EVALUATION OF SERUM CHOLESTEROL, AMINO TRANSFERASES, GAMMA GLUTAMYL TRANSFERASE AND CREATIVE KINASE IN RED YEAST RICE (MONACUS PURPUREUS-FERMENTED RICE) FED RATS

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ABSTRACT

BACKGROUND AND AIMS

The purpose of this study was to determine the efficacy of red yeast rice (Monascus purpureus-fermented rice) in lowering cholesterol in the blood. At the same time, alanine aminotranferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (γ-GT) were measured for notable side effects in the liver. Possible muscle damage was determined by measuring creatine kinase (CK).

METHODS

The cholesterol lowering effect in serum of red yeast rice-fed rats were studied over a 42-day feeding period. A total of 16 male Sprague-Dawley rats were randomised into 8 per group: control and treated. Treated rats were administered 1.35g/kg/day. Control rats were maintained on ordinary rat chow.

RESULTS

Serum cholesterol levels were significantly decreased by 19.13% in treated group compared to controls. This treatment also showed increase in serum ALT and AST activities by 41.90% and 21.53%, respectively. Mean CK activity in treated rats showed an increase by 32.32% when compared with control rats. γ-GT is the only enzyme that showed a decrease of 15.16% in sera of treated rats. Body weights of control and treated rats increased significantly by 10% end of feeding period but were not due to treatment.

CONCLUSION

Red yeast rice significantly decreased serum cholesterol level at a dosage of 1.35g/kg/day. However, the differences in serum enzyme activities between control and treated rats were not significant.

KEYWORDS

Red yeast rice, sprague-dawley rats, serum cholesterol, aminotransferases.

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INTRODUCTION: Red yeast rice or Monascus purpureus-fermented rice is the fermented rice product on which red yeast (Monascus purpureus) has been grown. It is widely used by the Chinese for centuries as part of traditional medicine; and as food preservative and food colouring.[1,2,3,4] Typical consumption ranges from 14 to 55 g per person per day as a staple diet in most Asian countries. The uses of red yeast rice were first recorded in the Tang dynasty (618-907).[4] According to Li et al. (1998), red yeast rice is traditionally produced by fermenting washed cooked non-glutinous rice in red wine mash, natural Polygonum grass juice and alum water.[5] In contrast, commercially manufactured extracts are produced by fermenting moistened premium rice with Monascus purpureus for 9 days at 25°C between pH 5 and 6 before being air-dried (Ma et al., 2000).[1]

The frequency of CHDs and cerebrovascular diseases are seeing an upward trend (Khor, 2001).[6] With more and more people diagnosed with CVDs it has become a norm to self-medicate other than by changing to a healthier lifestyle. Some subscribe to traditional medicine whereas some resort to buying treatment that is available over-the-counter. Amongst those is red yeast rice which has promising effect as an anti-hyperlipidemic agent. As red yeast rice has cholesterol-lowering effects it is generally consumed by those who are diagnosed with CVDs without knowing much about its efficacy and side effects.

Thus, this study was performed to determine the efficacy of red yeast rice (Monascus purpureus-fermented rice) in lowering cholesterol levels in the blood and to evaluate notable side effects in particular to the liver by measuring serum AST, ALT and γ-GT levels; and whether there were any possible muscle injury by measuring serum CK activity.

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MATERIALS AND METHODS: 16 pre-puberty male Sprague-Dawley (Rattus norvegicus) rats weighing between 149 to 167 g obtained and housed in Animal house of Santhiram Medical College, Nandyal. Institutional ethics committee's approval was obtained prior to start of the study. Water was provided ad libitum to prevent dehydration. 24-hour lighting and proper ventilation were maintained throughout the study.

The rats were randomised into 2 groups: control and treated. Those assigned to control group were fed with ordinary rat chow; about 7 g of chow were rationed to each group. Treated rats were given mixed pellets for 42 days. Mixed pellets were prepared by mixing red yeast rice (1.35 g/kg/day) with ordinary rat chow. The dosage was based on the study by Wei et al. (2003).[2] Additional ordinary rat chows were added to amount to about 7g of chow per rat. Treated rats were separated individually during feeding time to ensure each rat only eats its rationed mixed pellet. All 16 rats were weighed every 2 weeks over the six-week feeding period to monitor their weights. Behaviour changes, appetite and faeces were observed over the feeding period. Raw red yeast rice (Monascus purpureus-fermented rice) was from SP suppliers, Mumbai.

