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Anti-Diabetic Activity of Aqueous Extract of *Monascus purpureus* Fermented Rice in High Cholesterol Diet Fed-Streptozotocin-Induced Diabetic Rats

A. Rajasekaran, M. Kalaivani and R. Sabitha
KMCH College of Pharmacy, Coimbatore, India

Abstract: The present study was designed to investigate the hypoglycemic and hypolipidemic activity of aqueous extract of *Monascus* fermented Indian variety rice in high cholesterol fed-streptozotocin-induced diabetic rats. Wister rats were fed with high cholesterol diet for 2 weeks prior to intra-peritoneal injection with streptozotocin (50 mg kg^{-1}). The Indian variety rice IR-532-E-576 was fermented with *Monascus purpureus* for 10 days and sterilized. Aqueous extract of the fermented rice at two dose levels showed a significant decrease in fasting blood glucose level. The total cholesterol and triglycerides were also significantly reduced where as the HDL cholesterol levels were significantly increased, which confirmed the potent anti-diabetic property of the *Monascus purpureus* fermented rice in diabetic rats, which may be due to presence of statins.

Key words: *Monascus purpureus*, anti-diabetic, Indian rice, high cholesterol diet, statins

INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by insufficiency of insulin secretion from pancreatic beta cells/insulin resistance in peripheral tissues such as liver, adipose tissue and skeletal muscle (Sharma *et al*., 2008; Lee and Sohn, 2008). The World Health Organization reported that more than 180 million people worldwide have diabetes, which may be doubled by 2030. Most prevalent form of diabetes is non-insulin dependent diabetes mellitus (type 2) (Bhat *et al*., 2008). Type 2 diabetes comprises 90% of people with diabetes around the world and is due to excess body weight and physical inactivity.

Red Yeast Rice (RYR) is a fermented rice product produced traditionally by fermenting cooked rice kernels with yeast *Monascus,* had been used as preservative and coloring agent (Heber *et al*., 1999). The genus *Monascus* consist of important species like *Monascus purpureus*, *Monascus ruber* and *Monascus pilosus*, whose important characteristic is to produce secondary metabolites of polyketide structure (Juzlova *et al*., 1996) and yellow, orange and red pigments. *Monascus pilosus* and *Monascus purpureus* are used traditionally for its pigments, where as *Monascus ruber* was used for production of angkak, a fermented rice product with anti cholesterol activity (Wong and Koehler, 1981) and also as colorant in wine cheese and meat. Traditionally fermented RYR proved to contain many active constituents such as compounds resembling statins in its structure, unsaturated fatty acid, sterols and B-complex vitamins (Wang *et al*., 1997; Moghadasian and Frohlich, 1999).

Corresponding Author: M. Kalaivani, Department of Pharmaceutical Biotechnology, KMCH College of Pharmacy, Kalapatti Road, Coimbatore-641048, TN, India

Various studies also reported that RYR and statins decreases blood glucose levels in diabetes (Chang *et al*., 2006; Chen and Liu, 2006; Yee *et al*., 2004; Zhang *et al*., 2008). Investigation on anti-diabetic activity of *Monascus purpureus* fermented rice was not performed, hence the present work has been undertaken for investigating the anti-diabetic activity of aqueous extract of *Monascus purpureus* fermented Indian rice variety in experimentally induced high cholesterol diet fed -diabetic rats.

MATERIALS AND METHODS

The research project was done for one year in the academic year 2008-2009.

Microorganism

Fungal culture of *Monascus purpureus* MTCC 1090 (Microbial type culture collection) was obtained from the Institute of Microbial Technology, Chandigarh, India (Invoice No. MTCC/08/9/5369). Fungal culture was maintained routinely in a PDA (Potato dextrose agar) medium containing infusion of potatoes (20%), Dextrose (2%) and agar (2%) and subcultured every 30 days interval (Sayyad *et al*., 2007).

Preparation of Seed Culture

Seed cultures were prepared by transferring a loopful of spore from PDA agar plate into 500 mL Erlenmeyer flask containing 100 mL basal medium (100 g dextrose, 10 g peptone, 2 g KNO_3, 2 g $NH_4H_2PO_4$, 0.5 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2$ dissolved in 1000 mL distilled water, pH 6.0). The culture was incubated at 30°C for 48 h at 110 rpm (Wang *et al*., 2005). After incubation 5% seed culture was inoculated for solid state fermentation.

