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CHEMICO-BIOLOGICAL EFFECTS OF *MONASCUS* FERMENTED RICE (*ANGKAK*) IN HYPERLIPIDEMIC RATS: A COMPARATIVE ANALYSIS

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ABSTRACT: *Angkak* rice (red mold rice, red yeast rice, Chinese red rice) which is a product of solid fermentation was prepared from non glutinous rice (*Oryza sativa* L.). Five different *angkak* samples containing varied level of lovastatin, pravastatin and citrinin suspension at a dose of 1g/kg were administered to hyperlipidemic rats for 30 days. There was a significant decrease in serum total cholesterol (30.8%), total triglyceride (33.3%), high-density and low-density lipoprotein cholesterol (25.9% and 40.5% respectively) in *angkak* fed hyperlipidemic rats in comparison to untreated hyperlipidemic rats ($p<0.05$). No significant difference in serum total cholesterol, total triglyceride, high-density lipoprotein and low-density lipoprotein cholesterol in serum of hyperlipidemic rats treated with *angkak* 1 (lovastatin 3.410 mg/g, pravastatin 1.524 mg/g and citrinin 8.345 μg/g) and *angkak* 4 (lovastatin 3.331 mg/g, pravastatin 1.751 mg/g and citrinin 9.874 μg/g) in comparison to hyperlipidemic rats treated with standard lovastatin (10 mg/kg) was observed. Animals fed with *angkak* samples showed no significant difference in their serum creatinine kinase, serum creatinine level and liver enzymes in comparison to normal control rats.

INTRODUCTION: Atherosclerosis, a major risk factor for coronary heart disease and myocardial infarction, remains by far the major cause of death. High serum concentration of cholesterol, especially serum low-density lipoprotein cholesterol (LDLc) and lowering of high-density lipoprotein cholesterol (HDLc) are mainly responsible for the development of atherosclerosis[1]. *Angkak* (red mold rice, red yeast rice, Chinese red rice), a traditional Chinese medicine is produced from solid-state fermentation of cooked rice (*Orizae sativa* L. Gramineae) with *Monascus purpureus*, *M. ruber*, *M. anka* and *M. pilosus* [2-8].

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The *angkak* has long been recognized remedy culture as a medicine for improving food digestion, blood circulation, muscle pain, and has also been used for the treatment of dysentery [9]. The Chinese ancient pharmacopoeia, *Ben Tsao Gum Mu*, indicates the use of *angkak* in various disorders of cardiovascular system[10].

Recent chemical investigation and clinical observation now clearly shows that *angkak* has different secondary metabolites such as lovastatin[4], γ-aminobutyric acid (GABA) [11], dimerumic acid [12] and monascin [13]. *Angkak* is empirically recognized as safe in China, Taiwan, Thailand, Korea and Japan for centuries [14]. However, during the past 10 years, some researchers have uncovered and confirmed that few strains of *Monascus* could produce citrinin, a hepatonephrotoxin, which has been reported to be found mainly in *Aspergillus* and *Penicillium* genera [15] and might contaminate

angkak [16-18]. This raises the issue about safety of *angkak* as functional food.

There are several reports on antihyperlipidemic activity of *Monascus* fermented rice. However, effects of *Monascus* fermented rice (*angkak*) containing diverse levels of lovastatin and mycotoxin citrinin on serum biochemical parameters have not been elucidated so far.

The present work aims at investigating the changes in serum lipid profile during treatment period and biochemical parameters related to liver and kidney damage at the end of treatment period, caused by different *angkak* samples (produced by different solid-state fermentation) containing different levels of lovastatin and citrinin. Hyperlipidemic albino Wistar rats were used as animal model and lovastatin was adopted in this study as reference hypolipidemic drug for data comparison.

MATERIALS AND METHODS:

Preparation of *Monascus* fermented rice (*Angkak*): A total of five different *angkak* samples designated as *angkak* 1, *angkak* 2, *angkak* 3, *angkak* 4 and *angkak* 5 were produced by employing different fermentation methodologies according to the previously reported methodology[19]. *Angkak* 1 was produced by *Monascus purpureus* MTCC 369 in 14 d fermentation period. *Angkak* 2 was produced by *Monascus purpureus* MTCC 369 and *Monascus ruber* MTCC 1880 in 14 d fermentation period. *Angkak* 3 was produced by *Monascus purpureus* MTCC 699 in a solid medium containing 5% decanoic acid in 11 d fermentation period. *Angkak* 4 was produced by *Monascus purpureus* MTCC 369 in 18 d fermentation period. *Angkak* 5 was produced by *Monascus purpureus* MTCC 369 and *Monascus ruber* MTCC 1880 in 18 d fermentation period.

Analysis of Pravastatin, Citrinin and Lovastatin in *Monascus* Fermented Rice:
Pravastatin from fermentation broth was extracted according to the procedure given by, Ajaz et al., (2011) [20] with minor modification. To Fermented rice (5 g), 10 ml of distilled water was added and pH was adjusted to 6.5 with either acid (H_3PO_4) or alkali (aq. NaOH). The broth was diluted five-fold with absolute ethanol, filtered through 0.22 µm filter and analyzed by high performance thin layer chromatography HPTLC (Camag, Muttenz, Switzerland). The samples were spotted in the form of bands of width 5 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland). A constant application rate of 150 nL s^{-1} was employed with 11.7 mm space between two bands. The chromatography was carried out by a mobile phase consisting of toluene: ethylacetate: formic acid (3:2:1, v/v/v) in a twin trough glass chamber that was previously saturated for 20 min at 25 ^{O}C with the mobile phase.

