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Hypolipidemic activity of *Phellinus rimosus* against triton WR-1339 and high cholesterol diet induced hyperlipidemic rats

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ABSTRACT

Patients with the risk for atherosclerotic disease will be targeted to reduce the existing hyperlipidemia. The hypolipidemic activity of *Phellinus rimosus* was studied using triton WR-1339 and high cholesterol diet (HCD) induced models. The triton induced elevated lipid profile was attenuated by *P. rimosus* or standard drug atorvastatin. Similarly, administration of *P. rimosus* along with HCD significantly decline serum triglyceride, total cholesterol, low-density lipoprotein, with elevating the high-density lipoprotein. Thiobarbituric acid reacting substances in heart and liver significantly decreased; where as activity of enzymatic antioxidants and level of reduced glutathione were significantly increased. In both models, *P. rimosus* extract showed a significant ameliorative effect on the elevated atherogenic index as well as LDL/HDL-C ratio. The hypolipidemic activity of *P. rimosus* can be ascribed to its inhibitory effect on the liver HMG CoA reductase activity. The results suggest the possible therapeutic potential of this fungus as hypolipidemic agent.

1. Introduction

Hypercholesterolemia and hypertriglyceridemia are major risk factors either, alone or together for the development of coronary artery disease and the progression of atherosclerosis (García-Fuentes et al., 2000; Lusis, 2000). High levels of low-density lipoprotein (LDL) accumulate in the sub endothelial space of arteries and are highly atherogenic and toxic to vascular cells thereby leading to atherosclerosis (Lusis, 2000). Furthermore, free-radical-mediated peroxidative modification of polyunsaturated fatty acids of LDL and very-low-density lipoprotein (VLDL) is thought to be contributed in the progression of atherosclerotic lesions (Minhajuddin et al., 2005). However, high density lipoprotein cholesterol (HDL-C) is an anti-atherogenic fraction. Triacylglycerols (TAGs) may also be a risk factor, especially in individuals with diabetes (West et al., 1983).

In hyperlipidemic conditions, enzymatic as well as non-enzymatic antioxidative defence systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ascorbic acid and reduced glutathione (GSH) are altered leading to ROS mediated damage (Araujo et al., 1995). Oxidative stress is an early event in the evolution of hyperlipidemia, and it has been suggested that appropriate support for enhancing antioxidant supply in subjects with abnormally elevated lipid levels can attenuate the course of the disease. The cause of hyperlipidemia has been thought to be related to increased

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lipid synthesis, decreased lipid clearance from the blood or a combination of these two processes. Consequently, one method to lower blood lipid levels would be to inhibit the synthesis of cholesterol or triglyceride. Such agents have been developed and currently serve as therapeutics for hyperlipidemia. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol synthesis such as statins: These drugs effectively lower the serum cholesterol levels and are widely used to treat patients with hypercholesterolemia. These lipid lowering drugs that are used to treat hyperlipidemia are known to possess side effects. Therefore, there is need to have drugs with lipid lowering and antioxidant activities with no side effects. Natural products are the best claimed option.

Edible mushrooms have been prescribed in Oriental medicine due to their hypocholesterolemic effects (Sun et al., 2007). In general, the intake of edible mushroom reduces the cardiovascular risk (Mori et al., 2008). The hypocholesterolemic effect of edible mushrooms has been reported, in an early work by Kaneda and Tokuda (1966), they studied cholesterol lowering properties of *Lentinus edodes*, *Auricularria polytricha*, *Flammulina velutipes* and *Agaricus bisporus*. Additionally, investigations by Bobek et al. (1991) focused on the hypocholesterolemic effects of *Pleurotus ostreatus*, which revealed suppression of the activity of HMG-CoA reductase in both normocholestolemic and hypercholesterolemic animal models. Indeed, inhibitors of HMG-CoA reductase have been isolated from different mushrooms such as eritadenine from *L. edodes* (Chibata et al., 1969) and meviolin from *Pleurotus* sp. (Gunde-Cimerman, 1999).

Phellinus is a large and widely distributed genus of the family Hymenochetaceae (Donk). Some of the species of *Phellinus* are extensively studied in China, Japan and Korea especially *Phellinus linteus*, which has been considered to be an important traditional Chinese medicine (Ying et al., 1987). *Phellinus rimosus* is a parasitic host specific polypore mushroom often found growing on jack fruit tree trunks in Kerala. It is a less extensively studied species of the genus *Phellinus*. Investigations on the pharmacological activities of *P. rimosus* are fragmentary. Presence of antioxidant, anticancer and antidiabetic activities of *P. rimosus* has been reported (Ajith and Janardhanan, 2002, 2011; Rony et al., 2013). No studies evaluating the hypolipidemic effects *P. rimosus* have been reported so far. Therefore, this study was aimed to determine the hypolipidemic effects of *P. rimosus* in triton WR-1339 and high cholesterol diet induced hyperlipidemic rats.

2. Materials and methods

2.1. Chemicals

Hydrogen peroxide (H_2O_2), dihydrogen potassium phosphate anhydrous (KH_2PO_4), and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Merck India Ltd, Mumbai. Sodium azide (NaN_3), reduced glutathione (GSH), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), nitroblue tetrazolium (NBT), and riboflavin were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai. Folin's phenol reagent was purchased from Qualigens, Mumbai, India and Triton WR-1339 (Tyloxapol) from Sigma Chemical Company, Saint Louis, MO, USA. All other chemicals used were of analytical reagent grade.

2.2. Animals

Male Wistar albino rats (180 ± 25 g) were used for the study. The animals were purchased from Small Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India and were kept for a week under environmentally controlled conditions with free access to standard food and water *ad libitum*. The experiment was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India and approved by Institutional Animal Ethics Committee, Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala, India.

2.3. Preparation of the extract

Sporocarps of *P. rimosus* growing on the jack fruit tree trunks were collected from the out skirts of Thrissur, Kerala. The type specimen was deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai, India (HERB.MUBL.3171). The fruiting bodies of the mushroom were cut into small pieces, dried at 40–50 °C for 48 h and powdered. Hundred grams of powdered fruiting bodies of the mushroom were extracted with 70% (v/v) ethanol on a boiling water bath for 48 h. The extraction was repeated again. Extracts pooled and evaporated under vacuum and finally lyophilized. The residue (4%) was suspended in distilled water and employed for the experiments.

2.4. Hypolipidemic effect against Triton WR-1339 induced hyperlipidemia

Animals were divided into five groups consisting of six animals each and treated as follows. Group I treated with vehicle (distilled water) served as normal, in all other groups (II to V) hyperlipidemia was induced by a single intraperitoneal (ip) injection of triton WR 1339 (300 mg/kg body wt) dissolved in normal saline (pH 7.4) (Okazaki et al., 1990). Group II was kept as control, without any further treatment. Group III and IV were treated with *P. rimosus* extract 50 and 250 mg/kg body wt Group V was treated with atorvastatin 2.5 mg/kg body wt. The extract and atorvastatin were administered by oral gavage one hour before the triton administration. Animals were sacrificed 24 h after the administration. Blood was collected directly from the heart of each animal and the serum was separated and used for the estimation of serum lipid profile. Liver samples were removed and stored at −70° until analysis could be completed.

2.5. Hypolipidemic effect against high cholesterol diet induced hyperlipidemia

Animals were divided into five groups consisting of six animals each and treated as follows. Group I animals received chow diet and treated with vehicle (distilled water p.o) were kept as normal. All other groups of animals were fed with