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Effect of Red Yeast Rice and Coconut, Rice Bran or Sunflower Oil Combination in Rats on Hypercholesterolemic Diet

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ABSTRACT

Introduction: Dietary supplements provide a novel population based health approach for treating hyperlipidemias. Red yeast rice is known to have lipid lowering effects. Combination of red yeast rice with various oils is taken by different population around the world.

Aim: In this present work, we aimed to compare the effects of red yeast rice with different oil (coconut, rice bran and sunflower oil) supplementations on lipid levels and oxidative stress in rats fed on hypercholesterolemic diet.

Materials and Methods: A Randomized controlled study was conducted on 28 male Sprague Dawley rats. It included 4 arms-Control arm (hypercholesterolemic diet), Test arm A (hypercholesterolemic diet +Red yeast rice + Rice bran oil), arm B (hypercholesterolemic diet +Red yeast rice + Coconut oil) and arm C (hypercholesterolemic diet +Red yeast rice + Sunflower oil). At the end of one month, serum cholesterol, triglycerides, MDA and paraoxonase was measured. The mean values of analytes between the different groups were compared using student 't-' test.

Results: The rats fed with red yeast rice and rice bran oil combination showed significantly lower levels of serum cholesterol, triglycerides and MDA when compared to the controls. The serum paraoxonase levels were significantly higher in this group when compared to the controls. The rats fed with red yeast rice and coconut oil combination showed significantly lower serum cholesterol and MDA levels when compared to the controls. The mean triglyceride and paraoxonase levels did not show any statistically significant difference from the controls. The rats on red yeast rice and sunflower oil combination did not show any statistically significant difference in the lipid levels and oxidative stress parameters.

Conclusion: The food combination which had best outcome in preventing the development of hyperlipidemia and oxidative stress in rats fed with hypercholesterolemic diet was red yeast rice and rice bran oil. Combining red yeast rice with coconut oil and sunflower oil gave suboptimal benefits.

Keywords: Cholesterol, Hyperlipidemia, Lipid peroxidation, MDA, Paraoxonase, Triglyceride

INTRODUCTION

Cardiovascular disease is a major cause of mortality and morbidity worldwide. In India, rapid urbanization has led to rising trends in the prevalence of conventional risk factors of cardiovascular diseases [1]. Steadily increasing prevalence of Cardio vascular disease and its risk factors highlight the need to promote primary prevention strategies [1].

Hyperlipidemia is a major risk factor for cardio vascular diseases. A recent study states that prevalence of hypercholesterolemia in India is about 14% and hypertriglyceridemia is about 30% [2]. Though several pharmacological agents are used to lower cholesterol levels such as statins, niacin, resins and fibrates, each has its array of adverse effect [3]. Diet therapy could play a dominant role in the treatment of hyperlipidemia [4]. In addition to being inexpensive, it has the potential to become habitual and augment drug therapy [4]. Most importantly diet therapy can be adopted by the entire family and thus help in primary prevention of acquired hyperlipidemias [4]. Over years many lipid lowering dietary factors have come to light from various cultures across the world [5]. Many of these are being studied for their applicability and effectiveness in different populations [5].

Red yeast rice, a fermented rice product used widely in China and Japan, is known to have lipid lowering effects [6]. Red yeast rice is a promising therapeutic alternative in patients with hypercholesterolemia [5]. Incidentally in regions which traditionally use red yeast rice, rice bran oil is also widely used. Rice bran oil has also been shown to have cholesterol lowering properties [7]. The effect of these two could be additive.

In various regions of India, traditionally people have been using different cooking oils ranging from coconut oil, gingelly oil, mustard oil, to safflower and sunflower oil. The efficacy of the red yeast rice in lowering lipid levels and oxidative stress when combined with these widely used edible oils has not been studied.

AIM

In this present work, we aimed to compare the effects of red yeast rice with different oil (coconut, rice bran and sunflower oil) supplementations on lipid levels and oxidative stress in rats fed on hypercholesterolemic diet.

MATERIALS AND METHODS

A randomized controlled study was carried out in the animal facility of PSG Institute of Medical Sciences and Research (PSG IMSR), Coimbatore, during the period Apr 2014 to Aug 2014. The study was approved by the Institutional Animal Ethics Committee and the experiments were carried out according to the guidelines of Institutional Animal Ethics Committee, PSG IMSR. Twenty eight healthy 2-3-month-old male Sprague Dawley rats, weighing 200-300 gm, were included in the study. Irritable rats and overweight rats were excluded. These were housed in steel cages under controlled temperature (22±2°C) and humidity (55±5%) with a fixed 12 hours light–dark cycle. The rats were randomly divided into 4 groups - control (n=10), Group A (n=6), Group B (n=6) and Group C (n=6).

