Since ancient time, mushrooms have been cultivated worldwide for their taste, nutritional supplements and potential application as drugs. In present scenario, some bioactive constituents of mushrooms have attracted attention in the fields of chemistry, biochemistry, microbiology, and pharmacology. Mushrooms have received increasing attention from the researchers in food and pharmaceuticals. Nowadays there is an increasing public interest in the secondary metabolites from mushrooms for discovering new drugs or lead compounds.

In recent years, increased interest in human health, nutrition and disease prevention has enlarged consumer demand for functional foods. In fact, mushrooms have become attractive as functional foods and as a source of bioactive compounds. Mushrooms are world wide appreciated for their taste and flavour and are consumed both in fresh and processed form. Mushrooms constitute an integral part of the normal human diet; these are considered as valuable health foods since they are low in calories, fats and essential fatty acids and high in vegetable proteins, vitamins and minerals, but they contain appreciable amounts of dietary fibre, particularly important for the regulation of physiological functions in the human organism. Studies have demonstrated that the regular consumption of mushrooms or consumption of isolated bioactive constituents present in mushrooms is beneficial to health. Mushrooms may thus be considered as functional food or nutraceutical product. Mushrooms accumulate a variety of secondary metabolites such as phenolic compounds, polyketides, terpenes and steroids possibly involved in their medicinal effects and functional values.

The present investigation was undertaken to evaluate the therapeutic potential of *Hypsizygus ulmarius* aqueous extract (HUAE) in various models of toxicity. The objective of the study was also to assess the phytochemical constituents and the scavenging ability of aqueous extracts from fruiting bodies, mycelium and culture filtrate of *H. ulmarius* by different *in vitro* model systems.
The fresh fruiting bodies at various stages and mycelium were analyzed for enzymic, non-enzymic and phytochemical constituents. The fresh mycelium and fruiting bodies were found to be rich sources of enzymic, non-enzymic antioxidants and bioactive substances.

The various aqueous extracts were analysed for *in vitro* antioxidant scavenging assays. The antioxidant activities were determined by DPPH, ABTS, OH scavenging assay, reducing power, FRAP assay, chelating ability, total antioxidant activity by phosphomolybdenum assay and lipid peroxidation inhibition assay. All the analyses were done in triplicates and average values were taken.

DPPH radical is scavenged by the antioxidants through the donation of a proton forming reduced DPPH. EC$_{50}$ values of the DPPH radical scavenging activity of various extracts of mushroom ranged from 5.54 to 6.77 mg/ml. The better scavenging ability of might be due to more hydrogen donating components extracted by mushroom and mycelium.

ABTS assay measures the relative antioxidant ability to scavenge the radical ABTS$^+$ and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and chain breaking antioxidants. EC$_{50}$ values of the ABTS radical scavenging activity of various extracts of mushroom ranged from 6.55 to 7.55 mg/ml. The good antioxidant activity on ABTS radicals may be attributed to a direct role in trapping free radicals by donating hydrogen atom or electron.

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. EC$_{50}$ values of hydroxyl radical scavenging abilities of various extracts of mushroom ranged from 3.03 to 3.53 mg/ml. These results suggest that the extracts are capable of scavenging hydroxyl radicals and could prevent or ameliorate oxidative damage.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The EC$_{50}$ values of various extracts of mushroom in the ferrie cyanide (Fe$^{3+}$) reducing antioxidant power assay ranged from 13.93 to 32.00 mg/ml. The reducing properties of mushrooms are generally associated with the presence of reductones and they exert antioxidant action by breaking the free radical chain by donating hydrogen atoms. FRAP assay measures the antioxidant effect of any
substance in the reaction medium as reducing ability. The EC\textsubscript{50} values of various mushroom extracts in the FRAP assay ranged from 9.43 to 19.55 mg/ml.

Metal chelating activity is claimed as one of the antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. EC\textsubscript{50} values of the chelating abilities of various extracts of mushroom on ferrous ions ranged from 4.91 to 8.71 mg/ml. The various extracts exhibit good chelating activity on ferrous ions.

