as a source of antioxidant was found to recover from CCl₄-induced damage, as evidenced by the presence of normal hepatocytes with well-defined nuclei (Asha et al., 2007). Pretreatment with an extract of the flower of Cassia siamea, a potent antioxidant, has also been reported to preserve the hepatic architecture, with only a few areas of hemorrhage between columns of hepatocytes in rats exposed to CCl₄ (Kaur et al., 2006). In addition, administration of the antioxidant compound echinacoside has been reported to ameliorate CCl₄-induced liver injuries (Wu et al., 2007). So also, in the present study, administration of an extract of the oyster mushroom Pleurotus ostreatus, as a source of antioxidant, appeared to prevent CCl₄-induced tissue damage in all vital organs; histopathological studies revealed that the hepatocytes and sinusoidal spaces of the liver, renal corpuscles of the kidneys, trabeculae of the heart and cortical cells of the brain were all normal in architecture.

5. Conclusion

Administration of an extract of the oyster mushroom Pleurotus ostreatus as a source of antioxidant appeared to protect the liver, kidneys, heart and brain of Wistar rats from CCl₄-induced acute oxidative stress by preventing structural alterations such as disruption of the structure of hepatocytes and sinusoidal spaces of the liver, renal corpuscles of the kidneys, trabeculae of the heart and cortical cells of the brain. These observations also suggest that the liver is not the only target organ of CCl₄, for it appears to cause damage in other organs as well.
Summary and conclusion

The potentially reactive derivatives of oxygen, collectively referred to as reactive oxygen species (ROS) are superoxide anion radicals (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (OH'). They are continuously generated within the human body as a consequence of exposure to a plethora of exogenous chemicals in the ambient environment such as cigarette smoke, pesticides, air pollutants, radiation and xenobiotics such as carbon tetrachloride (CCl$_4$). ROS are also generated through a number of endogenous metabolic processes involving the mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation, fertilization and ageing. The harmful effects of free radicals, which manifest as damage to biological systems, are collectively termed “oxidative stress” when caused by ROS and nitrosative stress when caused by reactive nitrogen species (RNS). Oxidative stress has been implicated as a crucial etiological factor in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process (Hogg, 1998; Pong, 2003).

Although almost all organisms are equipped with antioxidant defense and repair systems that have evolved to protect them against oxidative damage, these systems are often inadequate to completely prevent oxidative stress-induced damage (Simic, 1988). Therefore, antioxidant supplements, or natural products containing antioxidants, may be used to help reduce oxidative damage to the human body. Supplementation of the diet with natural products, such as fruits and vegetables, may provide protection against various diseases, such as cancer and various cardiovascular and cerebrovascular diseases (Li et al., 2005).
Mushrooms have been part of the normal human diet for thousands of years and, in recent times, the amounts consumed have risen greatly, involving a large number of species. The genus *Pleurotus* comprises 40 different species that are commonly referred to as “oyster mushrooms”. They are ubiquitous, being found both in temperate and tropical parts of the world, and are now considered to be the second most important cultivable mushroom in the world. Until now, research has tended to focus on the dietary value of edible mushrooms; however, there is relatively little information pertaining to the antioxidant activity and the possible use of such mushrooms to neutralise oxidative stress. Hence, the aim of the present thesis was to evaluate the putative antioxidant activity of the mushroom *Pleurotus ostreatus*. The evaluation was done in five phases:

In the first phase of the study, the antioxidant activity of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*, was investigated. By employing various established *in-vitro* test systems, the ability of the extract to scavenge free radicals, inhibit lipid peroxidation, and chelate ferrous ions, was evaluated; the reducing power of the extract was also determined. Potential antioxidant components of the mushroom, *P. ostreatus*, were also assayed. The extract of *P. ostreatus* showed concentration-dependent antioxidant activity by virtue of inhibiting lipid peroxidation, scavenging hydroxyl and superoxide radicals, quenching DBO, reducing power and chelating ferrous ions when compared with different standards, such as L-ascorbic acid, butylated hydroxyl toluene (BHT) and ethylene diaminetetra acetic acid (EDTA). The mushroom extract was found to contain a perceptible quantity of total phenols, in addition to other constituents such as ascorbic acid, α-tocopherol, β-carotene and flavonoid compounds.
rutin and chrysin); all of these probably contributed to the observed antioxidant activity of the extract.

