

## Hypolipidemic and antioxidant activity of aqueous extract of *Monascus purpureus* fermented Indian rice in high cholesterol diet fed rats

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**Aim:** To evaluate hypolipidemic and anti-oxidant activities of *Monascus purpureus* fermented Indian variety of rice.

**Materials and methods:** Indian rice variety IR-532-E-576 was fermented with *Monascus purpureus* MTCC 1090 (Monascaceae) and extracted with water by boiling. Experimentally induced hyperlipidemia was produced by feeding rats with 2% cholesterol enriched diet for 15 days. Afterwards hypercholesterolemic rats were orally administered with *Monascus* fermented Indian rice extract at the dose of 1.2 and 2.4 mg/kg bw along with high cholesterol diet for 30 successive days in order to evaluate their expected hypolipidemic activity. Plasma lipid parameters such as total cholesterol, triglycerides, LDL (low density lipoprotein), VLDL (very low density lipoprotein), HDL (high density lipoprotein), atherogenic index, and oxidative stress markers such as lipid peroxidation, reduced glutathione, and superoxide dismutase, and catalase activities were measured in the plasma and liver respectively.

**Results:** The administration of *Monascus* fermented Indian rice extract retained lipid profiles and oxidative stress markers to about normal level and also produced a significant decrease in atherogenic index to about 98.07% and 99.53% for 1.2 and 2.4 mg/kg bw *Monascus* fermented Indian rice extract. It also showed better in vitro and in vivo antioxidant activity with  $IC_{50}$  of  $250 \pm 0.2 \mu\text{g}/\text{mL}$  and  $245 \pm 0.12 \mu\text{g}/\text{mL}$  by DPPH (diphenylpicrylhydrazyl) radical scavenging activity and metal chelating activity respectively.

**Conclusion:** The consumption of *Monascus* fermented Indian rice may act as a potent anti-hypercholesterolemic nutrient and powerful antioxidant.

**Key words:** Hypolipidemic, antioxidant, lipid peroxidation, DPPH

### Introduction

Cardiovascular diseases are the second largest cause of mortality worldwide. Increase in the cholesterol level is a major risk factor for progression of atherosclerosis, which is usually accompanied by the production of free radicals. Patients with cardiovascular disease showed significant increases in lipid peroxidation, which correlates with severity of hypercholesterolemia (1,2). Recent scientific research strategies have been focusing on the removal of reactive oxygen species (ROS).

The Chinese dietary supplement red yeast rice has been widely used in western countries to reduce the cholesterol level nutraceutically (3). Scientific investigations have also showed pharmacological effects of *Monascus* fermentate. Monacolin K, a cholesterol lowering agent, was also isolated and characterized from fermented rice products of *Monascus ruber* (4). In this present investigation, we studied antioxidant and hypolipidemic effects of *Monascus* fermented Indian variety of rice in high cholesterol fed rats.

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## Materials and methods

Fungal culture of *Monascus purpureus* MTCC 1090 (Microbial type culture collection) belonging to the family Monascaceae was obtained from the Institute of Microbial Technology, Chandigarh, India. Standard pellet diet for the experimental animals was obtained from Hindustan Lever Ltd., Bangalore, India.

### Experimental animals and diet

This study was approved by the Institutional Animal Ethical Committee of Kovai Medical Centre Research and Educational Trust (KMCRET), Coimbatore, India (Voucher no. KMCRET/PhD/1/2009). Male Wistar rats weighing 200-250 g were used for the study. The rats were maintained in accordance with internationally accepted ethical guidelines for the care of laboratory animals. Experimental rats given access to laboratory food and water ad libitum for 1 week and fed with high cholesterol diet (except the control group) prepared using 2% w/v cholesterol with standard pellet diet for 15 days except the control group.

