activity in different tissue like liver, small intestine, large intestine and kidney. Treatment of the rats with the different extracts resulted in fewer number of persistent nodules. Mean nodular volume decreased, nodular volume as a percentage of liver volume also decreased concomitantly. Treatment with aqueous extract resulted in increase of glutathione level and glutathione-S- transferase activity which again varied according to different tissue types like liver, small intestine, large intestine, kidney. Thus, *Polyporus grammocephalus* extracts demonstrate a chemoprotective effect against diethylnitrosoamine induced hepatocarcinoma in rats and a significant increase in glutathione level and glutathione-S- transferase activity.

Keywords: diethylnitrosoamine, hepatocarcinogenesis, persistent nodules, chemoprotection.

*Correspondent Author.

Introduction

Cancer is one of the leading causes of death in the world and despite considerable progress in development of tools for treatment and diagnosis, adequate treatment strategies are still lacking. Chemoprevention is a preventive strategy used to reduce the incidence of cancer by decreasing the toxic effects of carcinogens and delay the process of carcinogenesis. One of the approaches that have been made in recent years is to use natural products or dietary constituents which may counteract, prevent or delay the harmful effects of carcinogens. Extensive studies have revealed that a number of mushroom species have therapeutic properties for the prevention and/or treatment of cancer, viral diseases, hypercholesterolemia, and hypertension (1,2).

Several taxa of the family *Polyporaceae* of Basidiomycetes were reported to exhibit anti-tumor activities. *Polyporus giganteus*, *Ganoderma lucidum* (Fr.) Karsten, *Pleurotus pulmonarius*, *Laetiporus sulphureus* have shown to have anti- tumoregenic effect on sarcoma-180 and adenocarcinoma 755 cells (3-8). Most of these studies have indicated that polysaccharides isolated from these mushrooms are the principal bioactives responsible for their immunomodulatory and anti-tumour effect (9).*Polyporus garmmocephalus* is an edible fungi found widely in India. It has potent therapeutic properties by virtue of its anti-oxidant activity (10). Thus, it was thought worthwhile to explore the anti-tumour activity of the extract.

Changes in the activities of enzymes or isoenzymes that occur during experimental hepatocarcinogenesis and in transplantable tumors have gained marked attention in recent years (11). Hepatic pre-neoplastic foci induced by various carcinogens using different models have been extensively reviewed. (12) Some of the marker enzymes that appear in blood and other body fluids can be traced for early detection of hepatocarcinoma. In the liver, glutathione (γ-Glu-CysH-Gly) (GSH), glutathions-S-transferase (GST) can act as possible markers of pre-neoplastic hepatocytes (11, 13). The glutathione S transferases (GST) are a family of multi- functional enzymes which have important roles in the detoxification process. They catalyze the binding of glutathione(GSH) to endogenous and exogenous electrophilic compounds including drugs, toxins and carcinogens, allowing the products to be removed from the cell by the formation of less toxic metabolites. These enzymes are overexpressed in drug resistant especially cancer cell lines, hence they are regarded as a prognostic factor in cancer detection and treatment (14). GSTs are induced by a variety of chemoprotective agents and thus can prevent tumour formation (15)

It is now well known that many natural products or dietary chemicals can affect or modulate drug metabolizing enzymes which are responsible for inhibiting or retarding the multiple stages of carcinogenesis. Chemopreventive effects of the polysaccharides isolated from the mushrooms *Lentinus edodes* polysaccharides (LPS), *Ganoderma lucidum* polysaccharides (GPS) and *Coriolus versicolor* polysaccharides (CPS) were studied. It was found that increased glutathione S-transferase activity was most pronounced in *Ganoderma lucidum*.(16).Though a few species of the genus *Polyporus* were reported to exhibit antitumor activities, but no report has been found for the mushroom *Polyporus grammocephalus*.

Thus, in this study, an attempt was made to evaluate the chemopreventive effect of the extracts of *Polyporus grammocephalus* on antitumor activity by measuring persistent nodule growth and nodule volume in the liver.

