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# BIOLOGICAL STUDEIS ON RED RICE EXTRACT PRODUCED BY *MONASCUS PURPUREUS* FUNGUS

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**ABSTRACT:** ***Monacolin k (MK) was produced using Monascus purpureus fungus (DSMZ 1379) cultivation on broken rice medium moistened to 35% and incubated at 28°C for 17 days. The resultant materials had been analyzed as chemical composition and HPLC determination. The results indicated that MK in red rice yeast was 0.36 %. Also, protein, fat, ash and total carbohydrate contents were 20.43%, 6.84%, 0.933 % and 72.357 % respectively.***

***Biological investigation had taken place in three rat groups with concentrations of Monacolin k, 0.2%, 0.4% and 0.8% /ml extract/day orally treated beside two control groups. Data showed that Monacolin k extract improved lipid profiles by lowering plasma total cholesterol and triglyceride concentrations compared with the HF and NC groups. The plasma HDL-cholesterol concentration was higher in the Monacolin k extract treated groups than in the HF group, however, the ratio of HDL- cholesterol /Total-cholesterol was significantly increased by 37.58%, 61.67% and 79.95% of treated samples with 0.2, 0.4 and 0.8% MK/ml extract/day, respectively.***

***Histopathological diagnosis reported that there were a good prognosis of the tested liver and heart slides of the pretreated hypercholesterolemic experimental rats specially 0.2% and 0.4% MK/ml extract/day groups.***

***Key words:*** ***Monascus purpureus – Monacolin k -Fermented red rice-Lovastin- Angkak.***

## INTRODUCTION

**Increased levels of cholesterol and triglycerides are known to be risk factors for developing coronary artery diseases. Lipid-lowering agents that inhibit 3-hydroxy-3-methylglutaryl co-enzyme A (HMG coenzyme A reductase) are now prominent among the drugs of choice for treating hypercholesterolemia. It is another effective way to control cholesterol level with diet and food supplements. (Wei *et al.,* 2003).**

**Cultivation and production procedure of Monacolin k was reported by Blanc *et al.,* (1994). Slant culture of *Monsscus purpureus* was kept on potato dextrose agar (PDA) at 28°C for 10 days. A suspension of spores was used to inoculate 2L of medium, which incubated at 28°C on a rotary shaker for 3**

**days then the inoculum was transferred to 15L of synthetic medium and incubated at 28°C.**

**Meyer (1990) mentioned that, *Monascus purpureus* DSMZ 1379 is a red mold which may be cultivated on starch containing substrates. The solid state fermentation of rice by *Monascus* has a long tradition in East Asian countries.**

**Broder and Koehler (1980) reported that the optimum temperature to incubate *Monascus purpureus* on liquid medium that gave maximum pigment and biomass production was 28°C.**

**Madkour *et al*., (2000) applied *Monascus* pigments in beef burger as natural red colorant. Results indicated that, the burger and the red rice origin at the concentration of 0.32% dry biomass and 0.54% red rice had highly sensory evaluation scores.**

**Juzlova *et al.,* (1994), mentioned that *Monascus* pigments have been used as food colorants in the Far East for centuries. They are considered to be a possible substitute for synthetic food dyes. Also, the red pigments produced in solid state cultures by several species of the genus *Monascus* have been traditionally used in Asia for coloring and securing a number of fermented foods. Furthermore, *Monascus* pigments had therapeutic properties and were relatively high stable with respect to pH and temperature.**

**Martinková and Patakova (1999) reported that, *M. purpureus* extract produced from the fungal mycelium and applied in mice (in vivo) had non-toxic effect (mean lethal dose (LD50) >10 g/kg body weight) as administrated orally. Similarly, oral doses of up to 18 g red rice per kg body weight caused no toxic effect in mice.**

**Therefore, the main objective of this research is to study the effect of using different MK concentrations on reducing blood-lipid levels in animal models.**

## MATERIALS AND METHODS

### Materials

### Tested organism:

***Monascus purpureus* (red mold rice) was used for the production of Monacolin k. The strain was kindly provided by DSMZ (Deutsche Sammlung von Mikroorganismen, und Zell Kulturen, Braunshweig, Germany).**