### Preparation of *Monascus* fermented rice extract

The dried *Monascus purpureus* fermented Indian rice was crushed and used for the extraction. Rice powder (4 g) was mixed with 40 mL of sterilized distilled water and then boiled for 4 h. The extract was filtered, concentrated and used for further studies (5,6).

### Experimental design

Thirty animals were divided into 5 groups, each consisting of 6 animals.

Group 1: Received normal diet (control group).

Group 2: Fed with high cholesterol diet and received oral administration of 2 mL of sterile water.

Group 3: Fed with high cholesterol diet and received oral administration of *Monascus* fermented rice extract, 1.2 mg/kg bw in 2 mL of sterile water for 30 days.

Group 4: Fed with high cholesterol diet and received oral administration of *Monascus* fermented rice extract, 2.4 mg/kg bw in 2 mL of sterile water for 30 days.

Group 5: Fed with high cholesterol diet and received oral administration of reference hypolipidemic drug lovastatin, 10 mg/kg bw in 2 mL of sterile water for 30 days.

### In vivo evaluation of hypolipidemic activity

#### Effect of *Monascus* fermented rice on cholesterol levels (7)

The plasma triglycerides (TG), total cholesterol (TC), LDL-cholesterol, VLDL-cholesterol, and HDL-cholesterol were measured using assay kits (AGAPPE Diagnostics, Agappe Hills, Ernakulum, Kerala-683562, India).

The atherogenic index (AI), which is calculated using following formula, is a marker of plasma atherogenicity because it is increased in people with higher risk of cardiovascular diseases.

The atherogenic index (AI) was calculated as  $AI = (TC - HDL) / HDL$ .

### In vitro antioxidant activity

#### DPPH radical scavenging activity

The antioxidant activity of *Monascus* fermented Indian rice extract was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) (8-10). Extract at the concentrations 100-600 µg/ mL was treated with 2 mL of  $6 \times 10^{-5}$  mol L<sup>-1</sup> methanol solution of DPPH separately. Absorbance was measured at 517 nm immediately using UV-visible spectrophotometer and the decrease in absorbance was determined after 1 h for all samples using methanol as blank. Absorbance of the DPPH radical without antioxidant, i.e. the control, was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution. Percentage inhibition of the DPPH radical by the samples was calculated according to the following formula

$$\text{Inhibition (\%)} = (\text{control-test/control}) \times 100.$$

The IC<sub>50</sub> (concentration causing 50% inhibition of maximum effect as 100%) value of extract was calculated (µg/mL). The inhibition of DPPH activity was observed for quercetin as standard.

### Metal chelating activity

Metal chelating activity was observed to investigate the ferrous ion chelating ability of *Monascus* fermented Indian rice extract. Then 5 mL of extract in the concentration range of 100-500 µg/mL was mixed with 0.1 mL of 2 mM FeCl<sub>3</sub> and 0.2 mL of 5 mM ferrozine solutions. The absorbance at 562 nm was determined after 10 min. A complex of Fe<sup>2+</sup>/ferrozine showed strong absorbance at 562 nm. IC<sub>50</sub> value of extract was calculated. The metal chelating activity was observed for EDTA as standard (11,12).

### ABTS radical scavenging activity

ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) 2 mM and potassium persulfate 70 mM were prepared in distilled water. Then 200 mL of potassium persulphate and 50 mL of ABTS were mixed and used after 2 h (ABTS radical cation solution). To 500 mL of various concentrations of extract were added 300 mL of ABTS radical cation and 1.7 mL of phosphate buffer pH 7.4. For the control methanol was used, instead of the test compound, and the absorbance was measured at 734 nm and the total antioxidant activity was expressed as trolox equivalent (10,13,14).

### In vivo antioxidant activity

#### Preparation of tissue homogenate

For the estimation of non-enzymatic and enzymatic antioxidant activity, liver tissues were minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 5000 × g for 10 min and the resulting supernatant was used for enzyme assays.