All rats were sacrificed by performing intraperitoneal injection of about 0.1mL of 0.06mL/100g pentobarbitone sodium anaesthetic which corresponded to their mean body weights. Blood was withdrawn as soon as possible from the heart using 5mL latex free syringe. Collected blood samples were allowed to clot for 3 hours to ensure that sera obtained were free of coagulants. Centrifugation at 4000 rpm at 25°C for 5 minutes proceeds. Obtained sera were separated and evenly distributed into 3 eppendorf tubes. They were kept at -20°C until aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (γ-GT), creatine kinase (CK) and cholesterol were measured. 5 parameters were measured using Perkin Elmer Lambda 25 UV/Vis Spectrophotometer with 1cm light path cuvettes. The assay kits were purchased from RANDOX Laboratories, United Kingdom.

Data were presented as means±standard deviation; and standard error of means. Comparison of the 5 parameters between control and treated groups was carried out with unpaired student's t-test. Body weights of control and treated rats pre and post treatment were compared with paired student's t-test. Values of P<0.05 were considered statistically significant.

RESULTS: In this study, 16 male Sprague-Dawley rats were used as experimental models to show the lipid-lowering effects of red yeast rice. They were randomised into 2 groups of 8 each: control and treated.

Time	First week of treatment		Last week of treatment		Percentage of difference from first week of treatment (%)	P value[a]
	Mean±Standard Deviation (SD)	Standard Error of Mean (SEM)	Mean±Standard Deviation (SD)	Standard Error of Mean (SEM)		
Control (n=8)	156.190±6.690	2.731	166.785±10.982	4.484	+10.60[b]	0.0045
Treated (n=8)	158.533±8.642	3.528	168.698±8.872	3.622	+10.17 [b]	0.0045

Table 1: Comparison of body weights between control and treated rats

Units of values expressed in mean±standard deviation are in grams (g).
[a]indicates that the P values apply to the difference of body weights between first week of treatment and last week of treatment in each group and are significant (P<0.05).
[b]indicates that the positive sign shows a positive difference.

The rats were weighed in the first and last weeks of treatment as shown in Table 1. The results displayed in Table 1 showed that the body weights of each group increased about 10g throughout the six weeks. Each group significantly saw an increase in their mean body weight for P<0.005. In the first week, control group had a mean of 156.190±6.690 whereas treated group recorded a mean of 158.533±8.642. A raise to 166.785±10.982 and 168.698±8.872 was observed in control and treated groups, respectively.

Serum cholesterol levels in controlled rats were higher than of treated rats, as shown in table 2. It can be said that by administering 0.21g of red yeast rice, cholesterol levels in treated rats were 19.32 % lower than untreated rats and 29.69% lower if compared with the reference range. However, the P value calculated was not considered statistically significant (P>0.05). AST activity of treated rats at 37.038±13.607 U/L is contrasted with the mean of controlled rats which at 26.103±14.510 U/L. With an increase of close to 42% the difference was insignificant because P>0.05.

Controlled rats gave a lower reading than the reference range whereas higher AST activity was detected in treated rats as displayed by Table 2. There was an additional of 21.53% of enzyme activity in treated rats. Untreated rats had a mean serum AST activity of 119.611±58.282 U/L compared to 145.360±50.160 U/L in treated ones. The mean AST level in control group is lower than of the reference range. An elevation of about 22% did not qualify the result to be of statistically significant as P>0.05.

Mean.γ-GT levels were 4.754±2.485 U/L and 4.034±3.577 U/L in contraol and treated groups, respectively. Treated rats were found to have a lower mean value by 15.16% compared to control rats. However, the difference was not considered statistically significant because P >0.05 as shown in Table 2.