Lipid peroxidation, a process induced by free radicals, leads to oxidative deterioration of polyunsaturated lipids. LPO inactivates cellular components and therein plays a key role in oxidative stress in biological systems. EC\textsubscript{50} values of the LPO inhibition in rat liver homogenate of various extracts of mushroom ranged from 2.43 to 3.45 mg/ml. The various polysaccharide extracts could inhibit lipid peroxidation by scavenging the OH\textsuperscript{−} or O\textsubscript{2}\textsuperscript{−} radicals or by chelating the iron itself.

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex. Various extracts of mushroom exhibited higher absorbance indicating a high antioxidant activity.

The phenolic content of various extracts of HUAE varied from 1.98 to 3.87 mg/g CE and 4.50 to 8.77 mg/g GAE respectively. The flavonoid content varied from 3.04 to 7.03 mg/g CAE and 8.32 to 19.20 mg/g RE respectively. Phenolic compounds are known to be powerful chain breaking antioxidants. The antioxidant activity of mushroom extract is well correlated with the content of their phenolic and flavonoid compounds.

The various extracts from fruiting bodies of H. ulmarius showed concentration dependent antioxidant activity by virtue of scavenging DPPH, ABTS and OH radicals, inhibition of lipid peroxidation, reducing power and chelating ferrous ions. The various antioxidant mechanism of the mushroom extract may be attributed to strong hydrogen donating ability, metal chelating activity, scavengers of hydroxyl radicals and inhibition of lipid peroxidation. The mushroom extract was found to contain a perceptible amount of total phenols, flavonoids and polysaccharides all of which probably contributed to the observed antioxidant activity. The results of the
present study suggest that various extracts of the mushroom, could serve as an easily accessible item of food rich in natural antioxidants, as a possible food supplement or even as a pharmaceutical agent.

**Acute toxicity studies**

The current study was performed to determine the acute toxicity of aqueous extract of *H. ulmarius*. The rats were orally treated with 1000, 2000, 3000 mg/kg b.wt mushroom extracts for 30 days. After the treatment, clinical signs and body weight change were observed for 30 days. All the animals survived during the study and did not produce any sign of toxicity or death in rats during 30 days of observation.

Acute toxicity study revealed that *H. ulmarius* did not induce any toxic effects and changes in serum biochemical constituents, enzymes, lipid profile, antioxidants in liver, hematological parameters and histopathological alterations. The data suggest that the oral administration of HUAЕ could stand as an assurance for the medicinal use of this mushroom.

**Cardioprotective effect**

Myocardial infarction (MI) remains the major cause of death in the developed world and a major pathological issue worldwide despite the rapid advances that have been made in the treatment of coronary artery disease. Isoproterenol (ISO), a synthetic catecholamine and an important regulator of myocardial contractility and metabolism serves as a standard model to study the beneficial effect of many drugs on cardiac function. Cardioprotective effect of HUAЕ was evaluated in isoproterenol induced myocardial infarction in rats. ISO induced myocardial damage was indicated by increased activities of marker enzymes such as creatine kinase, aspartate transaminase, alanine transaminase, lactate dehydrogenase, levels of troponin-T and uric acid in the serum. The levels of lipid peroxidation products in heart were significantly increased and the activities of enzymic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase in the heart and non-enzymic antioxidants such as glutathione and vitamin C in heart were significantly decreased in ISO induced rats. A significant increase in the levels of hexose, hexosamine and sialic acid in the heart was observed in ISO induced rats. The activity of Na⁺/K⁺ ATPase was decreased
significantly and the activities of Ca$^{2+}$ and Mg$^{2+}$ ATPases were increased significantly in the heart of ISO-induced rats. Treatment with HUAE (500 and 1000 mg/kg b.wt) significantly decreased the marker enzymes in serum, increased the activities of enzymic and non-enzymic antioxidants, decreased the lipid peroxide, hydroperoxides, glycoprotein components, restored the biochemical constituents and ATPases when compared with ISO treated group. HUAE pretreatment showed significant protective effect on all the biochemical parameters supported by histopathological findings. Thus, HUAE protects the myocardium against isoproterenol induced oxidative stress.