#### Lipid peroxidation (LPO) in liver tissue

Lipid peroxidation in liver was estimated colorimetrically by using thiobarbituric acid reactive substances (TBARS). In brief, 0.1 mL of tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 mL of TBA-TCA-HCl (1:1:1) reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and kept in a water bath for 15 min and then cooled. The absorbance was measured spectrophotometrically at 532 nm. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA) and expressed as nm MDA/mg protein (10,15,16).

### Superoxide dismutase (SOD) activity in liver tissue

The activity of SOD was assayed by the method of Kakkar et al. (17). Tissue homogenate (0.5 mL) in 1 mL of water was added with 2.5 mL of ethanol and 1.5 mL of chloroform and then shaken for 1 min at 4 °C and lastly centrifuged. The enzyme activity in the supernatant was determined using an assay mixture containing 1.2 mL of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 mL of 186 µM PMS (phenazine methosulphate), 0.3 mL of 30 µM NBT (nitroblue tetrazolium), 0.2 mL of 780 µM NADH, appropriately diluted enzyme preparation, and water in a total volume of 3 mL. The reaction was started by the addition of NADH. After incubation at 30 °C for 90 s the reaction was stopped by the addition of 1 mL of glacial acetic acid. The reaction mixture was stirred vigorously and then treated with 4 mL of *n*-butanol. The intensity of the chromogen in the butanol layer was measured at 560 nm against butanol blank. A sample without enzyme served as control. One unit of the enzyme activity is defined as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 min under the assay conditions.

### Estimation of reduced glutathione (GSH) in liver tissue

GSH was determined by the method of Ellman (12). Tissue homogenate (0.5 mL) was precipitated with 2 mL of 5% TCA and centrifuged at 3200 × g for 20 min. The supernatant (1 mL) was taken after centrifugation and added to 0.5 mL of Ellman's reagent (2,2'-dinitro-5,5'-dithiobenzoic acid) and 3 mL of phosphate buffer (pH 8.0). Then the absorbance was measured at 412 nm. The values were expressed as mg/100 g tissue.

### Estimation of the catalase activity in liver tissue

Catalase was assayed colorimetrically at 620 nm and expressed as µmoles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein as described by Sinha (18). The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M pH 7.0 phosphate buffer, 0.1 mL of tissue homogenate (supernatant), and 0.4 mL of 2 M hydrogen peroxide. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio) and then the absorbance was measured.

### Statistical analysis

The data were analyzed using one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test.

### Results

Spores of *Monascus purpureus* were inoculated at the concentration of  $10^7$  spores/mL into 5 different varieties of pretreated Indian rice and fermented at 30 °C for 10 days. The fermented products were analyzed quantitatively for HMG CoA reductase inhibitor using lovastatin as a standard. From the results it was found that Indian rice variety IR-532-E-576 produced high concentration of statins on fermentation with *Monascus purpureus* and was used in our study.

#### In vivo evaluation of anti-cholesterolemic activity

##### Plasma lipid profile

The oral administration of 2% cholesterol in normal rodent diet increased plasma total cholesterol, triglycerides, LDL, and VLDL by 59.58%, 42.91%, 60%, and 43.59% respectively ( $P < 0.001$ ), as compared to the normal control. These significant rises were accompanied by a significant decrease in plasma HDL

cholesterol by 16.38% ( $P < 0.001$ ), as compared to the normal control (Figure 1). The atherogenic index compared to the normal control was also increased significantly to about 28.4% (Table 1).

