

EVALUATION OF DIETARY SUPPLEMENTATION OF *PLEUROTUS OSTREATUS* AND *CALOCYBE INDICA* IN SWISS MALE ALBINO MICE

JAWAHAR GANAPATHY¹, EMILIN RENITTA^{2*}

¹Department of Biotechnology, C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Gopal Vidyanagar, Surat - 394 350, Gujarat, India. ²Department of Food Processing and Technology, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore - 641 114, Tamil Nadu, India. Email emilinrenitta@karunya.edu

Received: 14 July 2018, Revised and Accepted: 27 August 2018

ABSTRACT

Objective: The present study deals with dietary supplementation of *Pleurotus ostreatus* and *Calocybe indica* in Swiss male albino mice in comparison with casein control diet.

Methods: The Swiss albino male mice were divided into four groups. Group 1 served as normal, Group II served as control mice, and Groups III and IV mice were fed with food supplement *P. ostreatus* and *C. indica* for 28 days. After 28 days, the animals were euthanized by cervical dislocation, and liver samples were used to analyze the following parameters such as superoxide dismutase (SOD), catalase, glutathione peroxidase, reduced glutathione (GSH), Vitamin C, and Vitamin E.

Results: The results of the present study indicated that dietary supplementation of *P. ostreatus* and *C. indica* significantly ($p < 0.05$) increased the antioxidants levels in experimental mice.

Conclusion: From the results, it was found that the mushrooms such as *P. ostreatus* and *C. indica* seem to be a promising source because of its antioxidant properties. Therefore, this medicinal mushroom could be used as a nutritional supplementation for human.

Keywords: Antioxidants, Dietary supplements, *Pleurotus ostreatus*, *Calocybe indica*.

© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i10.28497>

INTRODUCTION

Mushrooms are becoming a popular food in daily meal because of their nutritious and medicinal values. Edible mushrooms have been known for texture and flavor as well as medicinal and tonic attributes and utilized as a human food for centuries [1]. From the nutritional point of view, mushrooms contain appreciable amount of dietary fiber, particularly important for the regulation of physiological functions in human organism; furthermore, it contains a high amount of proteins and is excellent source of fibers, vitamins, and minerals [2]. These functional substances of mushrooms are able to lower cholesterolemia, modulate the immune system, and inhibit tumor growth [3].

Antioxidant is an important property by which living organisms can neutralize the toxic and cell-damaging molecules called free radicals which are generated during various metabolic reactions of the body [4]. Antioxidant in food plays an important role by reducing the risk for chronic diseases including cancer and heart disease. The fruiting bodies of mushrooms contain about 56.8% carbohydrate, 25.0% protein, and 12.5% ash on a dry weight basis [5]. The immune function throughout life can be influenced by nutrition. Possible ingredients for the development of functional foods that could contribute to optimal immune response include the antioxidant vitamins and trace elements (e.g., zinc, copper, manganese, magnesium, selenium potassium, B vitamins, and Vitamin D) [6]. Healthy nutrition and diet are gaining importance not only in the everyday life of human beings but also in the treatment of chronic diseases. Mushrooms are recognized worldwide as medicinal foods rich in nutrition by doctors. The Food and Drug Administration has officially designated mushrooms as "healthy foods" [7].

Oxidation is important to all living organisms for the production of energy to fuel biological processes. However, production of free radicals is involved in the cause of many diseases such as cancer, rheumatoid

arthritis, and atherosclerosis as well as in degenerative processes associated with aging [8]. Even though there is free radical damage, all organisms can be protected by enzymes such as superoxide dismutase (SOD) and catalase (CAT), or compounds such as ascorbic acid, tocopherols, and glutathione (GSH). The presence of antioxidants in human diet acts as protective agents to reduce oxidative damage caused in the human body [9]. The present study was carried out to investigate the nutritional and antioxidant level of the diet prepared with *Pleurotus ostreatus* and *Calocybe indica* in Swiss male albino mice.