The linear ascending chromatogram was developed up to 80% height of the TLC plate. The developed TLC plates were dried in current air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III under absorbance mode at 237 nm with a speed of 20 mm s^{-1} by keeping slit dimension at 4 mm × 0.45 mm.

Lovastatin and citrinin were extracted and analyzed according to the procedure given by Samiee et al., 2003; Panda & Ali, 2012 respectively [21-22].

Preparation of Drug Compounds:
After fermentation, *Monascus* fermented rice (*angkak*) was sterilized by autoclaving at 15 psi for 20 minutes and further dried by heating at 50 °C for 24 h and powdered. The moisture content of dried *angkak* was measured at 15 to 20 %. A viscous suspension of crushed and dried *angkak* was prepared by triturating with doubled distilled water to get a concentration of 50 mg/ml with 10% w/v polyethylene glycol (PEG 400) [23]. Lovastatin suspension was prepared by mixing 50 mg of pure lovastatin (Ranbaxy Laboratories, New Delhi, India) in 5 ml of double distilled water containing 10% *polyethylene glycol 400 (*PEG 400).

Animals and Diet:
Sixty female albino Wistar rats weighing 170 g to 185 g were obtained from Central Animal Facility, Jamia Hamdard, and all the rats were initially fed a standard commercial pellet diet for 5 days after delivery to our laboratory in order to accustom the rats to our experimental conditions. After 5 days of acclimatization, the rats were randomly divided

into 10 experimental groups. They were housed identically in 15 polypropylene cages (four in each cage) in an air-conditioned room 25 ± 2 °C under a 12 h light/12 h dark cycle with free access to food and water. Water was allowed *ad libitum*.

Preparation of High Cholesterol Diet:

High fat rat diet (**Table 1**) was obtained in powder form, from National Center for Laboratory Animal Science (NCLAS), National Institute of Nutrition (NIN), Hyderabad, India. Powdered high fat diet was mixed with water (30%), formed into pellets (10g each) and finally air-dried and stored in vacuum until the diet was consumed by rats.

TABLE 1: HIGH FAT RAT DIET FORMULA (PER 857.48 g OF DIET)

Components	Amounts
Casein	233 g
DL- methonine	0.07g
Starch	210 g
Sucrose	206 g
Cellulose	0.582 g
Safflower oil	0.291 g
Tallow	207 g
Mineral mix (AIN 93G)	0.524 g
Vitamin mix (AIN 93G)	0.020 g

* High fat diet contained 58% of energy in the form of fat

Study Design:

Rats were allocated into 3 main reference groups (normal controls, vehicle controls and hyperlipidemic).

Group1 represents normal controls comprised of 6 normal rats, fed a standard commercial pellet diet and left intact without any treatment.

Group 2 represents vehicle controls comprised of 6 normal rats fed a standard commercial pellet diet and received daily 4 ml of 10 % PEG 400, orally during treatment period. Hyperlipidemic group was subdivided into 8 subgroups (Group 3, 4, 5, 6, 7, 8, 9, 10) (6 rats in each subgroups) and the rats were fed a high fat diet for one month and fed a standard commercial pellet diet during treatment period.

Group 3 represents hyperlipidemic controls in which, rats fed high fat diet during disease induction period and left without any treatment.

Group 4 represents hyperlipidemic vehicle controls received daily 4ml of 10% PEG 400 orally during treatment period. Group 5 represents hyperlipidemic rats treated with oral dose (1g/kg) of *angkak* 1 sample during treatment period. Group 6 represents hyperlipidemic rats treated with oral dose (1g/kg) of *angkak* 2 during treatment period. Group 7 represents hyperlipidemic rats treated with oral dose (1g/kg) of *angkak* 3 during treatment period. Group 8 represents hyperlipidemic rats treated with oral dose (1g/kg) of *angkak* 4 during treatment period. Group9 represents hyperlipidemic rats treated with oral dose (1g/kg) of *angkak* 5 sample during treatment period. Group 10 represents hyperlipidemic rats treated with oral dose of lovastatin suspension (10 mg/kg), during treatment period.

During the feeding and treatment period, all animals used were handled according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Government of India and Institutional Animal Ethics Committee (356/06), Jamia Hamdard, Hamdard University, New Delhi, India.

Blood Collection, Measurement of Body Weight:

Diet intake was measured daily and body weight was recorded at every 10 days intervals. During experimental period blood samples were also collected at an interval of every 10 days, in order to check the effect of high fat diet for induction of hyperlipidemia. Blood samples were collected from the tail vein under light ether anesthesia after a fast of 12 h and immediately centrifuged at a speed of 4000*g* X (3 min, 4 °C) to obtain serum. The serum samples were stored at -20 °C until serum lipids and biochemical parameters were analyzed [24].