### Potato dextrose agar medium (PDA):

**Yeast and fungi were maintained on the PDA media (Oxoid, 1990) which has the following composition:**

| | |
|---|---|
| **Potato extract** | **4.0 gram (g)** |
| **Dextrose** | **20.0 g** |
| **Agar** | **15.0 g** |
| **Distilled water** | **1000 ml** |

## Broken rice medium:

**Fifty g of broken rice in 250 ml Erlenmeyer flasks were moistened to 35% moisture content then the media flasks were sterilized at 121$^{o}$C for 1h. (Martinkova *et al.;*1995).**

## Standard solution and High Performance Liquid Chromatography (HPLC) reagents.

**A Mevacor tablet (MSD, Rahway, USA) with nominal content of 20 mg mevinolin (Mk) per tablet was used as standard solution. Ethanol was used for the extraction of the crude Monacolin k. All solvents (acetonitrile, methanol and phosphoric acid) were of HPLC grade (E. Merck, Darmstadt, Germany).**

## Biological experiments.

## Experimental animals.

**Male albino rats (Wister strain), with an initial body weight of 150 g were obtained from the Organization of Biological Products and Vaccines, Helwan farm, Egypt.**

**Compositions of the experimental diets.**
**The formula of the experimental diets was illustrated in table (1).**
**Table 1. The composition of the experimental diets:**

| Composition | Normal diet % | Hyperlipidemic diet % |
|---|---|---|
| Corn starch | 65 | 50 |
| Casein | 15 | 15 |
| Corn oil | 10 | 10 |
| Cellulose | 5 | 5 |
| Salt mixture | 4 | 4 |
| Vitamin mixture | 1 | 1 |
| Cholic acid | - | 0.2 |
| Lard | - | 14.8 |

## Biological experiments kits.

**Total cholesterol, triglycerides, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were obtained from the biodiagnostic Co. Dokki, Egypt. While, High Density Lipoprotein Cholesterol (HDL-c) kit was obtained from the Biosystem Co. Spain.**

## Methods:

### Preparation of spores suspension:

***Monascus purpureus*, culture was inoculated onto slants of potato dextrose agar medium (PDA) and incubated at 28°C for l0 days (until heavy sporulation). Ten milliliters of sterile distilled water was pipeted aseptically onto each slant. The spores were dislodged by rightly scraping the aerial growth with the same pipette and the resultant suspension (about $l0^8$ spore /ml) was withdrawn and transferred to sterile glass according to the procedure of Hajjaj *et al.,* 2000.**

### Solid medium fermentation:

**The method described by Martinkova *et al*., (1995) was used as follows: Fifty g of broken rice was placed in 250 ml Erlenmeyer flasks and moistened with distilled water to 35% moisture content. The medium was sterilized at 121°C for 1h then the flasks were inoculated with *Monascus purpureus* and incubated at 28°C for 17 days.**

### Moisture and Ash content:

**Moisture and ash content of all tested samples were determined as described by AOAC (2003).**

### Crude protein:

**Total nitrogen was determined in samples using Kjeledahl method as described by AOAC (2003) and crude protein was calculated by multiplying the total nitrogen percentage by 5.87 according to Paulo *et, al.* (1994).**

### Total carbohydrates:

**Total carbohydrate was determined in the samples as described by Trevelyan and Harrison (1956).**

### HPLC determination of Monacolin k:

**The chromatographic determination of Monacolin k, either by a Beckmann Ultrasphere ODS (50×4.6 mm I.D., 5 µm) or a Waters NovaPak $C_{18}$ (150×3.9 mm I.D., 4 µm) column was used. The eluent was acetonitrile 0.1% phosphoric acid (50:50, v/v) solution flowing at 1.5 ml/min for both columns. The detection wavelength was 358 nm. Using the Ultrasphere column and 100µl injection volume. The detection limit for Monacolin k was 50 ng/ml for the fermentation sample. (Food Technology Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt).**

**Standard solution of Monacolin k was prepared by dissolving 20 mg (one tablet ) in 1ml of HPLC-grade water. The resulted solution was filtrated throw Wattman No.1 and centrifuged at 2000 rpm for 15 min and aliquots of the clear supernatant was injected.**