MATERIALS AND METHODS

Experimental animals

Swiss male albino mice (20–25 g, 8–10 weeks old) were purchased from the Agricultural University at Mannuthy (Thrissur, India). All mice were kept in a pathogen-free air-controlled room maintained at 24°C with a 50% relative humidity and 12-h light and dark cycle. All mice had *ad libitum* access to a control diet (Hindustan Lever Ltd., Mumbai) and filtered water. All animal experiments were approved and performed according to the regulations of the Institutional Animal Ethics Committee, Government of India (IAEC Reg. No: IAECK/KU/BT/13/03).

Grouping of mice

After 1 week period of acclimatizing, the mice were randomly divided into four groups of six mice each. Group 1 served as normal, Group II served as control mice, and Groups III and IV mice were fed with food supplement *P. ostreatus* and *C. indica* for 28 days.

Group I=Normal diet

Group II=Control (casein) diet

Group III=Test diet - I (*P. ostreatus*)

Group IV=Test diet - II (*C. indica*)

*Mode of administration - oral route.

Preparation of diets

The control (casein), *P. ostreatus*, and *C. indica* diets were prepared following the method of Vasconcelos [10]. The composition of control and test diets is given in Table 1; casein, mushroom powder, refined groundnut oil, vitamin mixture, salt mixture, dextrose, and corn starch were used as ingredients. Vitamin mixture and salt mixture were prepared by following the method of Mattison [11]. All the ingredients were mixed together with the addition of water and made into small balls (30 g) and stored at -18°C in airtight containers. Feeding trials were carried out for 28 days. Feed and water were given *ad libitum*.

Proximate composition analysis of the diets

The protein content of each diet was determined by Lowry's method [12]. The total carbohydrate analysis was carried out by anthrone method [13]. The lipid content was estimated by Soxhlet extraction method of Association Official Analytical Chemists [14].

Enzymatic and non-enzymatic antioxidant assay

At the end of 28th day, the mice were euthanized by cervical dislocation and the liver tissue recovered at necropsy was assessed for antioxidant activity such as SOD which was determined by following the method of Kakkar [15], CAT was performed by following the method of Sinha [16], glutathione peroxidase (GPx) was analyzed by following the method of Rotruck [17], GSH was estimated by following the method of Ellman [18], ascorbic acid (Vitamin C) was estimated by following the method of Omaye [19], and α -tocopherol (Vitamin E) was estimated by following the method of Meydani [20]. Protein content was estimated using the Lowry method [12]. All liver samples (once prepared as homogenate) were used for the assays.

Statistical analysis

All the values were expressed as mean \pm standard deviation. The data were statistically analyzed using one-way Analysis of Variance (ANOVA), and the significant differences among the test groups were evaluated by Duncan's multiple range test. The results were considered as statistically significant at $p < 0.05$. All the statistical analyses were made using the Statistical Package for the Social Sciences 20.0 software package.

Table 1: Composition of control and test diets

Ingredients	Control diet (g)	Test diet - I (g)	Test diet - II (g)
Casein	12.6	-	-
Mushroom powder	-	18.2	18.2
Refined groundnut oil	7.0	7.0	7.0
Vitamin mixture	1.0	1.0	1.0
Salt mixture	2.0	2.0	2.0
Dextrose	25.0	25.0	25.0
Corn starch	52.4	46.8	46.8
Salt mixture (percentage)			
K ₂ HPO ₄			30
KCL			9.4
MgSO ₄			14.8
FeSO ₄ .H ₂ O			1.4
Ca ₃ (PO ₄) ₂			27.4
MnSO ₄ .7H ₂ O			0.2
CaCO ₃			16.8
Composition of vitamin mixture			
Vitamin A (as palmitate)			25000 IU
Vitamin D ₃			200 IU
Vitamin B ₁₂			1.0 mcg
Vitamin E acetate			5.0 mg
Vitamin B ₂ (riboflavin)			1.5 mg
Vitamin B ₁ (thiamine mononitrate)			1.0 mg
Vitamin B ₆			1.0 mg

RESULTS

The findings of the present investigation are based on the dietary supplementation of casein, *P. ostreatus*, and *C. indica* diets in Swiss male albino mice. Fig. 1 shows the biochemical composition of test diets. Dieting trials showed that the mice consumed the control and formulated diets in more quantities and the consumption of test diets was little lower than control diet. There was no non-acceptance by the mice for the diets containing *P. ostreatus* and *C. indica* and absolutely no unhealthy symptoms of deficiency disease or abnormal toxicities were observed in the mice throughout the experimental period.