Monascus Fermented Rice Preparation

Five different types of Indian rice were obtained from local markets of Coimbatore, India and tested for its use as a substrate for preparation of *Monascus* fermented rice with high concentration of statin related compounds. Five hundred grams of rice was soaked in distilled water (1 L) at room temperature for 8 h and excess water was removed after soaking. The soaked rice was autoclaved for 20 min at 121°C in an autoclave. Substrates were cooled and then inoculated with 5% seed culture of *Monascus purpureus* separately and the inoculated substrates were incubated at 30°C for 10 days (Wang *et al*., 2005). The dried fermented rice was then sterilized and used for further study.

Preparation of *Monascus* Fermented Rice Extract

The dried *Monascus purpureus* fermented Indian rice was crushed to powder and used for extraction. Four grams of rice powder was mixed with 40 mL of sterilized distilled water and boiled for 4 h at 100°C. Extract was filtered through Buchner funnel, freeze-dried and stored at -20°C until use (Wong and Rabie, 2008; Jeon *et al*., 2004).

Estimation of Statin

Aqueous extract of fermented rice was adjusted to a pH of 6.5. One gram of extract was diluted (5 mL) with ethyl alcohol (90% v/v) and filtered through Whattman filter paper No.1 and absorbance was read at 238 nm using UV Spectrophotometer and compared with reference drug lovastatin (Ahamad *et al*., 2006).

Experimental Animals

This study was approved by the Institutional Animal Ethical Committee of Kovai Medical Centre Research and Educational Trust (Voucher No.KMCRET/M.Pharm/03/2008),

Coimbatore, India. The rats were maintained in accordance with internationally accepted ethical guidelines for the care of laboratory animals. Male *Wistar* rats weighing 200-250 g were used. Experimental rats were given free access to laboratory food and water. Experimental animals were subjected to euthanasia as per guidelines and pancreas was isolated for histopathological investigation.

High-Cholesterol Diet fed STZ- Induced Diabetic Rats

Wister rats (except control group) were fed with high cholesterol diet prepared by using 2% w/v cholesterol with regular rodent diet (standard pellet diet obtained from Hindustan lever Ltd., Bangalore, India). After 2 weeks with high cholesterol diet overnight fasted animals were administered with 50 mg kg^{-1} of STZ (Streptozotocin) intra-peritoneally. Animals were allowed for free access to food and water after STZ injection (Tan *et al*., 2005; Eliza *et al*., 2008). Hyperglycemia was confirmed by elevated glucose levels in plasma, determined at 72 h and then at 7th day after injection. Only rats found with permanent diabetes were used for the anti-diabetic study.

Oral Glucose Tolerance Test

The oral glucose tolerance test (Soltani *et al*., 2007) was performed in overnight fasted (18 h) normal rats. Rats were divided into three groups (n = 6) were administered with drinking water, 1.2 and 2.4 g kg^{-1} b.wt. of aqueous extract of *Monascus* fermented rice, respectively. Glucose (2 g kg^{-1} b.wt.) was fed 30 min after administration of extract. Blood samples were collected from tail vein before administration of glucose and at 30, 60 and 120 min after the oral glucose administration and then glucose levels were estimated using glucose strips and a glucometer (Accu-chek, Roche Diagnostics, US).

Experimental Design

Thirty animals were divided into five equal groups (n = 6) as follows:

- **Group 1:** Control group received normal diet
- **Group 2:** Fed with high cholesterol diet-STZ-induced diabetic rats received oral administration of 2 mL of sterile water
- **Group 3:** Fed with high cholesterol diet-STZ-induced diabetic rats received oral administration of *Monascus* fermented rice extract, 1.2 mg kg^{-1} b.wt. in 2 mL of sterile water for 30 days
- **Group 4:** Fed with high cholesterol diet-STZ-induced diabetic rats received oral administration of *Monascus* fermented rice extract, 2.4 mg kg^{-1} b.wt. in 2 mL of sterile water for 30 days
- **Group 5:** Fed with high cholesterol diet-STZ-induced diabetic rats received oral administration of reference anti-diabetic drug glibenclamide, 300 μg kg^{-1} b.wt. in 2 mL of sterile water for 30 days

Effect of *Monascus* Fermented Rice on Fasting Blood Glucose Level

Fasting blood glucose was measured before start of experiment (0 day) and after 30 days of treatment with *Monascus* fermented rice extract. For the determination of fasting blood glucose, on 0 day and after 30 days of treatment, all rats including control group were fasted overnight. The blood was collected from the tip of the tail vein of overnight fasted rats and the blood glucose was measured using glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, US). The results were expressed in terms of milligrams per deciliter of blood.