The effect on the activity of the biomarker enzyme GST and altered GSH content was evaluated in an attempt to understand the mechanism of action. Diethylnitrosoamine (DENA) was selected as the initiator carcinogen because of its low hepatotoxicity and high hepatocarcinogenic properties (17). After limited treatment with DENA large benign hepatomas appear in the rats which are equivalent to neoplastic nodules and highly differentiated hepatocarcinoma.

Materials and Methods

Chemicals

Benzene, Ethylacetate, Ethylmethylketone, and Methanol were of analytical grades and procured from the local suppliers. All other biochemicals, unless otherwise mentioned, were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Animals

Male Sprague-Dawley rats (110-120 g) were purchased from the Central Research Institute (Ayurveda), Kolkata. The animals were housed in polycarbonate cages (four rats per cage) in an air-conditioned room maintained at a temperature of 25°C ±1°C and R.H of 55±5% with 6am to 6pm photoperiod. They were supplied with semi-purified basal diet and double-distilled drinking water *ad libitum*. The animals were acclimatized a week before the commencement of the experiment. Guidelines for the care and use of the laboratory animals (National Institute of Health, USA) were followed during the experiment and approved by the Institutional Animal Ethics Committee (IAEC), University of Calcutta. Sanction no 506/01/a/CPCSEA

Fungal Material

The fruit-body (carpophore) of *Polyporus grammocephalus* (Macrofungi) was collected from Sandakfu districts of Darjeeling and identified by Botanical Survey of India (Govt. of India), Office of joint Director, P.O Botanic Garden, Howrah-711103.

Preparation of the extract

10kgs of the fresh material of *P.grammocephalus* was air dried and crushed into finely powdered form. 900gms of dried powder of the Mushroom was used for work. 1300ml of Benzene was added to the dried Mushroom power and soaked for 48hrs with occasional stirring. Repeated decantation and filtration yielded cold benzene extract. The residue of Mushroom powder obtained after filtration was collected and resuspended in 1300ml of benzene again, refluxed over water bath for 14hrs, repeatedly decanted, filtered and filtrate obtained was the hot benzene extract. Residue left again after filtration of Mushroom powder was collected and thoroughly dried over water bath to

remove the last traces of benzene. The same procedure of extraction was repeated with different solvents like ethyl acetate, Ethyl methyl ketone and Methanol and water extract respectively to obtain the corresponding extracts. Each of the solvent extracts was evaporated to dryness under reduced pressure to form dark semisolid mass. For the study regime, a known volume (0.5ppm) of each of the chosen dried extract mass of ethyl acetate, methanol and water was supplemented in double distilled, demineralised drinking water and supplied to the animals.

***Experimental regime*:**

a) For Persistent Nodule Growth

66 rats were divided randomly into six experimental groups with 10 rats in each group-A, B & C and 12 rats in each group – D, E & F. Animals of group A were treated with aqueous extract of the mushroom and served as the extract control group. Animals of group B served as untreated control. Rats of other groups C, D, E and F were injected with a single intra-peritoneal injection of DENA (Sigma,St Louis,Mo, USA) at a dose of 200mg/Kg body weight in 0.9% NaCl solution for initiation of hepatocarcinogenesis. Following a two week recovery period, Phenobarbital (PB) (Sigma) as a carcinogen promoter at a level of 0.05% was incorporated into the basal diet of groups C, D, E and F rats till the end of study for 20weeks. PB was withdrawn from the diet 4 days before the sacrifice to eliminate its effect. Rats of group C were kept as DENA treated control and rat of other three groups D, E, F, were treated with 0.5ppm of ethylacetate, methanolic and aqueous extracts of *Polyporous grammocephalous* supplemented in drinking water respectively. Daily food and water intake were noted and body weights of the rats from all groups were recorded every second day. The animals were sacrificed by decapitation 20 weeks after the commencement of the experiment under proper ether anaesthesia. Rats were kept under fasting overnight before they where sacrificed.