The oral supplementation of the extract of *Monascus* fermented rice (1.2 and 2.4 g/kg/day bw) to high cholesterol rats resulted in significant declines in plasma total cholesterol level by about 93.35% and 93.91% ( $P < 0.001$ ) respectively as compared to the high cholesterol fed rats (733 mg/dL). LDL, VLDL, and triglycerides also showed a significant decrease as compared to the high cholesterol fed rats to about 8.94%, 20.67%, and 9.77% ( $P < 0.001$ ) for 1.2 g/kg/day bw and about 43.90%, 33.36%, and 61.19% ( $P < 0.001$ ) for 2.4 g/kg/day bw dose of *Monascus* fermented Indian rice extract (Figure 1). Similarly atherogenic index was also reduced to about 98.07% and 99.53% for 1.2 g/kg/day bw and 2.4 g/kg/day bw dose of *Monascus* fermented Indian rice extract respectively (Table 1). It also elevated plasma HDL cholesterol by 27.5% and 35.45%, respectively.

##### In vitro antioxidant activity

The antioxidant properties of the *Monascus* fermented Indian rice extract were established by dose dependently scavenging various commercially

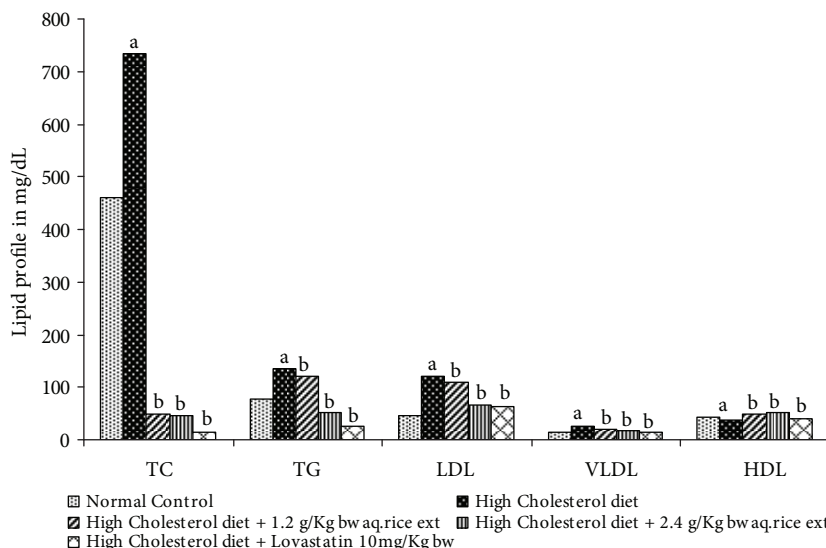


Figure 1. The effect of oral supplementation of *Monascus* fermented Indian rice extract to hypercholesterolemic rats for 30 successive days on plasma lipid profile. Values are mean  $\pm$  SD (n=6), a- $P < 0.001$  as compared with normal control, b- $P < 0.001$  as compared with high cholesterol diet group.

Table 1. Effect of *Monascus* fermented Indian rice extract on atherogenic index.

Groups	Atherogenic Index
Untreated	9.3 ± 0.4
High cholesterol diet	12.99 ± 0.2
High cholesterol diet + 1.2 g /kg bw aq. rice ext.	0.25 ± 0.2
High cholesterol diet + 2.4 g /kg bw aq. rice ext.	0.06 ± 0.3
High cholesterol diet + lovastatin 10 mg/kg bw	0.08 ± 0.1

Values are mean ± SD (n = 6)

available free radicals such as DPPH, ABTS, and reactive oxygen species, by chelating divalent iron. In this present investigation, free radical scavenging activity was evidenced in cell free assays. *Monascus* fermented Indian rice extract showed dose-dependent metal chelating activity with divalent iron and DPPH activity (Figures 2 and 3). DPPH radical scavenging assay and metal chelating activity showed IC<sub>50</sub> at 250 and 245 µg/mL, respectively (Table 2). This strong radical scavenging activity in vitro motivated the authors to investigate the biological significance of anti-oxidant activity of *Monascus* fermented Indian rice extract. Total antioxidant activity was also expressed as trolox equivalent and it was 13921 ± 23 µmol/Trolox/g.