**Hypolipidemic effect**

Obesity, characterized by the accumulation of excess body fat, has now become the leading metabolic disease and the largest health problem worldwide, particularly in developed countries. Hyperlipidemia has been incriminated as a contributory factor of atherosclerosis. The study evaluated the hypolipidemic effect of HUAE and a lipid lowering drug, lovastatin in rats fed a high fat diet for 4 weeks. At doses of 500 and 1000 mg/kg, oral administration of HUAE effectively reduced serum and hepatic total cholesterol, triglycerides, serum low density lipoprotein cholesterol, while increased the serum high density lipoprotein cholesterol. Furthermore, high cholesterol diet induced oxidative stress in rats but HUAE significantly increased the decreased activities of enzymic antioxidants and levels of non-enzymic antioxidants and decreased the raised malondialdehyde levels in liver. HUAE has lipid lowering effect similar to the drug lovastatin. Histopathological evaluation of the liver revealed that HUSE reduced the lipid accumulation in the liver. The study revealed that HUAE had significant health benefits and could be explored as a potentially promising food additive for the prevention of hyperlipidemic diseases.

**Antidiabetic effect**

Diabetes mellitus is a chronic metabolic disease and has been considered a major health risk in the world. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. The aim of the study was to determine the antidiabetic property of
HUAE in streptozotocin and nicotinamide induced diabetes in rats. The diabetic rats were treated orally with HUAE at the doses of 500 and 1000 mg/kg b.wt for 14 days. The levels of glucose, insulin, total protein, hemoglobin, glycated hemoglobin, liver glycogen, carbohydrate metabolic enzymes, hepatic function markers, lipid peroxides and antioxidants were analyzed. The levels of all biochemical constituents and activities of all enzymes were restored significantly compared to diabetic control rats due to treatment with HUAE extract. HUAE showed antihyperlipidemic activity as evidenced by significant decrease in serum total cholesterol, triglycerides, phospholipids, VLDL-C coupled with elevation of HDL-C levels in treated rats. The effect of HUAE was compared with glibenclamide, a well-known antihyperglycemic drug. The above findings were supported by histological observations of the liver and kidney. The present study indicates that the HUAE possessed significant antidiabetic effect. From the results it can be concludes that the H.ulmarius aqueous extract showed antidiabetic, antihyperlipidemic and antioxidant effect which could exert a beneficial action against pathological alteration caused by the presence of superoxide and hydroxyl radicals in streptozotocin induced diabetes.

**Hepatoprotective effect**

Liver diseases are considered to be serious health problems, as the liver is an important organ for the detoxification and deposition of endogenous and exogenous substances. An organic lipid hydroperoxide analogue, Tert-butyl hydroperoxide (t-BHP) is used as pro-oxidant to evaluate mechanisms involving oxidative stress in cells and tissues. The protective effect of HUAE on liver damage was evaluated using the model of tert-butyl hydroperoxide (t-BHP) induced acute hepatic damage in rats. Intraperitoneal administration of t-BHP resulted in significantly elevated serum and hepatic levels of AST, ALT, ALP and LDH compared to control rats. The activities of SOD, CAT, GPx, GST, GR, G6PDH were lowered in the liver of t-BHP administered rats. Elevated levels of lipid peroxides, hydroperoxides and lowered levels GSH, vit C were observed following t-BHP administration. Oral pretreatment with HUA (500 and 1000 mg/kg b.wt) and silymarin (30 mg/kg b.wt) significantly and dose-dependently lowered the serum and liver marker enzymes, increased the activities of
enzymic and non-enzymic antioxidants, decreased the lipid and hydroperoxides and restored the biochemical constituents when compared with t-BHP treated group. The activity of HUAE at the dose of 1000 mg/kg was markedly pronounced and comparable to the standard drug, silymarin. The histopathological evaluation of the rat livers showed that HUAE reduced the incidence of liver lesions induced by t-BHP in rats. On the basis of the results of this study, it can be speculated that HUAE protects liver against t-BHP induced hepatic damage in rats.

The contents of the extract not only protected the integrity of plasma membrane but, at the same time increased the regenerative and reparative capacity of the liver. The mechanisms for the protective effects of PEAE included well documented inhibitory effects and antioxidative actions. Beneficial effect of the mushroom extract may be due to the presence of some phenolic components that have membrane stabilizing effects. These results suggest that the compounds present in the mushroom extract efficiently works on the liver to keep it normally functioning and minimizing cell membrane disturbances and is able to alleviate significantly the hepatotoxicity induced by t-BHP in the rat.