Morphological study

After sacrifice, the livers were promptly excised, weighed and examined on the surface for subcapsular macroscopic liver lesions (neoplastic nodules). The grayish-white nodules could be easily identified on the reddish-brown liver. The nodules which approximated spheres, were measured in two perpendicular directions to nearest millimeter to obtain the average diameter of each nodules. The nodules were divided into three groups according to their respective diameter ($\geq$3, <3 ->1 and $\leq$1mm) as described by Moreno et al (18). From these diameters, individual nodule volumes were calculated and represented in table 2.

b) For GSH content and GST activity estimation:

After acclimatization 54 rats were randomly divided into three groups (1, 2, and 3) with 18 rats in each group. Each group was again divided into 3 sub groups (A, B, and C) with 6 rats in each sub group. A sub- group of all 1, 2 and 3 groups were treated as control. Rats of subgroup B of each 1, 2 and 3 groups received 0.2ppm of aqueous extract supplemented with drinking water for 4, 8 and 12 weeks. Rats of sub group C of each 1, 2 and 3 group received the 0.5ppm of aqueous extract supplement in drinking water for 4, 8 and 12 weeks respectively.

All rats received basal food daily and weight of the animals from each group were recorded every second day. All the animals were sacrificed by decapitation after their respective period of treatment. Animals were kept under fasting overnight before they were sacrificed.

Preparation of Cytosolic fractions from the tissues:

After the rats were sacrificed, liver, kidney, small and large intestine were excised immediately , washed with ice cold saline water, blotted dry and weighed. The tissues were separately homogenized in ice-cold 0.154 M KCl solution (pH 7.4) by means of a Teflon-glass homogenizer for 1 min to yield a 10% homogenate. The tissue homogenates were centrifuged at 9000xg for 20mins. Resultant supernatant was further centrifuged at 10,5000xg for 1hr in an ultracentrifuge at 4^{o}C. Supernatant obtained after second centrifugation forms the cytosolic fraction and was kept frozen at -20^{o}C until used for the enzyme assay.

GSH and GST Assays:

Total GSH concentration was measured using the method of Ellman (19). 1 ml of cytosol was mixed with 1ml of 4% 5-sulfosalicylic acid and the mixture was centrifuged at 1500g for 15 min. The supernatant was allowed to react with 0.1mM 5, 5'-dithio-bis (2-nitrobenzoic acid). The solution was kept at room temperature for 10 min and absorbance was noted at 412 nm in a Spectrophotometer (Shimadzu Model no. UV2550).

GST activity was measured following the method of Habig et al (20), using 1-chloro-2,4-dinitrobenzene (CDNB) and GSH as substrates. The reaction mixture of 1ml consisted of 0.1M potassium phosphate buffer (pH 6.5), 1mM GSH, 1mM CDNB and an aliquot of cytosol (0.6 to 0.mg protein/ml). The reaction mixture was incubated at 37°C for 5 min, after adding CNDB, the change in absorbance at 340nm was measured spectrophotometrically as a function of time. The specific activity of the enzyme was expressed in terms of formation of CDNB-GSH conjugate per mg of protein per min. Protein content of the cytosolic fraction was determined by the method of Lowry et al (21) using BSA as a standard.

Statistical Analysis:

All the data of persistent nodules, GSH and GST activity were statistically analyzed using student's t test Differences between the means were also evaluated by Student's t-test. The level of significance was set at $p < 0.05$.

Results

Food and Water intake:

During the entire period of the study no differences in food and water consumption were observed among the various groups of animals.

Mortality:

Two rats died from group C before the end of the study. None of the rats from any other groups died during the 20 weeks study period.

Body weight:

The final body weights of the carcinogen (DENA) control (group C) were slightly lower than that of the untreated normal control (group B). There were no significant differences in the body weights of the other extract treated rats of groups D, E, F when compared with the normal controls. Thus the extracts did not show any adverse effect on the growth of animals.

Effect of extracts on nodular growth:

To study the chemopreventive effect of the extracts on hepatic nodular neoplasia the appearance of hepatic nodules was evaluated. There were no visible hepatocyte nodules in the liver of normal control (group B) as well as the aqueous extract treated control group A.