In the second phase of the investigation, the putative antioxidant activity of the extract of *P. ostreatus* was evaluated in an experimental model of acutely-induced oxidative stress. This experimental state was created by exposure of Wistar rats to CCl₄, resulting in acute hepatotoxicity. The occurrence of CCl₄-induced acute oxidative stress in the liver was confirmed by noting a marked increase in the level of MDA and activities of liver marker enzymes such as SGOT, SGPT and SALP and activity of XDH, when compared to that of normal rats. Furthermore, a significant reduction was observed in the level of non-enzymatic antioxidants, such as reduced glutathione and vitamins C and E, and in the activities of primary and secondary antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (Gpx) and glutathione- S- transferase (GST), glutathione reductase (GR), ascorbate peroxidase (Apx) and glucose-6- phosphate dehydrogenase (G6PDH), respectively. In addition a decreased staining intensity of isozymes of antioxidant enzymes was also noted in the liver, kidneys, heart and brain of CCl₄-intoxicated rats. Administration of an extract of *P. ostreatus* appeared to protect the liver, kidneys, heart and brain of Wistar rats against CCl₄-induced acute oxidative stress (acute tissue toxicity) by reducing the intensity of lipid peroxidation and the activities of liver marker enzymes and XDH. In addition, administration of the extract brought about an increase in activities of enzymatic and the levels of non-enzymatic antioxidants and an increase in staining intensity of isozymes of antioxidant enzymes. This phase of the study is important in presenting data suggesting considerable promise for the mushroom *P. ostreatus* as a protective agent in CCl₄- induced acute oxidative stress in Wistar rats.
In the third phase of the study, the putative antioxidant activity of the *P. ostreatus* extract was evaluated in a chronic model of oxidative stress, that is, in aged Wistar rats. The occurrence of oxidative stress in the aged rats was revealed by a marked increase in the level of MDA, protein carbonyl (PCO) and activity of XDH and by a reduction in the activities of enzymatic and levels of non-enzymatic antioxidants. In addition, a decreased staining intensity of isozymes of antioxidant enzymes was also noted in major tissues (liver, kidneys, heart and brain) of aged rats when compared to young rats. However, when aged rats were treated with an ethanolic extract of *P. ostreatus*, there was a reduction in the levels of MDA and PCO and in activity of XDH and an increase in activity of enzymatic antioxidants and an increase in levels of non-enzymatic antioxidants in addition to increased staining intensity of the isozymes of the antioxidant enzymes. The observed alterations in the staining intensity of the isozymes of the antioxidant enzymes suggest the possible effect of *P. ostreatus* extract on the antioxidant defense system (ADS) gene. The results generated from this phase of the study strongly suggest that the mushroom extract conferred some degree of protection against oxidative stress in Wistar rats during ageing.

In the fourth phase of the investigation, the putative effect of the *P. ostreatus* extract on the gene expression of the antioxidant enzyme, catalase (CAT), during ageing was investigated. A decrease in the expression of the catalase gene in the liver and kidneys of aged rats, when compared to young rats was observed. Treatment of aged rats with the *P. ostreatus* extract caused a remarkable increase in the expression of the CAT gene in the liver and kidney tissues, when compared to the expression of the gene in these tissues of aged untreated rats. This result suggest that administration of
the mushroom extract to aged rats can upregulate the gene expression of the antioxidant enzyme catalase. This possibly contributes to the anti-ageing action of the extract of *P. ostreatus*.

In the final phase of the study, histoarchitectural changes were observed in various organs of the acute CCl₄-intoxicated rats, such as marked disruption of the structure of hepatocytes and sinusoidal spaces of the liver, renal corpuscles of the kidneys, trabeculae of the heart and cortical cells of the brain. These observations suggest that the liver is not the only target organ of CCl₄, and that it causes oxidative stress in other tissues such as the kidneys, heart, and brain. Only minimal disruption of the structure of the hepatocytes was noted in liver tissue of Group III rats (exposed to CCl₄ and mushroom extract); this minimal disruption of the hepatocyte structure was consistent with the results of the liver enzyme studies wherein Group III rats, SGOT, SGPT and SALP activities and MDA levels approximated to the levels seen in normal (Group I) rats.

In conclusion, administration of an extract of the oyster mushroom, *Pleurotus ostreatus*, appeared to protect the liver, kidneys, heart and brain of CCl₄-intoxicated and of aged Wistar rats against oxidative stress by reducing the intensity of lipid peroxidation and protein oxidation and by enhancing the activities of enzymatic and levels of non-enzymatic antioxidants. The increased gene expression of the antioxidant enzyme, catalase, in aged rats and the minimal histoarchitectural changes in acute CCl₄-intoxicated rats following administration of the mushroom extract represent notable findings of this thesis. The antioxidant principles identified, such as ascorbic acid, α-tocopherol, β-carotene and flavonoid compounds (rutin and chrysin), in the
mushroom extract possibly contributed to the observed effects. The results of the present investigation suggest that an ethanolic extract of the oyster mushroom, *P. ostreatus*, has potent antioxidant activity. Since this mushroom is easily available, it could readily be incorporated into the diet as a rich source of antioxidants; it could also conceivably be developed into a food supplement or pharmaceutical agent to treat oxidative stress-induced diseases.


Cimanga, K., Ying, L., De Bruyne, T., Apers, S., Cos, P., Hermans, N., Bakana, P.,
Tona, L., Kambu, K., Kalenda, D.T., Pieters, L., Vanden Berghe, D. and
Vlietinck, A.J. 2001. Radical scavenging and xanthine oxidase inhibitory
activity of phenolic compounds from Bridelia ferruginea stem bark. J.
Pharm.Pharmacol. 53:757-761.


in tumour promotion of mouse epidermal JB6 cells. In: Nyggard.O.F. (Ed.).
Proceeding of the Second Conference on Anticarcinogens and Radioprotectors,

analysis of the flavonoid content of commercial tomatoes, onions, lettuce and

Advances in Basic Science, Diagnostics and Intervention. (Eds.), World

Czinner, E., Hagymasi, K., Blazovics, A., Kery, A., Szoke, E. and Lemberkovics,
J. Ethanopharmacol. 77:31-35.

Davey, M. W., van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A. and
Smirnoff, N. 2000. Plant L-ascorbic acid: Chemistry, function, metabolism,

de Groot, H. and Rauen, U. 1998. Tissue injury by reactive oxygen species and

Quantitative analysis of flavan-3-ols in spanish foodstuffs and beverages.

Deiana, M., Aruoma, O.I., De Lousdes, P., Bianchi, M., Spencer, J.P.E., Kaur, H.,
Halliwell, B., Aeschbach, R., Banni, S., Dessi, M.A. and Corongiu, F.P.
1999. Inhibition of peroxynitrite dependent DNA base modification and
tyrosine nitration by the extra virgin olive oil-derived antioxidant

Modulation of the peroxisomal gene expression pattern by
ehydroepiandrosterone and vitamin D: therapeutic implications.
J Endocrinol.175:779-792.


xiv


xxxiii