**Monacolin k concentrations in tested samples were determined by calculating the percentage area under curves against the percentage area of standard curve solution. Results were calculated as mg Monacolin k/g fermented solid media.**

## Biological experiments:

**Twenty five albino rats, with an initial body weight of 150 g were housed in screen-bottomed aluminum cages in rooms maintained at 25±1°C with alternating cycles of light and dark of 12h duration. Rats were randomly allocated into two groups, the first group (normolipidemic) contained five animals and the second group (hyperlipidemic) contained twenty animals with a mark on their tails as a mean of differentiation. The normolipidemic group, rats were fed on normal diet. While the hyperlipidemic group, rats were fed on high fat diet (HF).**

**After arising the cholesterol level to ≥240 mg/dl, fifteen animals from the hypercholesterolemic rats was divided into three groups (five rats each), the three groups were treated orally (1 ml extract) by gastric tube with ethanolic *Monascus* fermented red rice extracts (crude Monacolin k) at 0.2, 0.4 and 0.8% MK/ml extract/day for 30 days (concentrations were prepared after HPLC determination), while the fourth group was fed on high fat diet (HF). The changes in body weight were recorded weekly. Blood samples were also obtained from the retro-orbital plexus of the eyes from all animals of each group at the end of experiment; the organs were excised immediately after bleeding for weight. Serum was obtained from blood samples by centrifugation at 1500 rpm for 15 min at ambient temperature. All serum samples were stored under -20 °C before usage. (Animal house, Horticultural Research institute-Agricultural Research Center, Egypt)**

**Enzymatic determination of cholesterol was carried out according to Allain (1974). Fully enzymatic determination of total triglycerides in plasma was measured colorimetrically at 546 nm, according to Fossati and Principe (1982), While, the HDL-c was determined according to the method of Burstein, *et al* (1980).**

## Organs histology:

**Autopsy samples were taken from the liver and heart of sacrificed rats in different experimental groups then fixed in 10% formol saline for twelve hours then decalcification was done. Serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used. Specimen were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 micron thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by Hematoxylin and eosin Stain as**

**described by Banchroft *et al.,* (1996), for histopathological examination through the light microscope.**

## Statistical analysis:

**All data were subjected to statistical analysis according to the procedure reported by Snedecor and Cochran (1980) and the statistical analysis system program (SAS, 1996). The analysis was carried out using the PROC ANOVA procedure. Duncan multiple ranges at 5 % level of significance were used to compare between means. Results followed by different alphabetical letters were significantly different, while Student t-test and factorial analysis were used with data obtained from plasma lipids profiles and liver function in rats.**

## RESULTS AND DISCUSSION

## Chemical composition of red yeast rice produced by *Monascus purpureus*.

**Data illustrated in Figure 1 showed the chemical composition of MK in red rice yeast was 0.36 %. It could be noticed that, the protein content was 20.43%, whereas the fat content gave 6.84%. Ash content was 0.933 %. On the other hand the total carbohydrate content was 72.357 %.**

**Data in Fig. 2 illustrated that Monacolin k (%) extracted from the red yeast rice produced by Monascus purpureus growing on broken rice medium with 35% moisture content at 28°C for 17 days was 0.36% this result is near to the result obtained by Heber *et al*., (1999), which was 5 mg in a 1.2 g of red yeast rice.**

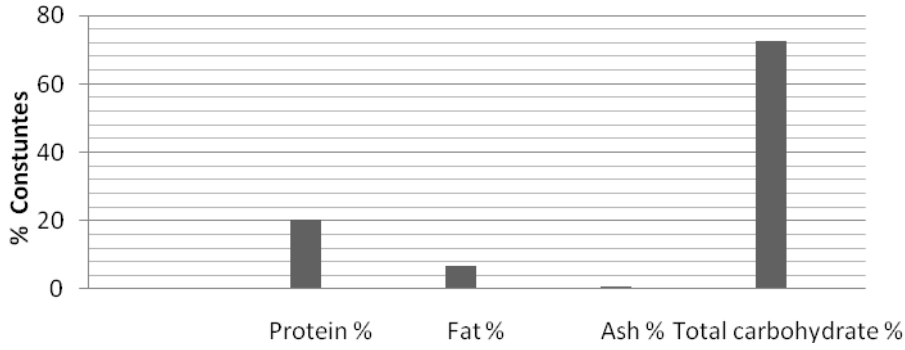



**Figure 1: Chemical composition (%) of red yeast rice produced by *Monascus purpureus* growing on broken rice medium moistened to 35% and incubated at 28°C for 17 days.**