Table1: Effect of different extracts on the rats development of persistent nodules in the liver of induced by DENA and promoted by PB

Group	Treatment	No. of rats with Nodules /total no. of rats	Nodule Incidence (%)	Total no. of Nodules	Average of Nodules Multiplicity
A	Aqueous Extract Control	-	-	-	-
B	Normal	-	-	-	-
C	DENA Control	10 /10	100	335	33.5± 2.5 [a]
D	DENA+ Ethyl acetate extract	6 /12	50	119	19.8±3.1[b]
E	DENA+CH_3OH extract	7 / 12	50.5	153	22.6±2.8[d]
F	DENA+ aqueous extract	4 / 12	30	25	8.5±0.9[c]

[a]Each value represents the mean ± S.E

[b]$p < 0.01$, [d]$p < 0.02$ and [c]$p < 0.001$ as compared to the DENA control group by student's t-test

Table I summarizes the incidence of nodules, total number of nodules and average number of nodule per nodule bearing liver of (DENA) treated groups in presence and absence of extract treatment. In comparison to 100% nodule incidence in DENA treated group C, nodule incidence of only 50%, 50.5% and 30% were observed in groups D, E and F which received ethyl acetate, methanol and aqueous extracts respectively. However, the aqueous extract (Gr. F) showed the best inhibitory effect.

Similarly, the average number of nodules per nodule-bearing liver (nodule multiplicity) was found to be smaller in the different extract treated groups D, E, F compared to DENA control group C. All the results obtained were statistically significant when compared to the DENA treated group i.e. for group D ($p < 0.01$), for group E ($p < 0.02$) and group F ($p < 0.001$).

Table II shows the size distribution of visible neoplastic nodules, mean nodule volume and nodule volume expressed as a percentage of liver volume of different group of rats. Nodules more than 3 mm in diameter were reduced considerably in groups D, E and F when compared to Gr. C. Mean nodular volume was found to be reduced in all the extract treated groups. Results obtained with all three extract treated groups D, E, F were statistically significant. Statistically significant results of groups D ($p<0.05$), group E ($p<0.05$), and group F ($p< 0.001$) were obtained in respect of nodular volume as percentage of liver volume as compared to DENA control group C by student's t-test

Table II. Effect of the extract on the size, distribution and growth of DENA-induced nodules in rats.

Group	No. of rats	Size of nodules (mm)			Mean nodular volume $(cm)^3$(mean ± SE)[a]	Nodular volume /liver volume (%) (mean ± SE)[b]
		≤1	>1-3	≥ 3		
Extract control	-	-	-	-	-	-
normal	-	-	-	-	-	-
DENA control	10	87	135	125	2.61±0.37[c]	70.3±4.9
$CHCl_3$ Extract -DENA	6	42	49	40	1.21± 0.12 [c]	50.8±5.7 [d]
Et-OH Extract + DENA	7	41	55	48	1.33± 0.34[d]	57.3± 2.8 [d]
Aq extract + DENA	4	13	11	7	1.30± 0.13[g]	39.9 ± 4.3 [g]

[a]Each value represents the mean ± S.E
[b]One gram of liver was assumed to occupy $1cm^3$ for this calculation.
[c]Each Nodular volume were calculated from the two perpendicular diameters on each nodule, [d]$p<0.05$, [c]$p<0.02$; [f]$p<0.01$ & [g]$p < 0.001$ as compared to DENA control group C by student's t-test.

.

Effect of the aqueous extract on GSH content

Since, the aqueous extract treated groups showed to be most effective in protecting against appearance of hepatic nodules and reduction in nodular volume(all manifestations of hepatic neoplasia) it was thought worthwhile to examine the effect of the aqueous extract on the GSH levels in the tissues and the activity of its metabolizing enzyme GST.

The effects of 0.2 ppm and 0.5 ppm aqueous extract supplementation in drinking water, increased the GSH content in different tissues of rats after 4, 8 and 12 weeks of study compared to the tissues of the untreated control groups. This is depicted in Table III.