Effect of *Monascus* Fermented Rice on Cholesterol Levels

Serum total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, Low Density Lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) were determined using Diagnostic kits purchased from AGAPPE Diagnostics, Ernakulum, Kerala, India.

Body Weight

Body weight of animals in each group was determined on 0 and 30th day and difference in weight was recorded.

Histopathological Investigation

Animals were sacrificed and pancreas was removed from rats, washed in 0.9% w/v Normal saline; small portion of the pancreas was quickly dissected and fixed in 25% formalin within 10-15 min. Then the tissue was processed for standard histopathological techniques i.e., dehydration through graded isopropyl alcohol, clearing through and impregnated in paraffin wax for 2 h. Then wax blocks were made, sections were cut using microtome and stained by haematoxylineosin method and photographed.

Statistical Analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) Tukey, followed by comparison of all pairs of column test using Graph pad prism 5.

RESULTS AND DISCUSSION

Spores of *Monascus purpureus* were inoculated at the concentration of 10^7 spores mL^{-1} into five different varieties of pretreated rice and fermented at 30°C for 10 days. After fermentation concentration of HMG CoA reductase inhibitors present in fermented rice was estimated UV spectrophotometrically using lovastatin as 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*. The concentration was found to be 11.6 μg mL^{-1} of aqueous extract. *Monascus* fermented IR-532-E-576 rice was used for further studies.

Oral Glucose Tolerance Test

The levels of blood glucose in control and aqueous extract of *Monascus* fermented rice supplemented groups demonstrated a significant change in plasma glucose level after administration of glucose (2 g kg^{-1} b.wt.). In treatment groups, plasma glucose level although reached the highest concentration (131.32 and 124.00 mg dL^{-1}, respectively) within 30 min which is significantly less than plasma glucose concentration (1257 mg dL^{-1}) at 30 min of control groups (Fig. 1).

Determination Fasting Blood Glucose Level in Normal and High Cholesterol Fed Diabetic Rats

There was a significant elevation in fasting blood glucose level in STZ and high cholesterol diet fed rats as compared to control groups. However, supplementation of aqueous extract of *Monascus* fermented rice for 30 days showed significant reduction of fasting blood glucose level (Fig. 2). Aqueous extract at concentration of 2.4 g kg^{-1} b.wt. showed significant effect on fasting blood glucose level in STZ induced high cholesterol fed diabetic rats.

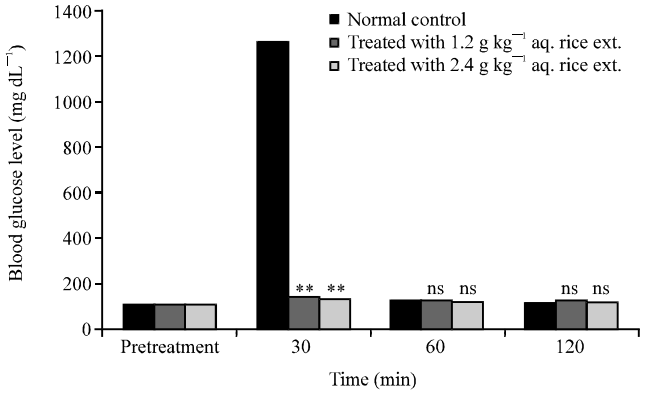

Fig. 1: Effect of the aqueous extract of *Monascus* fermented rice Oral Glucose Tolerance Test (OGTT) in high-cholestrol diet fed STZ-induced diabetic rats. Result are Mean±SD of n = 6. **p<0.05, ns: Non-significant as compared to control

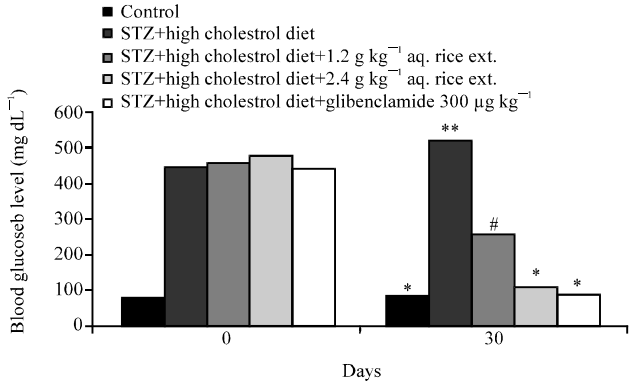

Fig. 2: Effect of the aqueous extract of *Monascus* fermented rice on blood glucose level in high-cholestrol diet fed STZ-induced diabetic rats. Value are Mean±SD of n = 6, *p<0.001, **p<0.05, #p<0.01 as compared to control