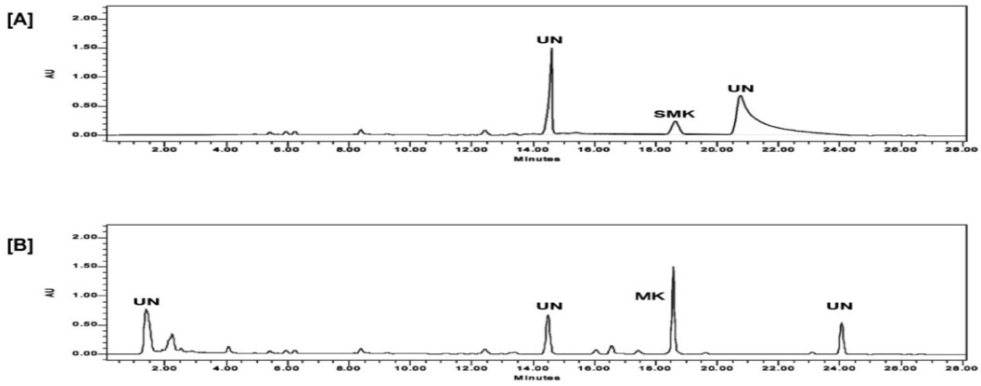



**Figure 2: HPLC diagrams of MK whereas (A) HPLC profile of standard Monacolin k solution. (B) HPLC profile of Monacolin k obtained from red yeast rice extract. SMK= Standard Monacolin k. MK = Monacolin k. UN= unanimous. AU= peak height**

## Biological experiment:

## Effect of red yeast rice (MK) extract at different concentrations on body weight.

**Table 2 showed that the initial body weights of the five groups were not significantly different, however, after feeding for 4 weeks; the body weights of High Feed (HF) group was significantly higher than the normal control (NC) group as well as MK treatments, we referred that to the consomption of high fat diet. This trend has also been reported by Almario *et al.,* (2001).**

**Table 2: Effect of oral administration of red yeast rice (MK) extract at different concentration on body weight gain.**

| Parameters | Normal control group (NC*) | High fat feed group (HF**) | Red rice yeast (MK) treatment (% of MK/ml extract/day) | | |
|---|---|---|---|---|---|
| | | | 0.2 | 0.4 | 0.8 |
| Body weight gain | | | | | |
| Initial | $149.8^{Aa}$ | $151.2^{Aa}$ | $150.9^{Aa}$ | $153.5^{Aa}$ | $149.4^{Aa}$ |
| Final | $170.7^{Ba}$ | $177.1^{Bb}$ | $169.2^{Ba}$ | $172.4^{Ba}$ | $171.6^{Ba}$ |

**Different superscript small characters mean significant differences between different treatments in the same storage periods ($p \leq 0.05$). Different superscript capital characters mean significant differences between different storage periods in the same treatment ($p \leq 0.01$). * Normal control group ** High fat fed group**

## Effect of red yeast rice (MK) extract at different concentrations on internal organs relative weight of male albino rats:

**Table 3 showed that, relative weights of liver were significantly higher in the HF group than in the NC and Monacolin k extract treated groups because of the fatty liver. However, the kidney and heart relative weights were not significantly different between all the groups. This trend has also been reported by Lee *et al.;* (2006).**