The liver is the main organ of oxidative and detoxifying processes as well as free radical reactions; in many diseases, biomarkers of oxidative stress are elevated in the liver at an early stage. The present results showed a significant increase in SOD level for *P. ostreatus* and *C. indica* diet-fed groups (Fig. 2).

The effect of normal, control, *P. ostreatus*, and *C. indica* diets on liver CAT of Swiss male albino mice is shown in Fig. 3. In the present study, CAT significantly showed an increase in activity in mushroom-fed diet (I and II) than normal diet.

GPx is an enzyme that catalyzes the reduction of hydroxyl peroxides by GSH. Its main function is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide (H₂O₂) to water. In the present study, GSH significantly showed an increase in activity in mushroom-fed diets than normal diet (Fig. 4).

The activity of GSH reductase is used as an indicator for oxidative stress. The effect of normal, control, *P. ostreatus*, and *C. indica* diets on liver-reduced GSH of Swiss male albino mice is shown in Fig. 5.

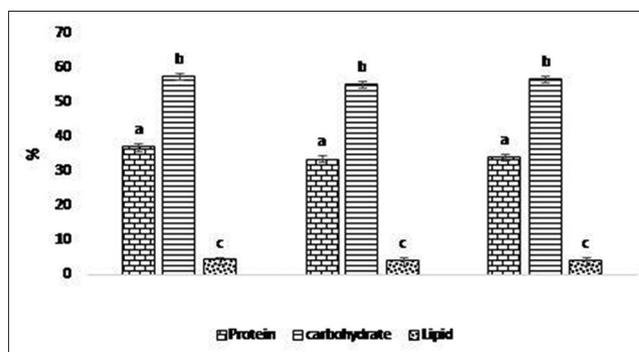


Fig. 1: Biochemical composition of diet. Values are \pm standard deviation for 6 mice each group. Bars not sharing a common superscript letter^(a,b,c) differ significantly at $p < 0.05$ Duncan multiple range test

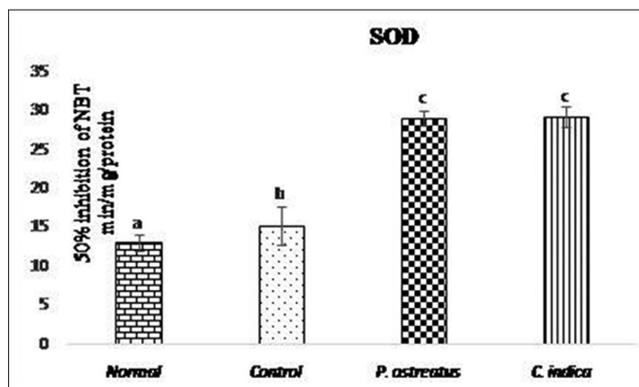


Fig. 2: Effect of normal, control, *Pleurotus ostreatus* and *Calocybe indica* diets on liver superoxide dismutase activity of Swiss male albino mice. Bars not sharing a common superscript letter^(a,b,c) differ significantly at $p < 0.05$ Duncan multiple range test

Vitamin C is an anti-inflammatory and helps to prevent the inflammatory diseases, including arthritis, fibromyalgia, and chronic fatigue. A significant increase in Vitamin C level was noticed in *P. ostreatus* and *C. indica* (Fig. 6) diet-fed mice.

There was a significant increase in the Vitamin E levels in mushroom diet-fed mice (I and II), and it may due to the protection against oxidative stress involving in the tocopherol radical (Fig. 7).