With reference to CK levels, Rats administered 0.21 g of red yeast rice daily saw a positive change of 32.23% when at 1078.470±585.600 U/L compared with the mean value 815.032±453.373 U/L of controlled rats. The reference point according to Bernadi et al (1996)[7] fell between the results. Despite of the increase, it is not considered significant for P>0.05 (Table2).

Parameter	Control (n=8)		Treatede (n=8)		Percentage of difference from Control (%)	P value
	Mean±Standard Deviation (SD)	Standard Error of Mean (SEM)	Mean±Standard Deviation (SD)	Standard Error of Mean (SEM)		
Cholesterol (mmol/L)	1.400±0.4212	0.1216	1.132±0.186	0.0588	-19.13[a]	0.0660[d]
ALT (U/L)	26.103±14.510	4.189	37.038±13.607	4.303	+41.90[b]	0.0844[d]
AST (U/L)	119.611±58.282	16.825	145.360±50.160	15.862	+21.53[b]	0.2793[d]
γ-GT (U/L)	4.754±2.485	0.7173	4.034±3.577	1.1311	-15.16[a]	0.5985[d]
CK(U/L)	815.032±453.373	130.877	1078.470±585.600	185.182	+32.32[b]	0.2624[d]

Table 2: Comparison of parameters between control and treated groups

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, gamma-glutamyl transferase; CK, creatine kinase.

[a]negative sign indicates a negative difference compared to controlled group.

[b]positive sign indicates a positive difference compared to controlled group.

[c]indicates that the P values apply to the difference between the control and treated groups.

[d]indicates that the P values are not considered to be statistically significant, P > 0.05.

DISCUSSION: The raw Monasus purpureus-fermented rice mixed with ordinary rat chow was used as an anti-hyperlipidemic drug in this short-term study. Each treated rat was fed with 0.21g of red yeast rice which corresponded to their mean body weight. The amount was based on a study by Wei et al. (2003) which used 1.35g/kg/day.[2]

Red yeast rice has been proven to effectively reduce cholesterol levels in animal and human. In a similar study periods: as much as 21.5% was reduced in patients by Lin et al. (2005)[8] whereas Lee et al. (2006)[9] claimed a 22% reduction in hamsters. Other studies conducted in a similar time-frame also saw significance reductions of 23% (Wang et al., 1997)[10] and 16.07% in the eight week of study by Heber et al. (1999).[4] There was no statistically significant difference between the treated and untreated rats in this study. Serum cholesterol in rats dosed with 0.21 g of red yeast rice per day was 19.13% lower than of untreated rats with a P>0.05. As the dosage used in this study (1.35 g/kg/day) was based on Wei et al. (2003), the efficacy of the dosage did not reach the same effect as of Wei et al. (2003).[2] The dosage used by Wei et al. (2003) is slightly higher than the recommended dose for a human which is 5g/kg body weight (Yang, Lin, Huang & Chou, 2005).[11]

Nevertheless, this study showed that red yeast rice has positive effects on cholesterol concentration in serum over a short period despite that the difference is not considered significant. This could be due to small sample size and duration. Another possible factor is that 0.21 g/rat is too small an amount to consider its significance. Likewise Li et al. (1998)[5] whose team did not obtain a significance value although there was a 39% reduction in cholesterol levels of rabbits dosed at 0.2 g/kg/day for 30 days but found that reductions were significant at 0.4 g/kg/day and 0.8 g/kg/day.

Heber et al. (1996),[4] Li et al. (1998)[5] and Wei et al. (2003)[2] advocated that the extent of its lipid-lowering effect is due to the combination of all the components found in red yeast rice and not solely because of monacolin K which is structurally similar to lovastatin. It was suggested that the other constituents play a minor role (Lin et al., 2005) [8]but is still important in determining its efficacy. Monacolin K is approximately 0.2% or about 5 mg making it a reasonable comparison to 20-40 mg of lovastatin (Heber et al., 1996).[4]

Although ALT and AST are called liver enzymes they are not confined in the liver. ALT is present in higher concentrations in liver making it a more specific marker than AST (Tolman, 2002)[12] as AST is more widely distributed but both are leaked out into the blood in hepatocellular injury. Apparently, ALT is a specific enzyme for liver function in rats not just in humans.[13]

According to Aniya et al. (1999), a certain Monascus product is able to confer liver protection by increasing the activity of glutathione-s-transferase and AST. In this study, AST levels in treated rats were found to be 21.53% higher than of controlled rats.[14] Red yeast rice can also lower the risk for developing hepatic fibrosis and hepatomegaly as seen in rabbits fed with 0.2 to 0.8 g (Li et al., 1998).[5] However, all these possibilities cannot be considered as the increase in AST levels is insignificant with P>0.05.