Table III: Effect of extract supplementation on GSH level in different Target Tissue of Rats. GSH contents expressed in µ mol/g tissue

Group	Treatment	Liver	Small intestine mucosa	Large Intestine mucosa	Kidney
Group;1:4wk					
A	Control	7.46± 0.13	6.49±0.11	4.38±0.11	5.25±0.37
B	Extract,0.2ppm	7.74±.04	6.21±0.17	4.04±0.27	5.78±0.06
C	Extract,0.5ppm	11.986±.23d	7.43±0.10	4.18±0.05	6.20±0.01[b]
Group:2:8wk					
A	Control	9.658±0.11	6.63±0.12	5.23±0.05	5.84±0.07
B	Extract;0.2ppm	11.99±0.24	9.47±0.10	6.17±0.02	7.30±0.07
C	Extract;0.5ppm	13.72±0.10d	10.54±0.08d	7.30±0.07[c]	9.04±.0.57[e]
Group;3:12w					
A	Control	10.64±0.09	8.62±0.11	5.73±0.11	6.99±0.86
B	Extract,0.2ppm	12.582±0.09[b]	9.16±0.15	6.29±0.05	9.10±0.91
C	Extract, 0.5ppm	17.72±0.11e	12.52±0.13d	8.35±0.06 [d]	10.22±0.12[d]

a of each value represents the mean ± S.E of six animals.
Significant differences from corresponding control values: b; $p<0.05$; c; $p<0.02$; d; $p<0.01$ and e; $p<0.001$

The liver is the main source of blood GSH but during toxic conditions GSH efflux takes place which ultimately reaches the kidney. In liver a statistically significant increase of ($p<0.05$) and ($p<0.001$) was found with both doses i.e 0.2ppm and 0.5 ppm extract supplementation, for 12weeks period of treatment respectively. Statistically significant result ($p<0.01$) was obtained for 4 and 8 weeks treatment only with 0.5ppm extract. GSH content in kidney also increased concomitantly with supplementation of 0.5 ppm of aqueous extract from 4 weeks to 12 weeks and the results obtained were all statistically significant ($p<0.05$). In small and large intestinal mucosa, increase in GSH level was marginal after 4 weeks of treatment. However statistically significant increase of ($p<0.01$) was found only with 0.5 ppm dose supplementation for 8 and 12 weeks.

Effect of the aqueous extract on GST activity

The effects of aqueous extracts on specific activity of cytosolic GST in various rat tissues are shown in table IV. In liver, statistically significant ($p< 0.05$) increase in GST activity was observed with 0.2ppm of extract supplemented for 12weeks of treatment. Similarly with 0.5.ppm dose supplementation, statistically significant results ($p<0.05$ and 0.01) were obtained for 4, 8 and 12weeks of treatment respectively when compared to the untreated control.

Table IV:
Effect of aqueous extract supplementation on specific Activity of Glutathione S-transferase (GST) in different targets tissue of rats GST activity (µ mol GSH conjugates formed /min/mg protein

Groups		Treatment	Liver	Small intestine mucosa	Large intestine mucosa	Kidney
Group1, 4weeks Subgroups	A	Control	1.95±0.12 [a]	0.92±0.2	0.87±0.06	1.20±0.10
	B	Extract,0.2ppm	2.00±0.29	0.98±0.17	0.82±0.08	1.40±0.17
	C	Extract,0.5ppm	2.25±0.01[b]	1.20±0.15	0.91±0.10	1.84±0.50
Groups2,8weeks Subgroups	A	Control	2.60±0.20	0.82±0.09	0.90±0.42	1.83±0.082
	B	Extract,0.2ppm	3.20±0.52	1.25±0.92	1.25±0.84	2.05±0.02[b]
	C	Extract,0.5ppm	4.14±0.42[c]	1.04±0.01[b]	1.90±0.02[b]	2.65±0.42
Group3,12weeks Subgroups	A	Control	2.40±0.52	1.48±0.32	1.21±0.48	2.05±0.10
	B	Extract,0.2ppm	3.80±0.22[b]	2.05±0.44	1.97±0.10	2.34±0.17
	C	Extract,0.5ppm	4.54±0.37[c]	2.63±0.042[c]	2.50±0.09[b]	2.64 ±0.19[b]

[a] of each value represents the mean ± s.e.m of six animals.
Significant differences from corresponding control values : [b]$p<0.05$ and [c]$p<0.01$

In the other tissues like small and large intestine and kidney, GST activity increased with all doses. In small intestine, statistically significant results ($p<0.05$ and 0.01) were found with 0.5ppm extract supplementation for 8 and 12 weeks respectively. In large intestine statistically significant results ($p<0.05$) was found with 0.5ppm extract supplementation for 8 and 12 weeks. Similarly in kidney statistically significant result ($p<0.05$) was found with both the doses i.e 0.2ppm extract supplementation for 8 weeks and 0.5ppm extract supplementation for 12 weeks.

