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Antioxidant activity of aqueous extract of *Monascus* fermented Indian variety of rice in high cholesterol diet fed-Streptozotocin diabetic rats, an in vivo study

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ABSTRACT

The present study was designed to investigate antioxidant activity of aqueous extract of *Monascus* fermented Indian variety of rice in high cholesterol diet fed- streptozotocin (STZ) induced diabetic rats. The Indian variety rice IR-532-E-576 was fermented with *Monascus purpureus* for 10 days and sterilized. High cholesterol fed-STZ-induced diabetic rats received aqueous extract of *Monascus* fermented Indian variety of rice at the concentration of 1.2 g / Kg of bw and 2.4 g / Kg bw daily for 30 days. The oxidative parameters like lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase activities were assessed. High cholesterol diet and STZ increased the level of LPO and reduced the level of SOD, GSH and catalase, where as on administration of aqueous extract of *Monascus* fermented Indian variety of rice reduced the level of LPO to almost normal and increased the levels of GSH, SOD and catalase dose dependently. The present study concluded that consumption of *Monascus* fermented rice can be good dietary supplement for diabetes and hyperlipidemia.

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1. Introduction

Oxidative stress has been one of the mechanisms for molecular and cellular tissue damage mechanisms. Oxygenated compounds, especially aldehydes such as melondialdehyde and conjugated dienes, are produced during the attack of free radicals to membrane lipoproteins and polyunsaturated fatty acids. Thus oxidative stress plays an important contributory role in the process of aging and pathogenesis of numerous diseases like diabetes, cancer, neurodegenerative diseases, and respiratory tract disorders [1]. Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose level caused by insulin deficiency, often combined with insulin resistance [2]. Diabetes mellitus leads to abnormalities in carbohydrate and lipid metabolism which results in excessive production of reactive oxygen species and oxidative stress [3, 4, 5, 6, 7]. Hyperlipidemia is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood. According to latest studies

hyperlipidemia leads to oxidative stress [8]. Enzymic antioxidants like superoxide dismutase and glutathione peroxidase and nonenzymic antioxidants play an important role in preventing the tissue damage due to the formation of free radicals. Usage of antioxidants helps in reducing risks of oxidative damages in diabetic and hyperlipidemic patients [9, 10]. RYR is a fermented rice product produced traditionally by fermenting cooked rice kernels with yeast *Monascus* sp. The use of red yeast rice in China dates back to first century. RYR was mentioned in ancient Chinese pharmacopoeia of medicinal food and herbs, where it is given as a medicine for digestion and revitalization [11]. It contains six pigments and other metabolic products from *Monascus* sp., such as alcohols, organic acids, and substances with a wide range of biological and therapeutic benefits, including anti-carcinogenic, anti-oxidative, and hypolipidemic activities [12]. As both diabetes mellitus and hyperlipidemia leads to oxidative stress, antioxidant benefits of RYR can be utilized in reducing the risks due to oxidative stress. In our present work antioxidant property of *Monascus* fermented Indian variety of rice was studied in diabetes induced-high cholesterol fed rats.

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2. Materials and Methods

2.1. Production of *Monascus fermented rice*

Monascus fermented rice was produced by solid state fermentation of an Indian variety of rice with *Monascus purpureus* at the concentration of 107 spores/ mL for 10 days at 30°C. Fungal culture of *Monascus purpureus* MTCC 1090 was obtained from The Institute of Microbial technology, Chandigarh, India.

2.2. Preparation of *Monascus fermented rice extract*

The dried *Monascus purpureus* fermented Indian rice was crushed and used for extraction with water by boiling at 100°C for 4 hours. Extract was filtered and used for further studies [13,14].

2.3. Evaluation of anti-oxidant activity

2.3.1. Experimental animal model

Male Wistar Albino rats weighing 200-250g were used in the present study. All rats were kept at room temperature of 22°C in the animal house. All animal procedures were performed after approval from the institutional animal ethical committee of Kovai medical centre research and educational trust (Approval no:KMCRET/M.Pharm/03/2008). All the animals were maintained in accordance with the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions.