**Anti-inflammatory effect**

Inflammation is a natural host defensive process in the innate immunity response. An inflammatory response implicates macrophages and neutrophils, which secrete a number of mediators responsible for the initiation, progression and persistence of the acute or chronic state of inflammation. The anti-inflammatory activity of HUAE at a concentration of 500 and 1000 mg/kg b.wt was evaluated in carrageenan induced acute inflammation in rats. Inflammation induced animals showed decreased level of protein and elevated levels of nitric oxide in serum. A significant increase in the level of lipid peroxide and hydroperoxides, with the depletion of antioxidants was observed in intoxicated rats. A significant decrease in the level of Hb, RBC count, PCV and increase in WBC and platelets was observed in carrageenan induced rats. The carrageenan induced acute
inflammation were significantly inhibited by HUAE extract as evident from the inhibition of the paw edema. The altered hematological and biochemical parameters were restored to near normal after treatment with HUAE. This study revealed the anti-inflammatory activity of HUAE. The extract showed significant dose dependent inhibition of acute inflammation as compared to that of the standard drug, diclofenac. The results suggest that anti-inflammatory activity of HUAE is possibly attributed to its free radical scavenging properties. The mechanism of the effect may be due to the presence of flavonoids and phenols and polysaccharides in the extract. In conclusion, the aqueous extract of *H. ulmarius* exhibited significant anti-inflammatory activity in mice. The extract also possessed significant antioxidative activity. However, the findings suggest the therapeutic potentials of the aqueous extract of this mushroom for the prevention and the control of inflammation and diseases mediated through oxidative stress.

**Phytochemical screening**

The preliminary phytochemical analysis of HUAE showed the presence of alkaloids, tannins, steroids, flavonoids, phenol, saponins, carbohydrates and proteins. The extract revealed the presence of phenols and flavonoids by HPTLC. The HPTLC profile showed the presence of four phenolic compounds, two flavonoid compounds and three organic acids in aqueous extract.

**Isolation and characterization of polysaccharides**

The polysaccharide was characterized by $^1$H NMR and $^{13}$C NMR. The total carbohydrate content in the fruiting body polysaccharide was found to be 42.61 mg/g. The IR, $^1$H NMR, $^{13}$C NMR spectrum strongly supports the expected neutral polysaccharide molecule which has been isolated.

To our knowledge, this is the first report to demonstrate the antioxidant activity and the wide application of this mushroom in the treatment of various diseases. This mushroom could be explored and investigated further in view of its potential as a therapeutic agent.

Therefore, HUAE could be a potential source of natural antioxidants, and the consumption of mushrooms might give certain level of health protection against oxidative damage. With the established antioxidant
activity of these mushroom extracts, the chemical characteristics of the antioxidative components in the extracts should be further investigated. Thus, more detailed work is necessary to isolate and quantify additional compounds from these extracts.

In conclusion, the results of the in vitro antioxidant measurement demonstrated that HUAE has antioxidant activities in all the assay systems. In vivo experiments showed that administration of mushroom extract appeared to protect liver, kidney and heart against oxidative stress. Phenolic compounds and polysaccharides provide a good source of dietary antioxidants that could offer potential protective effects against lipid oxidation. The promising antioxidant, cardioprotective, hypolipidemic, antidiabetic, hepatoprotective and anti-inflammatory effects demonstrated in this study may open new avenues in the treatment of various disorders and its complications. The findings of this study suggested that H. ulmarius could be explored as a novel and potential natural antioxidant for use in functional foods or medicine.

Basidiomycota species are still unknown, wild and cultured higher fungi represent a major source of novel metabolites. They may bring a paradigm shift in our medical way to maintain health in patients, not only focusing on treating the problem when it occurs, but also by preventing its occurrence. They generate extreme molecular diversity of the major types of compounds, notably polysaccharides. Bioactive metabolites from mushrooms will be indubitably used as pharmacological tools to investigate and better understand human physiology and physiopathology as well as mechanism of drug action. Mushroom metabolites defining new generations of pharmacologically active compounds, should definitely help and fill some of the weaknesses of current therapeutic arsenal and develop it against present and future therapeutic challenges.

**Suggestions for future research**

1. Isolation and characterization of phenolic compound from the fruiting bodies and mycelium.
2. Isolation and characterization of polysaccharides from the fruiting bodies and mycelium.
3. Free radical scavenging and therapeutic potential of isolated phenolic compounds and purified polysaccharides from the mushroom and mycelium.