Serum Lipid Analysis:

Concentrations of total cholesterol (TC), total triglyceride (TG), and high-density lipoprotein cholesterol (HDLc) in serum were determined by enzymatic colorimetric methods [25-26] using commercial kits (Bayer Diagnostic India Ltd., Baroda, India) by auto analyzer, Erba Chem – 5 plus (Transasia, India) [27]. Low-density lipoprotein cholesterol (LDL_C) was estimated by the equation proposed by Friedewald (1972) [28]. Serum atherogenic index was calculated from serum HDL_C and cholesterol levels using the following equation reported previously [29-30].

$$\text{Serum atherogenic index} = \frac{\text{TC - HDLc}}{\text{HDLc}}$$

LDLc = TC – HDLc - TG/5
LDLc – Low-density lipoprotein cholesterol,
HDLc – High-density lipoprotein cholesterol,
TG – Total triglyceride
TC – Total cholesterol

Liver Function, Serum Creatinine Kinase and Serum Creatinine Level:

At the end of treatment period, liver function test, i.e., serum albumin, bilurubin, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), serum creatinine kinase (CK), and creatinine were measured [31-36] in triplicate using commercial kits (Bayer Diagnostic India Ltd., Baroda, India) by auto analyzer, Erba Chem – 5 plus (Transasia, India).

Histopathological Examination:

At the end of treatment period, all the rats were sacrificed by an overdose of diethyl ether. The lungs, skeletal muscles, livers, and kidneys were carefully removed, cleaned of adhering matters and blotted on filter paper. Finally organs were immersed in buffered formalin stock (10%) and then the biopsies of organs were done. Histopathological sections of liver, kidney, lungs, and skeletal muscle tissues were stained by hematoxylin and eosin and examined for any damage by microscopic examination (Motic B1 series microscope with Motic images plus software version 2.0).

Statistical Analysis:

Values are expressed as mean ± standard error mean (SEM) n=6. Statistical significance was calculated by using one way analysis of variance followed by Dunnetts 't' test, with $p < 0.05$ to be considered as statistically significant.

RESULTS:

Quantification of Citrinin, Lovastatin and Pravastatin in *Monascus* Fermented Rice:

Angkak 1 was produced by *Monascus purpureus* MTCC 369 in a 14 d fermentation period and found to contain lovastatin at 3.410 mg/g and citrinin at 8.345μg/g and pravastatin at 1.524mg/g concentrations respectively. *Angkak* 2 was produced by *Monascus purpureus* MTCC 369 and *Monascus ruber* MTCC 1880 in a 14 d fermentation period found to contain lovastatin at 2.817 mg/g, citrinin at 9.552 μg/g and pravastatin at 1.344 mg/g concentration respectively. *Angkak* 3 was produced by *Monascus purpureus* MTCC 699 in a solid medium containing 5% decanoic acid in an 11 d fermentation period and found to contain lovastatin at 1.574 mg/g, citrinin at 0.028 μg/g and pravastatin at 0.721mg/g concentration respectively.

4 was produced by *Monascus purpureus* MTCC 369 in an 18 d fermentation period and found to contain lovastatin at 3.331 mg/g, citrinin at 9.874 μg/g and pravastatin at 1.751 mg/g concentration respectively. *Angkak* 5 was produced by *Monascus purpureus* MTCC 369 and *Monascus ruber* MTCC 1880 in an 18 d fermentation period and found to contain lovastatin at 2.002mg/g, citrinin *at* 11.382μg/g and pravastatin at 1.935 mg/g concentration respectively. The concentration of lovastatin, pravastatin and citrinin in five different *angkak* samples is shown in **Table 2**.

TABLE 2: LOVASTATIN, PRAVASTATIN AND CITRININ CONCENTRATION IN DIFFERENT *ANGKAK*.

Angkak type	Lovastatin (mg/g)	Pravastatin (mg/g)	Citrinin conc. (μg/g)
Angkak 1	3.4	1.5	8.3
Angkak 2	2.8	1.3	9.5
Angkak 3	1.5	0.7	0.03
Angkak 4	3.3	1.7	9.8
Angkak 5	2.0	1.9	11.3

Effect of *Angkak* on Serum Total Cholesterol (TC) and Total Triglyceride (TG) Levels:

The *angkak* produced by monoculture of *Monascus purpureus* and co-culture of *Monascus purpureus* and *Monascus ruber* was used as a hypolipidemia inducing drug compound in this study. **Table 3** and **Table 4** show the change of TC and TG levels during disease induction and treatment periods respectively. The TC levels and TG level gradually increased and reached at maximum level of 159.63 mg/dl and 176.29 mg/dl, respectively; on 30th day of disease induction period in group 3 animals whereas in control group 1 it remained at 88.29 mg/dl and 96.93 mg/dl, respectively (**Table 3).**

These results indicate that feeding high fat diet results in a significant increase in TC and TG levels

in contrast with normal control group1 and vehicle control group 2. Lovastatin, a cholesterol-lowering drug, was orally fed to the group

TABLE 3: LEVELS OF TOTAL CHOLESTEROL (TC), TOTAL TRIGLYCERIDE (TG), HDLc AND LDLc IN SERUM OF RATS IN VARIOUS GROUPS DURING DISEASE INDUCTION PERIOD.