DISCUSSION

Utilizing the promising functional foods has become an increasingly favored approach to the modulation of immune cell function [21]. Mushroom has a higher nutritional value such as proteins and vitamins similar to meat and eggs [22]. In the present era, the consumption of mushroom has been increased, and the consumption has been associated with the maintenance of health and in antioxidant defense in the prevention of many diseases. Mushroom diets are rich in total polyphenols, ascorbic acid, and vitamins which could be the main antioxidant compounds containing a wide variety of free radical scavenging molecules such as polyphenolic, triterpenoids, and steroids which have a strong antioxidant activity. It is reported that the total polyphenol was, naturally occurring antioxidant components, found higher in mushroom [23].

A significant increase could be due to the antioxidant enzyme which rapidly catalyzes the dismutation of superoxide anion and thus acts as the first-line antioxidant defense. There are many scientific research data that have provided a valid health supportive toward natural treatment for a variety of diseases [24,25].

CAT has no electron donor requirement during detoxification of H_2O_2 . CAT is a known antioxidant enzyme, and it has been involved in

protection against H_2O_2 ; its localization is limited to the peroxisome [26]. H_2O_2 is a non-radical reactive oxygen species with weak oxidizing activity. It diffuses through cell membranes rapidly and interacts with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and other free radicals [27]. The significant increase could be due to the antioxidant enzyme which is located in the cell's peroxisome, such as SOD naturally within the body. It converts H_2O_2 into water and oxygen, preventing the formation of carbon dioxide bubbles in the blood [28]. CAT also uses H_2O_2 to break down potentially harmful toxins in the body. CAT and SOD help to prevent free radical damage [29].

The significant increase could be due to the highly expressed scavenging and inactivating hydrogen and lipid peroxides, providing protection to the body against oxidative stress. GPx with other selenoproteins containing selenocysteine plays an important role in the GSH-dependent defense against peroxynitrite-mediated oxidations by serving as a peroxynitrite reductase [30].

GSH is a tripeptide with a gamma peptide linkage between the amine groups of cysteine and the carboxyl group of the glutamate side chain. It is an antioxidant, preventing damage to the important cellular components caused by reactive oxygen species such as free radicals and peroxides [31]. In the present study, the enhancement of GSH in *P. ostreatus* and *C. indica* mushroom diet-fed mice could be due to the tissue-specific efficiency of cysteine metabolism [32].

Vitamin C is also called as ascorbic acid, one of the important antioxidant acids, to maintain collagen in the skin, repair damaged tissue, promote healthy teeth and bones, and boost the immune system [33]. Vitamin C helps the body to absorb iron, which is also useful in treating iron deficiency and anemia [34]. Increased Vitamin C activity may due to the increase of GSH level because Vitamin C and GSH are synergistic antioxidants [35].

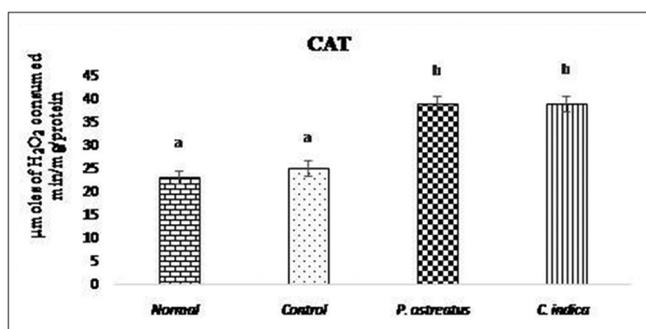


Fig. 3: Effect of normal, control, *Pleurotus ostreatus* and *Calocybe indica* diets on liver catalase activity of Swiss male albino mice. Bars not sharing a common superscript letter^(a,b,c) differ significantly at $p < 0.05$ Duncan multiple range test