Hypercholesterolemic diet [8] contained 1% cholesterol, 0.2% bile salts and 5% butter added to the base feed. The base feed used was commercial Laboratory animal feed preparation supplied by Sai Industries, Bangalore. Its composition was Carbohydrates 65%, crude protein 21.5%, crude fat 4.4 %, crude fiber 3.2%, total ash 5.1%, moisture 9.7%, calcium 1.4% and phosphorous 0.4%. In addition to the hypercholesterolemic diet, the test groups were

supplemented with red yeast rice and oil combination as specified in [Table/Fig-1]. Cholesterol and cholic acid were procured from Sigma-Aldrich chemicals. Commercially available butter, rice bran oil, coconut oil, sunflower oil and red yeast rice were used for preparing the diets. These were weighed and pulverized with the base feed, cooked in microwave oven to form pellets and sterilized by ethylene oxide fumigation. These diet pellets were fed to the rats ad libitum.

Composition	Control	Group A	Group B	Group C
Base feed	83.80 gm	78.80 gm	78.80 gm	78.80 gm
Butter	5 gm	5 gm	5 gm	5 gm
Cholesterol powder	1 gm	1 gm	1 gm	1 gm
Sodium Taurocholate	0.20 gm	0.20 gm	0.20 gm	0.20 gm
Rice flour	10 gm			
Red yeast rice		10 gm	10 gm	10 gm
Rice bran oil		5gm		
Coconut oil			5gm	
Sunflower oil				5gm

[Table/Fig-1]: Composition per 100 grams of diet.

At the end of one month, 1.5 ml blood was collected from the tail vein of rats under anesthesia. Serum triglycerides and total cholesterol were estimated by enzymatic colorimetric assay using Agappe kits in the Merck semiautoanalyser. Serum paraoxonase activity was estimated by spectrophotometric method using p-nitrophenyl acetate as substrate [9]. Lipid peroxidation was estimated spectrophotometrically using thiobarbituric acid [10].

STATISTICAL ANALYSIS

Results were expressed as mean and standard deviation. The mean values of analytes between the different groups were compared using student t-test. The p-value of less than 0.05 was considered significant.

RESULTS

Both the control and test group rats were given hyper-cholesterolemic diets. But the test groups were given red yeast rice and rice bran oil/coconut oil/sunflower oil combination in addition to the hypercholesterolemic diets. The protective effect of these food combinations against development of hyperlipidemia and oxidative stress was studied.

Study groups	Serum cholesterol (mg/dL)	Serum triglyceride (mg/dL)	Serum MDA µmol/L	Serum paraoxonase nmol/mL/min
Control (Hypercholes-terolemic diet)	86±1.414	109±18.33	2.94±0.258	105.26±9.174
Group A	69.5±4.123	63.5±14.27	1.81±0.556	134.73±15.30
Group B	78.1±3.81	69.6±52.25	1.69±0.5727	97.45±9.030
Group C	61±13.92	82.6±16.90	2.07±0.6022	103.6±5.041

[Table/Fig-2]: Mean and Standard deviation of the analytes in the control and test groups.
Group A - Hypercholesterolemic diet + Red rice and Rice bran oil; Group B - Hypercholesterolemic diet + Red rice and Coconut oil; Group C - Hypercholesterolemic diet + Red rice and Sunflower oil.

The mean serum cholesterol, triglyceride, MDA and paraoxonase levels in the control and test groups are shown in [Table/Fig-2]. The p-value of 2 tailed student t-tests comparing the control and various test groups are shown in [Table/Fig-3].

The rats fed with red yeast rice and rice bran oil showed significantly lower levels of serum cholesterol, triglycerides and MDA. The serum paraoxonase levels were significantly higher in this group when compared to the controls. The rats fed with red yeast rice and coconut oil combination showed significantly lower serum cholesterol and MDA levels when compared to the controls. The mean triglyceride and paraoxonase levels did not show any statistically significant difference from the controls.

Groups compared	Serum cholesterol	Serum triglyceride	Serum MDA	Serum paroxanase
Group A vs. control	0.0061	0.0043	0.0139	0.0195
Group B vs. control	0.0348	0.2585	0.0180	0.2624
Group C vs. control	0.0528	0.0686	0.0534	0.7274

[Table/Fig-3]: p-values showing comparison of analytes between test and control groups.
Group A - Hypercholesterolemic diet + Red rice and Rice bran oil; Group B - Hypercholesterolemic diet + Red rice and Coconut oil; Group C - Hypercholesterolemic diet + Red rice and Sunflower oil

The rats on red yeast rice and sunflower oil combination did not show any statistically significant difference in the lipid levels and oxidative stress parameters.

DISCUSSION

Dietary factors known to have lipid lowering effects are consumed in combination with indigenous food substances. Combination of red yeast rice with various oils is taken by different population around the world. There is paucity of data on which of these combinations works best in lowering lipids and oxidative stress.

Base line total cholesterol values for these experimental animals were not measured. But the hypercholesterolemic status was established using previous studies with similar experimental model [11-13].