Discussion

The results of our present study demonstrates a particular model of DENA induced hepatocarcinogenesis in rats, promoted by PB which on supplementation with different extracts of the mushroom *Polyporus grammocephalus* during the entire period of study of 20 weeks, greatly reduced the incidence, multiplicity and size of visible PNs. Another striking observation in the study showed that PNs more than 3mm in size developed in much lesser number in the extract treated rats compared to the DENA treated control animals. The nodular volume as well as the percentage of liver volume was also found to decrease concomitantly. Although it is evident that not all the hepatocyte nodules become cancerous during the life span of the animals, numerous studies support the concept that the nodules are the precursors of hepatic cancer (22). Hence inhibition of nodular growth and reduction of their sizes by supplementation with different extracts from the mushroom *Polyporus grammocephalus* has reduced the efficiency of nodule formation capacity of DENA which may in turn indicate protective action of the extracts against chemical induced hepatocarcinogenesis.

Moreover the food and water intake and changes in body weight among the treated and untreated groups are similar. This signifies that the treatment with the extract for 20 weeks does not produce any toxic effect.

To get an insight into the mechanism of chemopreventive action of *Polyporus grammocephalus* extract, a similar treatment with two doses of aqueous extract was undertaken to observe the modifying effect on activities of xenobiotic-metabolising enzymes such as glutathions-S-transferase (GST). It is known that in the liver glutathione (γ-Glu-CysH-Gly) (GSH) and related enzymes like glutathions-S-transferase (GST) can act as possible markers of pre-neoplastic hepatocytes (10, 12).
GSTs catalyze the conjugation of GSH to a variety of electrophilic compounds, including carcinogens, as well as endogenous reactive compounds (13). These conjugates thus become less toxic than the original toxic compound and can be easily excreted from the body. Hence elevation of GSH level signifies better detoxification of electrophilic compounds including carcinogens.

The anticarcinogenic potentials of a number of xenobiotics and dietary constituents are known to enhance the endogenous GSH pool (22, 23). A strong correlation exists between induction of GST activity, especially in liver and digestive tract, and protection against chemically induced carcinogenesis (24, 25). GST detoxifies a variety of electrophiles generated during oxidative stress (a common manifestation in cancer) by conjugating them with GSH and plays an important role in detoxification of carcinogens. Although liver has the most important role in detoxification of different chemicals, the other part of the gastrointestinal tract, mainly small and large intestine also play important role in protecting other tissues by metabolic activity. It is known that the part of the GIT is also of paramount importance since it represents a portal entry for exogenous chemicals like carcinogens. Though the exact mechanism has not been elucidated, but results suggest that extracts of *Polyporus grammocephalus* have the potential to provide protective action against DEN-PB induced changes in hepatocytes possibly through the inhibition of nodular growth. The results of our present study strongly suggest that extracts of *Polyporus grammocephalus* may be important for caner prevention.

References

1. Breene W.M., Nutritional and medicinal value of specialty mushrooms, J. Food Prod 1990; 53: 883-894
2. Tong SC, Birmingham IM, Pai SH, Immunomodulatory substances of fungal origin, J. Immunol. Immunopharmacol 1991;11: 115–122
3. Wasser SP. Review of medicinal mushroom advances: Good news from old allies. Herbal Gram 2002;56: 2833.
4. Kim BK, Shim MJ, Kim HW, Woo MS, Choi EC. Antitumor Constituents of Polyporus giganteus. Archives of Pharmacal Research 1987; 10: 179 – 183.
5. Kim BK, Chung HS, Chung KS, Yang MS. Studies on the antineoplastic components of Korean Basidiomycetes. Korean Journal of Mycology 1980; 8: 107 –113.
6. Kang CY, Shim MJ, Choi EC, Lee YN, Kim BK. Studies on antineoplastic components of Korean basidiomycetes mycelial culture and an antineoplastic component of *Ganoderma lucidum*. Korean Biochem. J 1981; 14: 101–102.