2.4. Induction of diabetes

Diabetes was induced in overnight fasted adult Wistar Albino rats weighing 200-250g by single intra peritoneal injection of 60 mg/kg of STZ dissolved in citrate buffer (pH 4.5). Hyperglycemia was confirmed by elevated glucose levels in plasma, determined at 72 hours and then on day 7 after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as >126mg/dL. Only rats found with permanent NIDDM (except for control group) were selected for the antioxidant study and are fed with high cholesterol diet.

2.5. Experimental design

Thirty animals were divided into five groups, each consisting of six animals. Group 1 animals were non diabetes induced, received normal diet (Control group). Group 2 animals were diabetes induced fed with high cholesterol diet and received oral administration of 2 mL of sterile water. Group 3 and 4 were diabetes induced, fed with high cholesterol diet and received oral administration of *Monascus fermented rice* extract at the dose of 1.2 and 2.4 mg/kg bw respectively in 2 mL of sterile water for 30 days. Group 5 was diabetic induced and fed with high cholesterol diet and received oral administration of reference hypoglycemic drug with antioxidant activity glibenclamide 10 mg/kg bw in 2 mL of sterile water for 30 days. In group 2,3,4 and 5 diabetes was induced by administration of streptozotocin at dose 60mg/kg body weight, intra peritoneally.

2.6. In vivo antioxidant activity

2.6.1. Preparation of tissue homogenate

The tissues were weighed and 10%w/v tissue homogenate was prepared by mincing and homogenizing the tissues in 0.1 M

phosphate buffer (pH 7.5). After centrifugation at 10,000 rpm for 10 minutes, the clear supernatant was used for the estimation of non-enzymatic and enzymatic antioxidants.

2.6.2. Estimation of LPO in liver tissue

Lipid peroxidation in liver tissue was estimated colorimetrically by TBARS and hydroperoxides. 0.1 mL of tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (TBA 0.37%, 0.25N HCl and 15% TCA) and placed in water bath for 15 min and cooled. The absorbance of clear supernatant was measured against reference blank at 535nm spectrophotometrically. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of MDA and expressed as nm MDA/g protein [15,16].

2.6.3. Estimation SOD in liver tissue

The activity of SOD was assayed by the method of Kakkar et al [17]. 0.5 mL of tissue homogenate was diluted with 1ml of water. To this mixture, 2.5 mL of ethanol and 1.5 mL of chloroform (all reagents chilled) were added and shaken for 1 min at 4°C and then centrifuged. The supernatant was taken. The assay mixture containing 1.2 mL of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1ml of 186 µM PMS, 0.3 mL of 30 µM NBT, 0.2 mL of 780 µM NADH, appropriately diluted enzyme preparation and water in a total volume of 3 mL. Reaction was started by the addition of NADH. After incubation at 30°C for 90 s the reaction was stopped by the addition of 1mL glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4mL of n-butanol. The intensity of the chromogen in the butanol layer was measured at 560nm against butanol blank. Assay mixture devoid of enzyme served as control. One unit of the enzyme activity is defined as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute under the assay conditions.

2.6.4. Estimation of GSH in liver tissue

GSH was determined by the method of Ellman (1959) [18]. 0.5mL of tissue homogenate was precipitated with 2mL of 5% TCA and centrifuged at 3200Xg for 20 minutes. After centrifugation 1mL of the supernatant was taken and added to 0.5mL of Ellman's reagent (2,2'-dinitro-5,5'-dithiobenzoic acid) and 3mL of phosphate buffer (pH 8.0). Then the absorbance was measured at 412nm. The values were expressed as mg/100 g tissue.