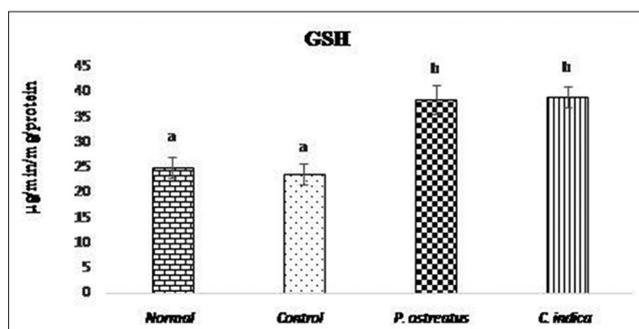


Fig. 5: Effect of normal, control, *Pleurotus ostreatus* and *Calocybe indica* diets on liver glutathione activity of Swiss male albino mice. Bars not sharing a common superscript letter^(a,b,c) differ significantly at $p < 0.05$ Duncan multiple range test

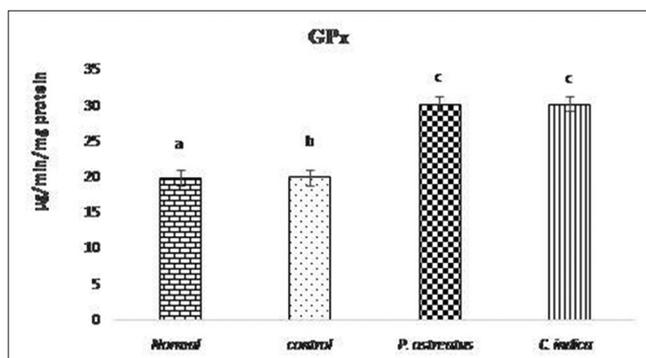


Fig. 4: Effect of normal, control, *Pleurotus ostreatus* and *Calocybe indica* glutathione peroxidase activity of Swiss male albino mice. Bars not sharing a common superscript letter^(a,b,c) differ significantly at $p < 0.05$ Duncan multiple range test

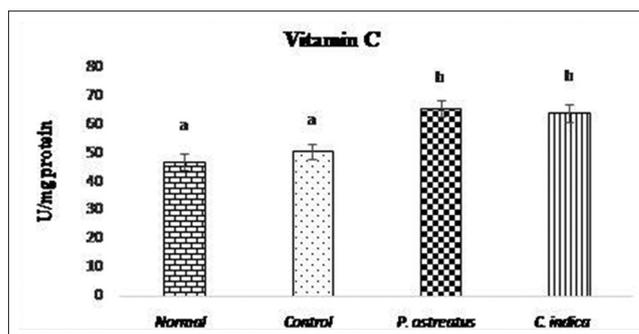


Fig. 6: Effect of normal, control, *Pleurotus ostreatus* and *Calocybe indica* diets on liver Vitamin C activity of Swiss male albino mice. Bars not sharing a common superscript letter^(a,b,c) differ significantly at $p < 0.05$ Duncan multiple range test

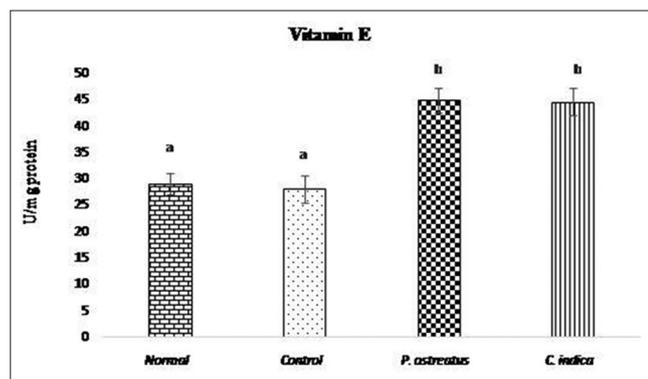


Fig. 7: Effect of normal, control, *Pleurotus ostreatus* and *Calocybe indica* diets on liver Vitamin E activity of Swiss male albino mice. Bars not sharing a common superscript letter^(a,b,c) differ significantly at $p < 0.05$ Duncan multiple range test

Vitamin E also protects the body from oxidation and normal aging process; the damage induced by diabetes and neutralizes the free radicals; when enough Vitamins E is present in the body, the unstable free radicals get their electrons from the Vitamin E molecules and leave the healthy molecules alone, thus causing less damage to tissues [36]. Decreased activity in respect of casein diet may due to the lipid soluble chain-breaking antioxidants in the biological system protecting cell membrane from peroxidative damage [20].