Animal groups	1	2	3	4	5	6	7	8	9	10
Serum total cholesterol (TC) mg/dl										
Initial day	82.36±3.2	83.19±2.2 [n]	82.19±3.1 [n]	83.64±2.6 [n]	81.17±1.5 [n]	76.25±2.5 [n]	83.21±1.7 [n]	79.48±2.5 [n]	82.29±1.9 [n]	77.29±0.3 [n]
10th day	85.48±2.9	83.27±3.5 [n]	114.43±1.9 [a]	118.39±5.1 [a]	116.34±3.2 [a]	119.24±4.2 [a]	111.23±2.9 [a]	105.73±3.7 [a]	115.84±3.6 [a]	114.27±2.5 [a]
20th day	85.67±1.6	88.37±4.2 [n]	138.92±5.3 [a]	142.32±4.2 [a]	135.27±6.3 [a]	137.67±4.7 [a]	132.64±2.7 [a]	142.72±4.2 [a]	147.35±2.6 [a]	143.92±3.9 [a]
30th day	88.29±3.8	88.51±1.3 [n]	159.63±8.2 [a]	163.59±7.1 [a]	154.21±5.8 [a]	153.84±6.3 [a]	157.29±4.2 [a]	161.53±5.5 [a]	163.61±4.9 [a]	163.83±5.8 [a]
Serum total triglyceride (TG) mg/dl										
Initial day	93.64±2.7	92.58±1.1 [n]	97.29±2.4 [n]	95.48±3.5 [n]	93.74±2.5 [n]	94.92±2.2 [n]	99.61±1.3 [n]	94.18±3.6 [n]	96.33±3.6 [n]	93.62±1.5 [n]
10th day	93.84±1.4	95.28±1.5 [n]	123.74±3.2 [a]	128.63±5.3 [a]	122.49±6.3 [a]	132.28±1.4 [a]	122.83±3.4 [a]	127.05±2.2 [a]	121.96±2.6 [a]	127.39±2.5 [a]
20th day	94.73±2.6	95.26±2.5 [n]	158.83±4.7 [a]	159.21±5.2 [a]	153.39±5.3 [a]	153.38±3.7 [a]	149.63±6.8 [a]	156.74±5.4 [a]	158.47±8.4 [a]	154.78±3.7 [a]
30th day	96.93±3.2	99.26±6.3 [n]	176.29±6.4 [a]	173.56±7.3 [a]	169.23±5.8 [a]	168.75±8.2 [a]	165.96±7.3 [a]	168.57±7.3 [a]	172.37±5.3 [a]	171.64±7.4 [a]
Serum HDLc Level mg/dl										
Initial day	22.90±1.3	21.74±2.3 [n]	22.00±2.2 [n]	23.29±1.5 [n]	23.18±3.1 [n]	23.37±0.2 [n]	23.53±1.6 [n]	22.26±2.4 [n]	24.75±3.4 [n]	24.89±2.6 [n]
10th day	22.17±2.3	21.84±1.5 [n]	21.03±2.6 [n]	21.94±1.4 [n]	23.19±1.3 [n]	21.39±2.3 [n]	21.46±1.3 [n]	22.94±2.7 [n]	22.45±2.3 [n]	21.83±1.7 [n]
20th day	22.27±1.6	22.58±1.3 [n]	18.75±1.3 [a]	17.87±2.3 [a]	20.85±2.4 [a]	17.94±1.5 [a]	17.28±0.9 [a]	19.38±1.5 [a]	19.82±0.3 [a]	19.36±3.4 [a]
30th day	23.48±2.9	23.93±1.6 [n]	18.32±0.4 [a]	18.73±1.5 [a]	17.82±1.8 [a]	17.12±0.4 [a]	16.39±1.4 [a]	18.23±0.3 [a]	18.29±1.1 [a]	17.28±1.4 [a]
Serum LDLc Level mg/dl.										
Initial day	43.71±2.7	44.83±2.5 [n]	44.32±3.1 [n]	44.26±2.3 [n]	41.42±1.1 [n]	42.86±2.3 [n]	43.68±2.4 [n]	40.34±1.3 [n]	43.24±1.3 [n]	41.67±1.1 [n]
10th day	46.59±1.5	43.74±2.4 [n]	69.62±2.6 [a]	72.24±1.3 [a]	72.64±2.3 [a]	74.24±2.7 [a]	66.64±3.4 [a]	55.38±2.5 [a]	66.99±2.1 [a]	63.92±3.2 [a]
20th day	43.94±2.6	45.78±2.6 [n]	84.39±4.2 [a]	91.65±3.4 [a]	87.72±3.1 [a]	85.74±2.5 [a]	88.14±3.2 [a]	97.92±3.2 [a]	93.76±4.2 [a]	97.64±1.3 [a]
30th day	46.76±1.8	44.28±1.5 [n]	104.03±1.8 [a]	108.28±2.5 [a]	105.54±4.3 [a]	102.97±1.7 [a]	108.78±4.9 [a]	110.48±3.2 [a]	111.43±4.1 [a]	114.52±2.7 [a]

Data are mean ± S.E.M, obtained from six rats per group

[n] No significant difference as compared to normal control group1 rats

[a] $p<0.05$ as compared with normal control group 1 rats at corresponding time

TABLE4: LEVELS OF TOTAL CHOLESTEROL (TC), TOTAL TRIGLYCERIDE (TG), HDLc AND LDLc IN SERUM OF RATS IN VARIOUS GROUPS DURING TREATMENT PERIOD.