7. Chung KS, Choi EC, Kim BK, Kim YS, Park YH. Studies on the constituents and culture of Korean basidiomycetes antitumor polysaccharides from the cultured mycelia of some basidiomycetes. Archives of Pharmacal Research 1982; 5: 17–19.
8. Kang CY, Lee CO, Chung KS, Choi EC, Kim BK. An antitumor component of Laetiporus sulphureus and its immunostimulating activity. Archives of Pharmacal Research 1982; 5: 39–43.
9. Zhanga M, Cuia SW, Cheungb PCK, Wan Q. Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. Trends in Food Science & Technology 2007; 18 :4-19.
10. Rai M, Biswas G, Acharya K. Antioxidant and Nitric Oxide Synthase Activation Properties of *Polyporus grammocephalus* Berk. International Journal of Biomedical and Pharmaceutical Sciences 2007; vol 1, number 2: 160-163.
11. Sato K. Glutathione transferases as markers of preneoplasia and neoplasia. Adv. Cancer Res 1990; 52: 205–255.
12. Farber E. The sequential analysis of liver cancer induction. Bio-chem.Biophys Acta 1980; 605: 149–166.
13. Fiala S, Mohindru A, Kettering WG, Fiala AE, Morris HP. Glutathione and gamma glutamyl transpeptidase in rat liver during chemical carcinogenesis. J. Natl. Cancer Inst 1976; 57: 591–598.
14. Hayeshi R, Mutingwende I, Mavengere W, Masiyanise V, Mukanganyama S. The inhibition of human glutathione S-transferases activity by plant polyphenolic compounds ellagic acid and curcumin, Food and Chemical Toxicology 2007; 45: 286–295
15. Lieberman R. Chemoprevention of prostate cancer: current status and future directions. Cancer Metastasis Rev 2002; 21:297–309.
16. Hyung SK, Sam K, Byung ML. In vitro chemopreventive effects of plant polysaccharides (Aloe barbadensis Miller, Lentinus edodes, Ganoderma lucidum and Coriolus versicolor) Carcinogenesis 1999; 20: 1637-1640.
17. Scherer E, Emmelot P. Kinetics of induction and growth of enzyme-deficient islands involved in hepatocarcinogenesis. Cancer Res 1976; 36: 2544–255.
18. Moreno FS, Rizzi MBSL, Dagli MLZ, Penteado MVC. Inhibitory effects of ß-carotene on preneoplastic lesions induced in Wister rats by the resistance hepatocyte model. Carcinogenesis 1991; 12: 1817–1822.
19. Ellman G., Lysko H. A precise method for the determination of whole blood and plasma sulfhydryl groups. Anal. Biochem. 1979; 93:98–102.
20. Habig WH, Pabst MJ, Jakoby WB, Glutathione S-transferase: the first enzymatic step in mercapturic acid formation J. Biol. Chem 1974; 249: 7130–7139.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem 1951; 193: 265–275.
22. Farber E, Cameron R. The sequential analysis of liver cancer induction. Biochim Biophys Acta 1980; 605: 149–166.
23. Benson AM, Cha YN, Bueding E, Heine HS, Talalag P. Elevation of extrahepatic glutathione S-transferase and epoxide hydralase activities by 2(3)-tert-butyl-4-hydroxyanisole. Cancer Res 1979; 39: 2971–2977.
24. Sparnins VL, Venegas PL, Wattenberg LW. Glutathione S-transferase activity by compounds inhibiting chemical carcinogenesis and by dietary constituents. J. Natl. Cancer Inst 1982; 68: 493 – 496.
25. Newmark HL. Plant phenolics as inhibitors of mutational and precarcinogenic events. Can. J. Physiol. Pharmacol. 1987; 65: 461–466.
26. Sparnins VL, Wattenberg LW. Enhancement of glutathione S-transferase activity of the mouse forestomach by inhibitors of benzo(a)pyrene-induced neoplasia of the forestomach. J. Natl. Cancer Inst 1981. 66: 769–771.