2.6.5. Estimation of Catalase in liver tissue

The reaction mixture for the estimation of catalase activity contained 1.0 mL of 0.01M pH 7.0 phosphate buffer, 0.1 mL of tissue homogenate (supernatant) and 0.4 mL of 2M H₂O₂ in a total volume of 1.5 mL. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratios). Then the absorbance was measured colorimetrically at 620 nm and it is expressed as µmoles of H₂O₂ consumed/min/mg protein as described by Sinha 1972 [19].

3. Result

The results of antioxidant activity were showed in table 1. LPO, GSH, SOD and Catalase serve as markers in studying the antioxidant activity. Diabetes induced, high cholesterol fed rats

Table 1 Effect of oxidative stress markers in control, hyperlipidemic and *Monascus* fermented Indian rice extract treated diabetic rats.

Groups	Control	STZ+Cholesterol Treated	<i>Monascus</i> Indian rice extract 1.2g/kg bw	<i>Monascus</i> Indian rice extract 2.4g/kg bw	Glibenclamide 300µg/kg bw
Lipid Peroxidation nmol MDA released /mg protein.	1.24±0.24	1.98±0.35 ^a	1.32±0.12 ^{ms}	1.36±0.15 ^b	1.54±0.39 ^c
SOD units/mg protein	4.4±0.41	2.9±0.7 ^a	3.6±0.12 ^{ms}	3.9±0.91 ^b	3.4±0.3 ^c
GSHµg reduced GSH utilized /min/mg protein	28.2±1.8	14.3±3.1 ^a	19.2±1.10 ^b	24.27±0.14 ^b	18.0±2.4 ^c
Catalase nmol of H ₂ O ₂ decomposed/min/mg protein	39.2±1.7	26.3±4.8 ^a	31±0.79 ^b	35.03±0.79 ^b	30.2±4.1 ^c

Values are mean ± SD (n=6); a-p<0.001, b-p<0.05, c-p<0.01; MDA-Malondialdehyde, SOD-Superoxide Dismutase, GSH-Reduced glutathione.

rats.further SOD, GSH and Catalase were found to be decreased by 34, 97 and 33% respectively. The rats treated with *Monascus* fermented rice extracts at the concentration of 1.2 and 2.4 g/kg/day bw showed 33 and 31% reduction in LPO. The present study showed that treatment with *Monascus* fermented Indian rice extract at the dose of 1.2 and 2.4 g/kg bw/day resulted in significant increase in GSH level to about 34 and 70% respectively. Antioxidant enzymes SOD and Catalase were also increased in their level to about 24 and 18% with supplementation of *Monascus* fermented Indian rice extract at the dose of 1.2 g/kg bw/day, respectively and about 34 and 33% at the dose of 2.4 g/kg bw/day, respectively.+

4. Discussion

STZ induces type 1 diabetes mellitus in the test animals resulting in hyperglycemia. High cholesterol diet leads to hyperlipidemia. Thus the test animals which are both hyperglycemic and hyperlipidemic are prone to the development of oxidative stress. The oxidative stress can be marked by LPO, GSH, SOD and Catalase levels. Oxidative stress results in increased LPO which plays an important role in several pathologies like atherosclerosis, diabetes, wound healing, liver disorder, inflammation etc [20]. The diabetic induced, high cholesterol fed animals showed increased LPO than in control animals. The animals treated with *Monascus* fermented rice extracts showed reduced LPO than diabetes induced animals fed only with high cholesterol food. This is better than the animals receiving the standard drug having hypoglycemic and antioxidant properties (glibenclamide) [21]. GSH, SOD and Catalase protect the cell constituents from oxidative damage. Oxidative stress may lead to reduction in glutathione or inactivation of superoxide dismutase and catalase [22]. Concentration of these markers were found to be increased in animals treated with *Monascus* fermented Indian variety of rice extracts, which is also better than the standard drug glibenclamide. Thus the antioxidant activity of *Monascus* fermented Indian variety of rice was evident with the decreased LPO and increased SOD, GSH and Catalase in *Monascus* fermented Indian variety of rice extract treated rats.

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