CONCLUSION

Mushrooms are an excellent food that can be used in a well-balanced diet containing all essential nutrients which are necessary for therapeutic benefits, easy way to escalate overall immunity, for their functional compounds, and low-fat content provides valuable nutrients to the human. The results of the present study demonstrated that the dietary supplementation with *P. ostreatus* and *C. indica* mushrooms enhances the nutritional value of the diet and antioxidants levels. *P. ostreatus* and *C. indica* diet consumption may increase innate immunity against many diseases. A regular intake of mushrooms can maintain our body in good physical condition, fine fettle, de-stress, and enhance the immune responses of the human body, thereby increasing resistance to disease and, in some cases, regression of a disease state. Nutritional and therapeutic benefits revealed that the *P. ostreatus* and *C. indica* are suitable for making nutraceuticals and would help to decrease the malnourishment suffered by millions across the world.

AUTHORS' CONTRIBUTIONS

Authors have contributed equally.

CONFLICTS OF INTEREST

We (authors) have no conflicts of interest.

REFERENCES

- Isabel CF, Ferreira PB, Miguel VB, Lillian B. Free-radical scavenging capacity and reducing power of wild edible mushrooms from Northeast Portugal: Individual cap and stipe activity. *Food Chem* 2007;100:1511-6.
- Barros L, Baptista P, Correia D, Casal S, Oliveira B, Ferreira IC. Fatty acid and sugar compositions and nutritional value of five wild edible mushrooms from Northeast Portugal. *Food Chem* 2007;105:140-5.
- Zhang M, Cu IS, Cheung P, Wang Q. Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and activity. *Trends Food Sci Technol* 2007;18:4-19.
- Swagiary A, Nath P, Basumatary B, Brahma D. Phytochemical, antioxidant, and trace element analysis of anthelmintic plants of North-East India. *Int J Pharm Pharm Sci* 2017;9:228-32.
- Ouzouni PK, Koller WD, Badeka AV, Riganakos KA. Volatile compounds from the fruiting bodies of three *Hygrophorus* mushrooms