Elevated levels of transaminases are commonly observed in statin-related studies and are dose-dependable (Lin et al., 2006).[5] Its effect is reversible (Tolman, 2002)[12] and it does not necessarily indicate liver dysfunction (Cohen et al., 2006)[15] yet it does not rule out the possibility. In previous statin-treated animal studies, ALT levels were raised without damaged livers (Tolman, 2002).[12] Moreover, the frequency of elevated levels of ALT was similar to placebo patients and statin-treated patients (Cohen et al., 2006).[15] However, the same cannot be implied from the outcome of this study because of the insignificant value obtained although there is an increase by 42% in treated rats.

The only option to consider for any possible hepatocellular injury is to perform liver index analysis and liver biopsy as performed by Lee et al. (2005).[9]

Increases in serum γ-GT is seen in 50-70% cases of acute myocardial infarction but the source of increase remains unclear as activity in the muscles are not measurable (McIntyre & Rosalki, 1994).[16] It is predominantly used to measure liver diseases in humans. Serum γ-GT activity was not even detected in treatment-free rats (Bernadi et al., 1996)[7] but was measured at 4.754±2.485 U/L for untreated rats and 4.034±3.577 U/L for treated rats. Even Pisipirogos et al. (1999)[12] detected γ-GT activity in untreated rats. Interestingly, the enzyme level was 15.16% lower in red yeast rice-treated models of this study. A study done by Heber et al. (1996)[4] also saw a significant decrease in γ-GT levels by 14.81% as opposed to Pispirigos et al. (1999)[13] whose team found that gamma-GT activity was significantly high in their study on statin-treated rats.[12] The result of this study looks tipped towards the fact that red yeast rice consumption may not increase serum γ-GT levels which could support the fact that it does not lead to muscle damage but the decrease lacked significance (P>0.05).

Statins are infamous for causing elevated levels in CK activity usually during the first phase of treatment and with an increase in dosage (Lin et al., 2005)[8] for very high concentrations of this enzyme are linked to myositis and rhabdomyolysis (Thompson et al., 2003).[17] Rats fed with red yeast rice have a higher mean CK activity by 32.32% than of controlled rats. There is evidence that red yeast rice is linked to rhabdomylosis (Prasad et al., 2002);[18] excessive concentrations of CK activity, pain and muscle weakness and myotoxicity (Vercelli et al., 2006) to the extent that muscle coenzyme Q_{10} levels were lowered.[19] From this study, treated rats have higher creatine kinase levels by 32.32% but is not considered statistically significant (P>0.05). The study was limited by the sample size and the time-frame.

CONCLUSION: Cholesterol, alanine aminotranferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT) and creatine kinase (CK) were measured in serum of the 6 treated rats and 5 untreated rats. The rats were fed at a dose of 0.21g/rat/day based on 1.35 g/kg/day by Wei et al. (2003) during this 6-week study. Cholesterol levels dropped 19.13%. Both AST and ALT increased 21.53% and 41.9% respectively but γ-GT saw a reduction of 15.16%. An elevation of 32.32% in CK activity was seen in red yeast rice-treated rats. However, all these values are statistically insignificant because P>0.05. Therefore, possible side effects on the liver and muscle cannot be ascertained as well as the efficacy of the amount of red yeast rice used.

Further work: This study can be improved were measured on a larger sample size with multiple doses over a longer study period. HDL, LDL and triglycerides levels to be measured. Histophathological study and a set of full liver function tests could have been performed to further ascertain the extent of hepatoxicity. Examining organs like the brain and muscle tissues may uncover unknown effects. Statins have been found to have pleitropic properties. Whether red yeast rice affects similarly can be explored on animal models.

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