Table 1: Effect of aqueous extract of *Monascus* fermented rice on cholesterol levels in high-fat diet fed STZ-induced diabetic rats

Groups	TC	TG	HDL	LDL	VLDL
1	87.00±1.01	94.72±2.3	32.20±0.7	25.87±1.3	183.00±0.63
2	129.30±2.5*	121.50±2.1*	21.40±3.8*	111.40±4.3*	24.21±2.6*
3	113.00±2.4#	115.50±3.7	26.31±2.8#	68.55±1.2	30.55±1.3#
4	98.09±2.1#	97.95±2.2#	39.84±1.2#	45.29±3.8#	20.91±0.3#
5	86.00±1.35**	93.87±5.8**	43.52±0.4**	42.13±0.5**	18.71±1.30

**Values are Mean±SD of n = 6. *p<0.001, **p<0.05, #p<0.01

Determination of Cholesterol Levels in Normal and High Cholesterol Fed Diabetic Rats

There was a significant decrease in the level of serum HDL-cholesterol and a significant increase in the levels of total cholesterol, triglycerides and LDL-cholesterol in diabetic rats (p<0.001) when compared to normal rats. Administration of aqueous extract of *Monascus* fermented rice brought back the levels of serum lipids to near normal (Table 1). Aqueous extract of *Monascus* fermented rice at the dose of 2.4 g kg^{-1} b.wt. showed significant effect

when compared to the dose at 1.2 g kg^{-1} b.wt. ($p<0.01$) and the effect was almost equivalent to standard drug glibenclamide.

Effect of *Monascus* Fermented Rice on Body Weight in Normal and High Cholesterol Fed Diabetic Rats

The effect of *Monascus* fermented rice extract on changes in body weight. In high cholesterol fed diabetic rats there was a significant decrease in body weight (8.33 g) when compared to normal rats (Table 2). Oral administration of aqueous extract of *Monascus* fermented rice significantly increased the body weight (6 g) when compared to diabetic rats. Aqueous extract of *Monascus* fermented rice at the dose of 2.4 g kg^{-1} b.wt. showed similar effect as that of standard anti-diabetic drug glibenclamide. Significant decrease in body weight was also observed in diabetic rats, which was reversed on administration of aqueous extract on *Monascus* fermented rice.

Histopathological Evaluation

The histological sections of the pancreas were observed to know the effect of *Monascus* fermented rice on high cholesterol fed-diabetic rats. In pancreatic sections of diabetic rats, the islets were less and their shape was destroyed compared to control group (Fig. 4). In treatment groups, there were more islets (Fig. 5, 6) and they were comparable to normal rat islets (Fig. 3) and standard anti-diabetic drug glibenclamide treated rats (Fig. 7), although there were individual differences. The relative distribution of pancreatic islet cells was similar to control rats.

Diabetes mellitus, particularly type 2 diabetes is highly prevalent now days, where the predominant cause of death in diabetic patients is vascular complications due to dyslipidemia and hypercholesterolemia. 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors (statin were designed for lowering cholesterol synthesis, which also benefits in prevention of

Table 2: Effect of aqueous extract of *Monascus* fermented rice on body weight in High-Fat Diet fed STZ-induced diabetic rats

	Body weight (g)		
Groups	0th Day	30th Day	Difference between 0 and 30th day
1	256.67±2.33	275±1.33*	18.33
2	220±2.79	210±0.59*	10
3	198±1.5	209±2.1	11
4	208±2.7	224±3.2*	16
5	221±3	235±2.39*	14

Values are Mean±SD of n = 6. *$p<0.001$

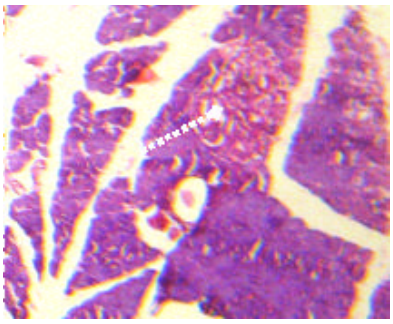

Fig. 3: Section of pancreatic tissue of control rat showing normal architecture

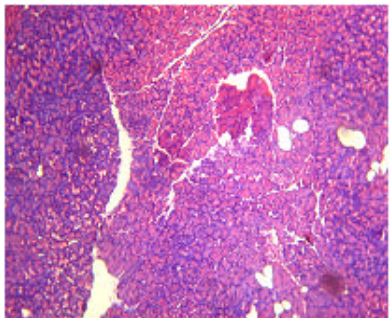

Fig. 4: Section of pancreatic tissue from a diabetic rat showing degenerative changes in islets and decrease in number of β cells