- species from Northern Greece. *Int J Food Sci* 2009;44:854-9.
- Sullivan R, Smith J, Rowan N. Medicinal mushrooms and cancer therapy: Translating a traditional practice into Western medicine. *Perspect Biol Med* 2006;49:159-70.
- Babu D, Rao G. Antioxidant properties and electrochemical behavior of cultivated commercial Indian edible mushrooms. *J Food Sci Technol* 2013;50:301-8.
- Thekkuttuparambil A, Ajithkainoor K, Janardhanan KK. Indian medicinal mushrooms as source of antioxidant and antitumor agents. *J Clin Biochem Nutr* 2007;40:157-62.
- Lillian B, Soraia F, Paula B, Cristina F, Miguel V, Isabel CF. Antioxidant activity of *Agaricus* sp. mushrooms by chemical, biochemical and electrochemical assays. *Food Chem* 2008;111:61-6.
- Vasconcelos I, Maia A, Siebra E, Oliveira J, Carvalho A, Melo V, et al. Nutritional study of two Brazilian soybean (*Glycine max*) cultivars differing in the contents of anti-nutritional and toxic proteins. *J Nutr Biochem* 2001;12:55-62.
- Mattison JA, Roth GS, Lane MA, Ingram DK. Dietary restriction in aging non-human primates Interdiscip Top Gerontol 2007;35:137-58.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.
- Sadasivam S, Manickam A. *Biochemical Methods*. 2nd ed. New Delhi: New Age International; 2002.
- Association Official Analytical Chemists. *Official Methods of Analysis of AOAC International*. Gaithersburg: AOAC International; 2005.
- Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 1984;21:130-2.
- Sinha KA. Colorimetric assay of catalase. *Anal Biochem* 1972;47:389-94.
- Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hosekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. *Science* 1973;179:588-90.
- Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70-7.
- Omaye ST, Turbull TD, Sauberlich HC. *Selected Method for the Determination of Ascorbic Acid in Animal Cells, Tissues and Fluids*. New York: Methods Enzymol Academic Press; 1979.
- Meydani M. Vitamin E and prevention of heart disease in high risk patients. *Nutr Rev* 2000;58:278-81.
- Dayong W, Munkyong P, Zhuyan R, Donald S, Meydani NS. Dietary supplementation with white button mushroom enhances natural killer cell activity in C57BL/6 mice. *J Nutr* 2007;137:1472-7.
- Selvi P, Uma DP, Suja S, Murgan S, Chinnaswamy P. Comparison of non enzymic antioxidant status of fresh and dried form of *Pleurotus florida* and *Calocybe indica*. *Pak J Nutr* 2007;6:468-71.
- Ali K, Ilkay K, Huseyin G, Keles K. Antioxidant properties of wild edible mushrooms. *J Food Process Technol* 2011;3:2-6.
- Lull C, Wichers H, Savelkoul H. Anti inflammatory and immune modulating properties of fungal metabolites. *Med Inflamm* 2005;9:63-80.
- Pacher P, Beckman J, Liaudet L. Nitric oxide and Peroxynitrite in health and disease. *Physiol Rev* 2007;87:315-424.
- Jayakumar T, Ramesh E, Geraldine P. Antioxidant activity of the oyster mushroom (*Pleurotus ostreatus*) on CCl(4)-induced liver injury in rats. *Food Chem Toxicol* 2006;44:1989-96.
- Shamsi TN, Parveen R, Sajida A, Ahamed A, Fatima S. Assessing the therapeutic role of joshanda: Phytochemical, antioxidant, anti-inflammatory and antimicrobial activities. *Int J Pharm Pharm Sci* 2018;10:122-8.
- Kovacic P, Pozos RS, Somanathan R, Shangari N, Brien PJ. Mechanism of mitochondrial uncouplers, inhibitors, and toxins: Focus on electron transfer, free radicals, and structure-activity relationships. *Curr Med Chem* 2005;12:2601-23.
- Lee HY, Eum WS, Kim DW, Lee BR, Yoon CS, Jang SH, et al. Isolation and identification of an antioxidant enzyme catalase stimulatory compound from *Ganoderma lucidum*. *J Biochem Mol Biol* 2003;36:450-5.
- Kern JC, Kehrer JP. Free radicals and apoptosis: Relationships with glutathione, thioredoxin and the BCL family of Proteins. *J Biosci* 2005;10:1727-38.
- Pompella A, Visviki SA, Paolicchi A, De T, Casini AF. The changing faces of glutathione a cellular protagonist. *Biochem Pharm* 2003;66:1499-503.
- Yang B, Hill CE. Nifedipine modulation of biliary GSH and GSSG/conjugate efflux in normal and regenerating rat liver. *Arch Gastrointest Liver Physiol* 2001;281:85-94.
- Jacob RA. Passive smoking induces oxidant damage preventable by Vitamin C. *Nutr Rev* 2000;58:239-41.

34. Kojo S. Vitamin C: Basic metabolism and its function as an index of oxidative stress. *Curr Med Chem* 2004;11:1041-64.
35. Inga K, Maria M, Mirosława S, Anna G. Effect of storage on the content of polyphenols Vitamin C and the antioxidant activity of orange juices. *J Food Compos Anal* 2007;20:313-22.
36. Jawad A, Muhammad AA, Waqas A, Chaudhry MA, Jamil S. Effect of different substrate supplements on oyster mushroom (*Pleurotus* spp.) Production. *Food Sci Technol* 2013;1:44-51.