### Evaluation of in vivo antioxidant activity

Lipid peroxidation serves as a marker for cellular oxidative stress and also a factor for atherosclerosis and cancer (5). The oxidative stress marker studies revealed that the LPO level was increased by 21.52% in 2% cholesterol fed rats, as compared to normal rats, where as SOD, GSH, and catalase levels were decreased to about 0.33%, 35%, and 48.17% as compared to normal animals (Table 1). TBARS concentration in plasma decreased to about 40.28% and 34.28% with supplementation of *Monascus* fermented Indian rice extract at the dose of 1.2 and 2.4 g/kg/day bw, respectively.

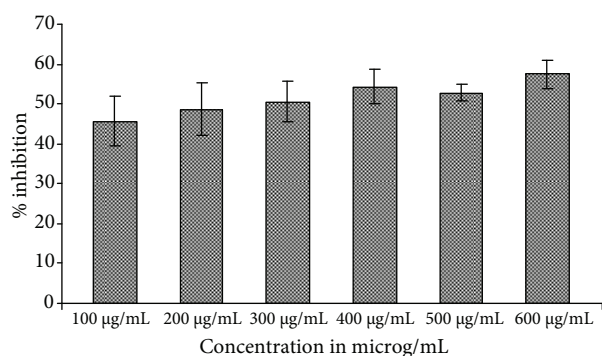


Figure 2. The dose dependent DPPH activity of *Monascus* fermented Indian rice extract.

Data represent percentage of DPPH radicals scavenged at 517 nm, mean ± SEM (n = 3).

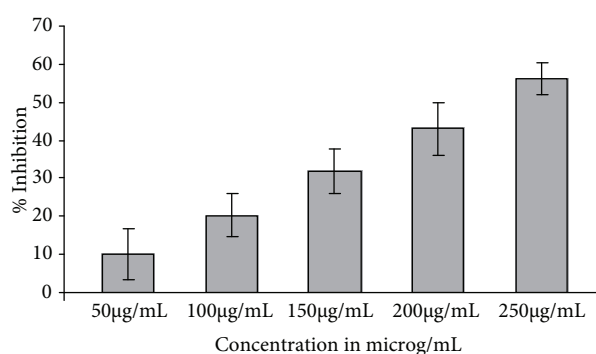


Figure 3. The dose dependent metal chelating activity of *Monascus* fermented Indian rice extract.

Data represent ferrozine complex formed at 562 nm, mean ± SEM (n = 3).

Table 2. The in vitro antioxidant activity of *Monascus* fermented Indian rice extract.

Antioxidant activity	<i>Monascus</i> fermented Indian rice extract IC <sub>50</sub> µg/mL	Standard IC <sub>50</sub> µg/mL
DPPH radical scavenging activity	250	13
Metal chelating activity	245	35

Glutathione-dependent antioxidant system consisting of reduced glutathione (GSH) and an array of functionally related enzymes play a fundamental role in cellular defense against reactive free radicals and other oxidant species (19). The present study showed that *Monascus* fermented Indian rice extract showed a significant increase in GSH level to about 45.87% and 56.19% for *Monascus* fermented Indian rice extract at the dose of 1.2 and 2.4 g/kg/day bw, respectively effect on the activity of liver GSH (Table 2). Dietary intake of *Monascus* fermented Indian rice may have increased the activity of antioxidant enzymes. Other antioxidant enzymes SOD and catalase were also supported similarly by showing an increase in their level to about 48.7% and 134% with supplementation of *Monascus* fermented Indian rice extract at the dose of 1.2 g/kg/day bw, respectively, and about 74.22% and 158.6% at the dose of 2.4 g/kg/day bw, respectively (Table 2).