Animal groups	1	2	3	4	5	6	7	8	9	10
Serum total cholesterol (TC) mg/dl										
Initial	88.29±3.8	88.51±1.3	159.63±8.2	163.59±7.1	154.21±5.8	153.84±6.3	157.29±4.2	161.53±5.5	163.61±4.9	163.83±5.8
10th day	89.30±2.3	90.10±2.3	157.42±4.2	162.59±5.3	129.40±2.4 [a,b]	139.29±6.4 [a]	149.36±3.5 [a,c]	132.34±5.3 [a,b]	137.38±4.2 [a]	135.28±4.7 [a]
20th day	92.04±1.3	92.49±2.4	155.95±6.3	158.42±6.6	114.38±3.6 [a,b]	128.74±4.9 [a]	142.11±2.9 [a,c]	121.33±2.4 [a,b]	126.11±3.7 [a]	113.38±3.6 [a]
30th day	91.64±2.4	93.56±3.2	156.49±6.9	158.27±5.3	108.10±1.6 [a,b]	114.90±5.3 [a]	140.21±3.7 [a,c]	106.39±3.8 [a,b]	111.85±2.4 [a]	105.93±4.4 [a]
Serum total triglyceride (TG) mg/dl										
Initial	96.93±3.2	99.26±6.3	176.29±6.4	173.56±7.3	169.23±5.8	168.75±8.2	165.96±7.3	168.57±7.3	172.37±5.3	171.64±7.4
10th day	94.39±2.4	97.15±3.5	171.09±5.8	169.39±6.8	142.07±6.7 [a,b]	154.28±7.4 [a]	162.44±6.3 [a,c]	143.63±5.5 [a,b]	157.29±6.9 [a]	131.09±6.9 [a]
20th day	97.16±3.2	97.29±2.3	172.26±4.8	170.21±8.5	119.03±4.4 [a,b]	136.11±5.7 [a]	149.28±5.3 [a,c]	118.44±5.2 [a,b]	126.58±7.3 [a]	114.17±7.5 [a]
30th day	97.88±1.7	98.25±3.6	173.37±7.5	168.24±6.9	112.95±3.6 [a,b]	118.25±3.5 [a]	142.87±4.4 [a,c]	111.21±2.5 [a,b]	114.28±2.5 [a]	104.23±5.3 [a]
Serum HDLc Level mg/dl										
Initial	23.48±2.9	23.93±1.6	18.32±0.4	18.73±1.5	17.82±1.8	17.12±0.4	16.39±1.4	18.23±0.3	18.29±1.1	17.28±1.4
10th day	22.94±1.4	22.74±1.5	18.28±1.4	19.11±1.2	20.38±2.2 [a,b]	18.37±1.5 [a]	17.28±1.7 [a,c]	20.19±2.1 [a]	19.93±1.6 [a]	20.94±1.5 [a]
20th day	24.63±2.4	23.76±0.4	19.25±2.2	18.39±0.7	22.25±1.3 [a,b]	19.29±2.1 [a]	17.26±0.4 [a,c]	22.11±1.4 [a]	20.36±1.4 [a]	22.64±2.5 [a]
30th day	21.85±1.5	22.95±2.4	18.39±1.5	18.29±1.2	24.95±2.4 [a,b]	21.83±0.7 [a]	18.39±1.4 [a,c]	23.28±0.9 [a]	21.27±2.3 [a]	24.05±2.3 [a]
Serum LDLc Level mg/dl										
Initial	46.76±1.8	44.28±1.5	104.03±1.8	108.28±2.5	105.54±4.3	102.97±1.7	108.78±4.9	110.48±3.2	111.43±4.1	114.52±2.7
10th day	48.42±2.1	45.93±1.6	106.92±3.5	110.65±3.6	79.60±3.4 [a,b]	91.64±3.2 [a]	98.59±3.2 [a,c]	84.34±4.6 [a]	85.92±2.6 [a]	86.38±1.9 [a]
20th day	48.38±2.8	50.22±2.2	101.24±2.7	107.98±4.9	66.34±2.2 [a,b]	83.28±2.5 [a]	95.94±2.2 [a,c]	72.57±2.4 [a]	75.44±3.6 [a]	65.26±2.5 [a]
30th day	51.64±3.1	51.91±2.6	103.46±3.1	105.32±3.5	58.56±1.8 [a,b]	67.42±2.3 [a]	92.26±3.6 [a,c]	62.18±1.9 [a]	65.54±2.8 [a]	59.29±1.6 [a]

Data are mean ± S.E.M, obtained from six rats per group

[a] $p<0.05$ as compared with groups 3 rats at corresponding time

[b] no significant difference as compared with groups 10 rats at corresponding time

[c] $p<0.05$ as compared with groups 5 rats at corresponding time

to establish a positive control group. The results after the 20th day and in the 30th day of treatment show that lovastatin significantly lowered the plasma TC levels of hyperlipidemic rats ($p<0.05$). As shown in **Table 4,** the serum level of TC and TG was significantly less in the *angkak* fed groups (Group 5, 6, 7, 8 & 9) than in the hyperlipidemic controls group 3. From these observations it can be concluded that *angkak* fermented by *M. purpureus* under monoculture and by *M. ruber* and *M. purpureus* under co-culture reduced TC and TG levels in serum. Feeding *angkak* 1 for 10, 20 and 30

days shows an 82.20%, 73.63% and 69.07 % decrease in the TC levels, and 83.03%, 69.09% and 65.14% decrease in the TG levels, respectively, compared to the hyperlipidemic control Group 3. *Angkak* 3, which contained a low amount of citrinin and a low amount of lovastatin, was fed to animal group 7. It showed significant decrease in TC and TG levels in comparison to the hyperlipidemic controls Group 3. However percentage decrease in TC and TG level was less in comparison to the Group 5 animal fed with *angkak* 1. *Angkak* 1 and *angkak* 4 showed a difference of 2 mg/dl and 1mg/dl in lowering of TC and TG levels respectively. Similarly *angkak* 2 and *angkak* 5 showed a difference of 4 mg/dl lowering of TC and TG levels in both.