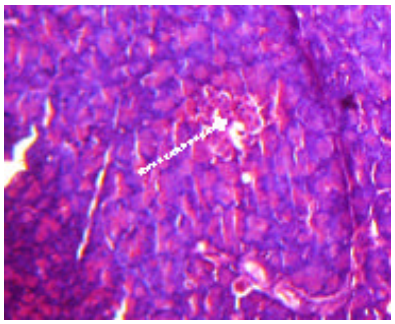

Fig. 5: Section of pancreatic tissue from a diabetic rat treated with aqueous extract of *Monascus* fermented rice at the dose of 1.2 g kg^{-1} b.wt. Showing initial stages of regenerating islets

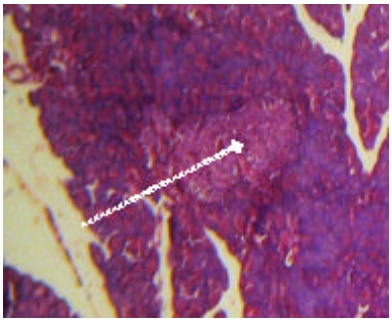

Fig. 6: Section of pancreatic tissue from a diabetic rat treated with aqueous extract of Monascus fermented rice at the dose of 2.4 g kg^{-1} b.wt. Showing apparently normal aechitecture

induced diabetic rat is one of the animal models for diabetes mellitus (Reed *et al*., 1999) were used in the study.

The present study discussed the production of *Monascus* fermented rice from Indian variety of rice and investigated the effect of aqueous extract of *Monascus* fermented rice in

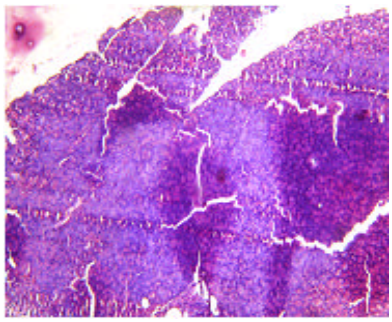

Fig. 7: Section of pancreatic tissue from a diabetic rat treated with glibenclamide 300 μg kg^{-1} showing decrease in the number of β-cells

vascular events or baseline lipid levels in diabetic patients and other complications such as nephropathy and retinopathy (Ludwig and Shen, 2006). The high cholesterol diet-STZ high cholesterol fed- STZ induced diabetic rats. Diabetes mellitus due to administration of STZ may be by destruction of the islets of langerhans of the pancreas (Kavalali *et al*., 2002; Shirwaikar *et al*., 2006) also proved in histopathological investigation of our study, shows prominent destruction of islets of langerhans when compared with normal control rat pancreas. Over production and decreased utilization of glucose by tissues are the fundamental cause of diabetes (Chattopadhyay, 1993), which was reflected in our study also. When aqueous extract of *Monascus* fermented rice was administered to glucose loaded normal rats fasted overnight, hypoglycemia was observed within 30 min.

In present study the difference between initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation of blood glucose in diabetic rats. Administration of aqueous extract of *Monascus* fermented rice showed significant decrease in level of blood glucose and significant regeneration of islets of langerhans of pancreas. The possible mechanism by which *Monascus* fermented rice extract brings hypoglycemic action may be by increasing pancreatic secretion of insulin from regenerated beta cells as said by Shirwaikar *et al*. (2006) in their study. Histopathological investigations also supported findings of our study.

An abnormality in lipid profile is one of the common complications in diabetes mellitus (Soltani *et al*., 2007). The administration of aqueous extract of *Monascus* fermented rice showed significant decrease in LDL, Total Cholesterol, Triglycerides, VLDL and significant increase in HDL supports the reports of Soltani *et al*. (2007).

In the present study diabetic rats showed reduced islet cells, which were restored to almost normal upon treatment with aqueous extract of *Monascus* fermented rice. In this study anti- diabetic activity may be due to presence of statins, further investigations are in progress to isolate individual compounds in aqueous extract of *Monascus* fermented rice and also to elucidate detailed mechanism of hypoglycemic effects in Diabetes mellitus.

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