## Discussion

In Chinese traditional medicine, red yeast rice has been used as a medicine for digestion and revitalization (20) and also as preservative and coloring agent. In our prior study, when we were interested in the effect of *Monascus* fermented Indian rice on sugar metabolism, we found that it has a potential lipid lowering activity in diabetic animals. To confirm this we studied the effect of *Monascus* fermented Indian rice on high cholesterol induced animal model. It is well known that increases in TC,

TG, and LDL are primary risk factors for vascular diseases, and high serum level of HDL is a protective factor against the same. The experimental data in this study shows that all the changes in plasma lipid profile induced by high cholesterol diet can be resisted by *Monascus* fermented Indian rice extract which correlates with the study of other researchers also (21,22). In accordance with the results of this study, high HDL level may compete with LDL receptor sites on arterial smooth muscle cells, thus inhibiting the uptake of LDL (23). Increase in HDL may prevent oxidation of LDL because lipids in HDL will get oxidized first before those in LDL (24).

The present study also showed anti-oxidant potential of *Monascus* fermented Indian rice, which also contributes to prevention of oxidation of LDL (Table 3). The scavenging activities on DPPH, metal chelating and ABTS radical were demonstrated in cell-free assays. The findings of strong radical scavenging activity by extract of *Monascus* fermented Indian rice led us to investigate further in vivo by measuring lipid peroxidation and GSH levels and SOD and catalase activities. As shown in the study, *Monascus* fermented Indian rice extract decreases elevated level lipid peroxidation to about normal, whereas the activity of antioxidant enzymes GST, SOD, and catalase were increased. The present results indicate that the antioxidant activity of *Monascus* fermented Indian rice extract function through the induction of antioxidant enzymes and the reduction of free radical formation, decomposition of hydrogen peroxide, quenching active singlet oxygen and by trapping and quenching radicals (25).

Table 3. Effect of oxidative stress markers in control, hyperlipidemic, and *Monascus* fermented Indian rice extract treated rats.

Groups	Untreated	Cholesterol treated 1.2 g/kg bw	<i>Monascus</i> Indian rice extract 2.4 g/kg bw	<i>Monascus</i> Indian rice extract 10 µg/kg bw	Lovastatin
Lipid Peroxidation nmol MDA released /mg protein.	2.88 ± 0.68	3.5 ± 0.61 <sup>a</sup>	2.09 ± 0.01 <sup>c</sup>	2.32 ± 0.43 <sup>c</sup>	2.59 ± 0.01 <sup>b</sup>
% Change		-21.52%	+40.48%	+34.28%	+26%
SOD units/mg protein	11.51 ± 0.5	7.72 ± 0.73 <sup>c</sup>	11.48 ± 0.72 <sup>a</sup>	13.45 ± 0.73 <sup>a</sup>	11.57 ± 0.31 <sup>b</sup>
% Change	+0.33%	-48.7%	-74.22%	-49.87%	
GSH µg reduced GSH utilized/ mg protein	17.42 ± 0.91	8.72 ± 0.30 <sup>c</sup>	12.72 ± 0.30 <sup>a</sup>	13.67 ± 0.43 <sup>a</sup>	12.92 ± 0.45 <sup>b</sup>
% Change	+35%	-45.87%	-56.19%	-48.16%	
Catalase nmol of H <sub>2</sub> O <sub>2</sub> decomposed/min/mg protein	25.7 ± 0.58	13.32 ± 1.2 <sup>c</sup>	31.2 ± 1.8 <sup>a</sup>	34.45 ± 0.07 <sup>a</sup>	28.5 ± 0.63 <sup>b</sup>
% Change	+48.17%	134%	-158.6%	-113.9%	

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.

## Conclusion

The present study clearly demonstrated that *Monascus* fermented Indian rice extract exhibits the free radical scavenging activity and the metal chelating activity. Furthermore, in hypercholesterolemia rats *Monascus* fermented Indian rice extract significantly increases HDL and decreases LDL levels in plasma. It also decreases LPO and increases antioxidant enzymes SOD, GSH, and catalase, thereby delaying the onset of atherosclerosis. In future, more studies are to be

conducted in order to confirm these results in humans and to study toxicological properties, if any.

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