Effects of *Angkak* on Serum High-Density Lipoprotein (HDLc) and Low-Density Lipoprotein (LDLc) Levels:

The effects of *angkak* on the serum HDLc and LDLc levels of rats are shown in **Table 4.** The HDLc levels were significantly higher in the hyperlipidemic Group 3 control group animals than in the Group 1 normal control animals. LDLc is one of the indicators of atherosclerosis, and, therefore, the influence of different *angkak* on the LDLc lowering effect needed to be investigated further shows in **Table 4**. Group 5 animals fed with *angkak* 1 show a significant decrease in serum LDLc levels in comparison to Group 3 animals of at corresponding time ($p<0.05$)**.** *Angkak* 3 induce a minor decrease in serum LDLc levels in Group 7 animals. However, there is no significant difference in lowering of LDLc levels between positive control Group 10 and animals of Group 5.

Calculation of Serum atherogenic index (SAI) after the treatment period was another measure for evaluation of the hypolipidemic efficiency of different *angkak* samples. SAI of different groups of animals is shown in **Table 5**. Results show that there is no significant difference between Group1 animals of and hyperlipidemic rats treated with *angkak* 1 in Group 5 animals of, with *angkak* 4 in Group 8 animals and standard drug (lovastatin) treated Group10 in animals.

Liver Function, Serum Creatinine Kinase Level and Serum Creatinine Analysis:

Since *angkak* contains the mycotoxin citrinin, a hepatotoxic and nephrotoxic agent; safety of different *angkak* samples was also evaluated. Liver function test of rats belonging to different groups showed no significant difference in serum albumin, bilurubin, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) and alkaline phosphatase (ALP) levels (**Table 6**). However, animals fed with different type of *angkak* showed no significant difference in their serum creatinine kinase and serum creatinine level in comparison tonormal control rats but animals fed with standard lovastatin showed an elevated level of serum creatinine kinase (**Table 6**).

This may be possibly due to damaged skeletal muscle. Similarity in serum creatinine kinase and serum creatinine level between all animal groups fed with *angkak* and animals of normal control group shows that there was no myopathy or nephropathy in the animals.

TABLE 5: EFFECTS OF VARIOUS ANGKAK SAMPLES ON SERUM ATHEROGENIC INDEX (SAI) OF HYPERLIPIDEMIC RATS

Animal groups	Serum atherogenic index
Normal control - group1	3.21±0.4
Vehicle control - group 2	3.45±0.3
Hyperlipidemic controls - group 3	7.38±0.2
Hyperlipidemic vehicle controls - group 4	7.50±0.8
Hyperlipidemic rats treated with angkak 1 - group 5	3.39±0.5 [a, b]
Hyperlipidemic rats treated with angkak 2 - group 6	4.29±0.4
Hyperlipidemic rats treated with angkak 3 - group 7	6.51±0.5
Hyperlipidemic rats treated with angkak 4 - group 8	3.45±0.6 [a, b]
Hyperlipidemic rats treated with angkak 5 - group 9	4.31±0.3
Hyperlipidemic rats treated with lovastatin - group 10	3.37±0.7 [a, b]

[a] $p<0.05$ as compared with groups 3 rats at corresponding time
[b] no significant difference as compared with groups 1 rats

TABLE 6: EFFECT OF ANGKAK ON LIVER PROFILE, SERUM CREATININE KINASE AND SERUM CREATININE LEVEL ON DIFFERENT ANIMAL GROUPS

Group	Creatinine kinase (U/L)	Albumin mg/dl	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Bilirubin mg/dl	Serum creatinine mg/dl
1	577±72 [a]	3.76±0.45	164.34±15.35	53.21±5.92	23.98±2.16	1.444±0.43	1.74±0.49
2	621±94 [a, n]	3.96±0.53 [n]	156.38±16.48 [n]	65.10±7.82 [n]	24.38±2.93 [n]	1.362±0.56 [n]	1.35±0.33 [n]
3	593±98 [a, n]	2.98±0.15 [n]	137.89±21.36 [n]	46.28±10.73 [n]	26.64±1.56 [n]	1.947±0.32 [n]	1.58±0.23 [n]
4	584±79 [a, n]	3.10±0.64 [n]	167.19±11.38 [n]	52.38±9.75 [n]	25.73±2.84 [n]	1.843±0.63 [n]	1.73±0.37 [n]
5	629±76 [a, n]	4.04±1.02 [n]	154.25±18.46 [n]	49.38±8.52 [n]	27.18±3.13 [n]	1.170±0.23 [n]	1.65±0.23 [n]
6	637±83 [a, n]	3.65±0.84 [n]	144.28±17.84 [n]	40.83±9.15 [n]	26.93±1.81 [n]	1.038±0.65 [n]	1.81±0.23 [n]
7	612±82 [a, n]	3.95±0.54 [n]	143.65±26.27 [n]	49.17±7.85 [n]	26.10±1.03 [n]	1.563±0.38 [n]	1.63±0.29 [n]
8	641±93 [a, n]	3.28±0.62 [n]	155.11±15.93 [n]	52.39±8.45 [n]	24.38±2.22 [n]	1.384±0.57 [n]	1.73±0.37 [n]
9	603±84 [a, n]	3.08±0.39 [n]	128.34±17.48 [n]	38.06±7.11 [n]	28.45±2.74 [n]	1.563±0.28 [n]	1.79±0.35 [n]
10	869±59	3.54±0.27 [n]	139.21±13.58 [n]	42.53±6.35 [n]	25.37±2.57 [n]	1.910±0.17 [n]	1.69±0.29 [n]