These studies were done on the Sprague Dawley rats weighing 200-300 gm and hypercholesterolemic diet (containing cholesterol and lard) was given for one month. In these studies, the mean plasma/ serum total cholesterol level of rats on normal diet ranged from 50-60 mg/dl (standard deviation ranged from 2.6-5.5 mg/dl) and that of rats on hypercholesterolemic diet ranged from 87-88 mg/dl with standard deviation varying from 1-10 mg/dl. This was in agreement with the serum total cholesterol level (86±1.414 mg/dl) seen in the control rats on hypercholesterolemic diet in our study. The current study shows that rats fed with red yeast rice and rice bran oil combination had lower lipid levels, MDA levels and higher levels of beneficial paraoxonase enzyme in their serum. This combination had the best outcome among all the test groups. Combining red yeast rice with coconut oil had significant lowering effect on total cholesterol and MDA levels but did not alter the triglyceride and paraoxonase levels. Lipid lowering effect of red yeast rice seems to be affected negatively by the concurrent use of sunflower oil. To the best of our knowledge no study has compared the effect of these food combinations.

Quite a few population and animal studies have reported the lipid lowering effects of red yeast rice. Double blinded randomized control trials showed red yeast rice or its extract lowered the total cholesterol and LDL- cholesterol levels when compared with placebo [14,15]. A randomized controlled animal trial has shown that red yeast rice prevents the development of obesity, hyperlipidemia and fatty liver in mice fed with high fat diet [16]. Thus, red yeast rice has therapeutic potential not only in the treatment but also in the prevention of hyperlipidemia and obesity. Animal studies have shown that extracts of red yeast rice, reduced the levels of lipid peroxidation in experimental models. Administration of aqueous extract of red yeast rice of Indian variety reduced the level of lipid peroxidation and increased the levels of reduced glutathione, superoxide dismutase and catalase dose dependently in high cholesterol diet fed- streptozotocin (STZ) induced diabetic rats [17]. Red yeast rice was able to lower the increase of serum total cholesterol, LDL cholesterol and blood malondialdehyde in a rabbit model of experimental atherosclerosis [18].

Animal studies comparing the effect of sunflower oil and coconut oil on lipid profile have shown that there is no significant difference in the serum total cholesterol and triglyceride levels between groups using sunflower oil and coconut oil [19,20]. In

a population study, no difference was observed when the lipid profile and antioxidant enzyme levels were compared between individuals using sunflower oil and coconut oil as cooking medium, [21]. However, rabbits fed with coconut oil showed decreased lipid peroxidation when compared to those fed with sunflower oil [19].

Rice bran oil has also been studied for its lipid lowering effect [7,22]. In a randomized control trial with cross-over study design including 14 subjects with hyperlipidemia, using rice bran oil as cooking oil significantly reduced serum cholesterol and triglycerides when compared with the use of sunflower oil [22]. Studies have also shown that supplementation of rice bran oil decreases the oxidative stress in streptozotocin induced diabetic rats on high fat diet [23,24].

Many population and animal studies using red yeast rice shows a consistent fall in cholesterol and triglyceride levels. In line with these studies, we expected a fall in lipid levels and oxidative stress in all the test groups when compared to the controls since all these groups were receiving red yeast rice. But we found that only the group fed with red yeast rice and rice bran oil combination showed lower lipid levels, MDA levels and higher levels of beneficial paraoxonase enzyme in their serum when compared with the controls. The group on red yeast rice and coconut oil showed lower cholesterol and MDA levels and the group on red yeast rice and sunflower oil did not show any change in these parameters when compared with the controls. These findings indicate that the rice bran oil accentuated, whereas coconut oil and sunflower oil attenuated the beneficial effects of red yeast rice. Our study offers new insights about the effect of various edible oils on lipid lowering property of red yeast rice.

Red yeast rice contains monacolins, sterols, isoflavin as well as mono unsaturated fatty acids. Monacolins are naturally occurring inhibitors of 3-Hydroxy 3-Methyl Glutaryl CoA (HMG CoA) reductase, the key enzyme in cholesterol synthesis [6]. The anti-atherogenic effect of rice bran oil is due to modulating enzymes and genes involved in lipid metabolism including ATP Binding Cassette protein A1, Apo A1 and Peroxisome Proliferator-Activated Receptor α [25]. Its antioxidant effect is due to the upregulation of gene PON1 gene, which codes for the enzyme paraoxonase 1, and increases its blood levels [25].

LIMITATIONS

Measurement of LDL, HDL was not done in our study due to financial constraints. However, serum paraoxonase activity was measured which is known to positively correlate with the HDL concentration [26]. Other commonly used edible oils like gingelly oil and mustard oil were not included in this study.

CONCLUSION

With increasing prevalence of hyperlipidemias in general population, role of dietary therapy in prevention has become important. Effects of various diet based supplements in combination with native foods need to be studied carefully before recommending these for population. The food combination which had best outcome in preventing the development of hyperlipidemia and oxidative stress in rats fed with hypercholesterolemic diet was red yeast rice and rice bran oil. Combining red yeast rice with coconut oil and sunflower oil gave suboptimal benefits.

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