Data are mean ± S.E.M, obtained from six rats per group

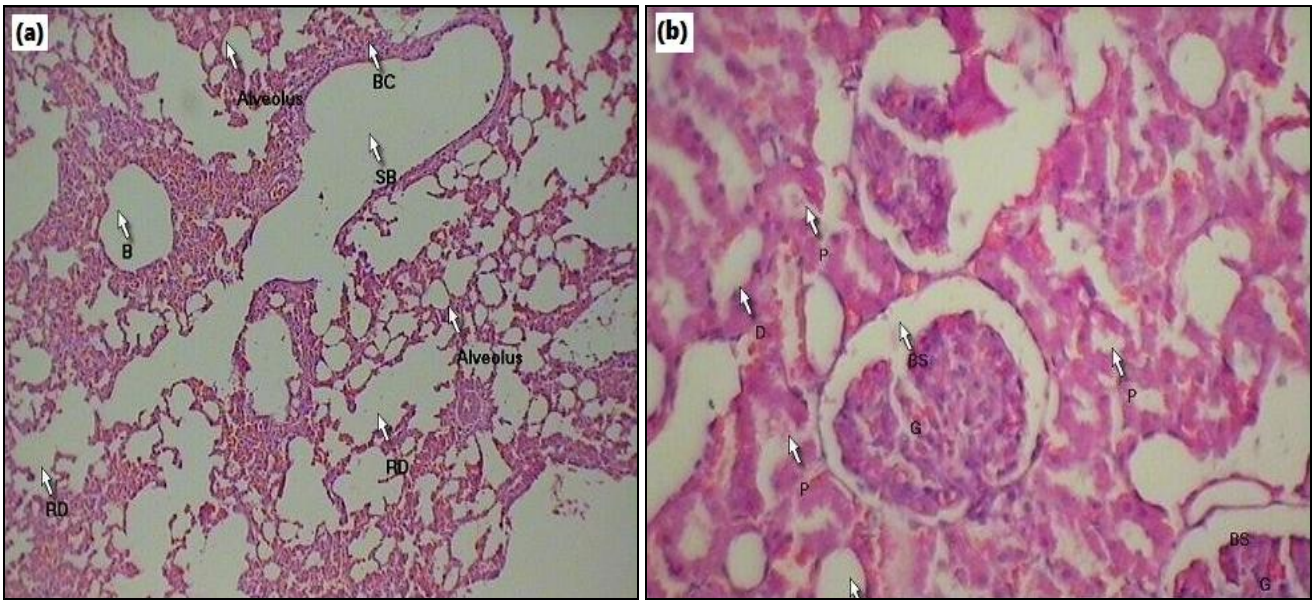
[a] $p<0.05$ as compared with group 10 rats

[n] no significant difference as compared with group 1

Histopathological Examinations of Organs:

The rats were sacrificed after the 30th day of treatment. The liver tissue, lungs tissue, kidney tissue and skeletal muscle tissue were removed and collected and a biopsy was carried out for each tissue. Microscopic examination during biopsy did not show any significant damage to lungs, liver, skeletal muscle tissue and kidney tissue of different animal groups. Microscopic examination of lung tissue of animals belonging to Group 9 showed no dilation of air space, destruction of septal walls. The bronchiole wall also did show infiltration by acute and chronic inflammatory cells (**Fig. 1a**). This implies that respiratory system was not damaged by presence of high citrinin in *angkak* sample 5. There was no sign of damage to skeletal muscle tissue in Group 5 animals of and histology of skeletal muscles of animals belonging to Group 3, 5 and 10. Histopathological examination of renal cortex and renal medulla in animals of group 9 shows normal glomerulus, Bowman's space, proximal tubule and distal tubules (**Fig. 1b**).

This implies that urinary system is not damaged by the presence of high citrinin in *angkak* 5. Histopathological examination of liver lobule and liver portal area of animals belonging to Group 9 showed very low amount of connective tissue in portal triads. This implies that liver cirrhosis is absent. Moreover, mononuclear inflammatory cells are not seen in portal area. This indicated that there was no damage to the hepatocytes (**Fig. 1c**).



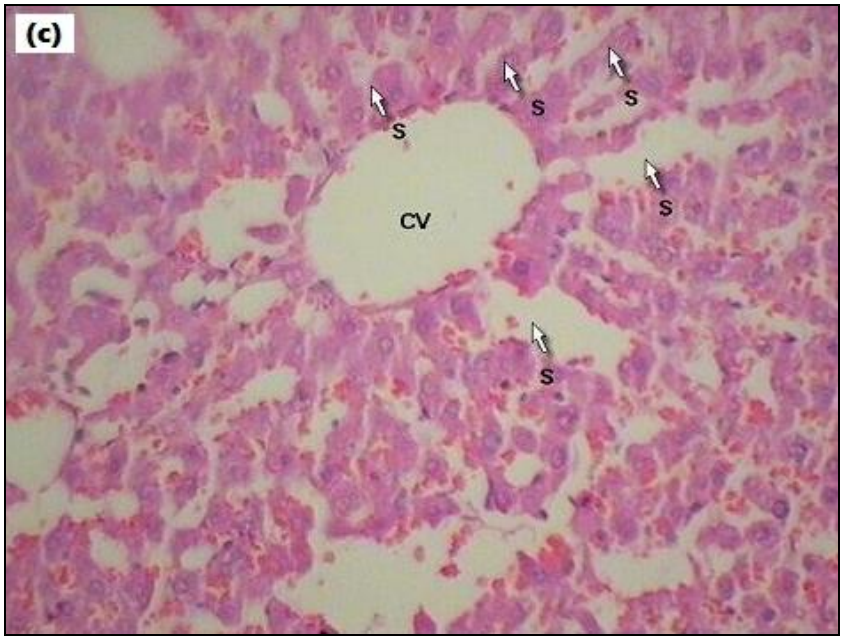


FIG.1: HISTOPATHOLOGICAL SECTION (100X) OF LUNGS OF RAT BELONGING TO GROUP 9, WHERE B, BC, SB, RD REPRESENTS BRONCHIOLE, BRONCHIAL CARTILAGE, SMALL BRONCHUS AND RESPIRATORY DUCT, RESPECTIVELY (a). Histopathological section (400 X) of renal cortex of rat belonging to group 9, where G, BS, P, D represents glomerulus, Bowman's space, proximal tubule and distal tubules, respectively (b). Histopathological section (400 X) of liver lobule of rat belonging to group 9, where CV and S represents central vein and sinusoids, respectively (c).

DISCUSSION: Lovastatin and pravastatin, competitively inhibits the activity of the rate-limiting enzyme of cholesterol biosynthesis 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the reduction of HMG-CoA to mevalonate during cholesterol biosynthesis [37-38]. Endo et al., 1979 demonstrated that *Monascus* sp. formed a cholesterol-lowering agent, monacolin K, which was proven to possess the identical structure with lovastatin and produced by *Monascus pilosus*, *Aspergillus terreus*, *Monascus ruber*, *Monascus purpureus* and *Penicillium* species [2, 5, 38, 40]. Some hyper producing strains of *Aspergillus terreus* have been reported to produce high amount of lovastatin under submerged fermentation [41]. *Angkak* fermented by *Monascus* has been regarded as a functional food having a cholesterol-lowering effect, since monacolin K is identical to lovastatin.

Previous studies have shown that *Monascus* fermented rice may lower total cholesterol (TC) by 13 to 26%, low-density lipoprotein cholesterol (LDL-C) by 21 to 33%, and triglycerides (TG) by 13 to 34%, in humans and also confirmed in animal models [42]. In this study *angkak* produced by monoculture of *M. purpureus* MTCC 369 significantly reduced serum TC, TG, and LDLc with minor changes in the HDLc levels. The results of the present study showed that *angkak* 1 and *angkak* 4 significantly decreased TC and TG levels. Feeding rats with *angkak* 3 which, contained a low level of lovastatin as well as low level of citrinin showed a decrease in TC, TG and LDLc in comparison to hyperlipidemic controls, but percentage decrease in comparison to groups fed with *angkak*1, *angkak* 2, *angkak* 4 and *angkak* 5 was less. Moreover, Serum atherogenic index (SAI) of different animal groups fed with *angkak* 1, *angkak* 2, *angkak* 4 and *angkak* 5 after the treatment period showed no significant difference when compared to groups fed with pure lovastatin. The anticholesterol effect of *angkak* is mainly due to presence of monacolin K (an analogue of lovastatin) and pravastatin. In combination of lovastatin and pravastain showed synergistic hypocholesterolemic activity [43] which support our study result.

Based on the liver profile and serum creatinine levels, hepatotoxic and nephrotoxic effects of all *angkak* samples were unobserved in experimental animals irrespective of citrinin levels. Presence of antioxidant compound (a free radical scavenger) dimerumic acid [12, 43] and an anti inflammatory agent monascin [13] in *angkak*, repaired the damaged

tissues. The serum creatinin kinase of all the groups remain same. Moreover, myopathy caused by statin [44] was not observed in animal fed with *angkak*. It may be due to the presence of low amount of lovastatin and pravastatin in *angkak* in comparison to animal fed with standard drug lovastatin (10 mg/kg).

CONCLUSION: In the present investigation it was observed that *angkak* 1 and *angkak* 4 at a dose of (1g/kg) had a very similar effect on the lipid profile in animals model that the standard drug lovastatin at a dose of 10 mg/kg could elicit. However, in *angkak*1 and *angkak* 4 the maximum lovastatin concentrations were only 3.41 mg/g and 3.331 mg/g respectively, suggesting that the presence of pravastatin in *Monascus* fermented rice, helped in lowering of serum total cholesterol, total triglyceride, and low-density lipoprotein cholesterol due synergistic activity. *Angkak* could be used as functional food for lipid management. Moreover, downstreaming of pure lovastatin from fermented medium was not required as solid fermented materials can be consumed directly after sterilization.

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