**Table 3: Effect of red yeast rice (MK) extract at different concentrations on internal organs relative weight of male albino rats:**

| Parameters | Normal control group (NC*) | High fat feed group (HF**) | Red rice yeast (MK) treatment (% of MK/ml extract/day) | | |
|---|---|---|---|---|---|
| | | | 0.2 | 0.4 | 0.8 |
| Relative weight of visceral organs (final) [% of body weight] | | | | | |
| Liver | 3.33[a] | 4.15[b] | 2.95[a] | 3.25[a] | 3.34[a] |
| Kidney | 0.68[a] | 0.71[a] | 0.70[a] | 0.71[a] | 0.73[a] |
| Heart | 0.40[a] | 0.50[a] | 0.44[a] | 0.40[a] | 0.42[a] |

**Different superscript small characters mean significant differences between different treatments in the same storage periods ($p \leq 0.05$). Different * Normal control group ** High fat fed group**

**Table 3 showed that, relative weights of liver were significantly higher in the HF group than in the NC and Monacolin k extract treated groups because of the fatty liver. However, the kidney and heart relative weights were not significantly different between all tested groups. Those results are in good agreement with those reported by Lee *et al.;* (2006).**

## Effect of red yeast rice (MK) extract at different concentrations on lipid profile parameters:

**Concentrations of plasma lipids are shown in Table 4. Monacolin k extract treated groups at concentrations 0.2, 0.4 and 0.8% MK/ml extract/day are significantly lower in plasma total cholesterol concentration by 27.64%, 32.84% and 44.8% compared to the HF group. Also, triglyceride concentrations were significantly lower by 41.98%, 47.69% and 53.36% when compared to the HF groups, respectively. On the other hand the HDL-cholesterol concentrations were significantly elevated in the Monacolin k extract treated groups compared to the NC or HF group. The ratio of HDL-C/Total-C exhibited the highest value in the Monacolin k extract treated groups and the lowest value in the HF group. For this reason, atherogenic index (AI) was significantly higher in the HF group than in the NC and Monacolin k extract treated groups.**

**From the previous results, it could be noticed that Monacolin k extract improved lipid profiles by lowering plasma total cholesterol and triglyceride concentrations compared with the HF and NC groups. The plasma HDL-cholesterol concentration was higher in the Monacolin k extract treated groups than in the HF group, however, the ratio of HDL- cholesterol /Total-cholesterol was significantly increased by 37.58%, 61.67% and 79.95% of treated samples with 0.2, 0.4 and 0.8% MK/ml extract/day, respectively when compared with the HF group, these findings are in agreement with the results obtained by, Li *et al* (1998).**

## Effect of red yeast rice (MK) extract at different concentrations on liver function parameters:

**Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) plasma levels were performed to assess liver function. As can be observed in Table 4, alterations were not detected in the animal group treated with the HF diet while those treated with Monacolin k extracts were significantly decreased in AST and ALT enzymes during 4 weeks, when compared to the control group.**

**Table 4: Effect of oral administration of red yeast rice (Mk) extract on plasma lipids profiles and liver function in rats.**

| Parameters | NC[a] | HF[b] | Red rice yeast (Mk) treatment (% of MK/ml extract/day) | | |
|---|---|---|---|---|---|
| | | | 0.2 | 0.4 | 0.8 |
| Total cholesterol (mg/dl) | 150.16 ± 0.32 | 166.02** ± 0.23 | 120.13*** ± 0.27 | 111.5*** ± 0.15 | 82.82*** ± 0.03 |
| Triglycerides (mg/dl) | 70.88 ± 0.06 | 107.63** ± 0.02 | 62.44 ± 0.05 | 56.3* ± 0.03 | 50.2** ± 0.02 |
| HDL-cholesterol (mg/dl) | 48.19 ± 8.68 | 45.29 ± 4.35 | 62.31** ± 2.6 | 73.22** ± 5.2 | 81.5*** ± 3.1 |
| LDL-cholesterol (mg/dl) | 94.7*** ±2.1 | 144.2** ±6.5 | 88.8*** ±9.2 | 88.7*** ±4.9 | 90.6*** ±29.1 |
| HDL-cholesterol/ Total cholesterol (%) | 32.09 ± 0.46 | 27.28** ±0.45 | 51.87*** ±0.41 | 65.67*** ±0.26 | 98.41*** ±0.13 |
| AI[c] | 2.12 ±0.045 | 2.67** ±0.060 | 0.926*** ±0.01527 | 0.523*** ±0.006 | 0.016*** ±0.006 |
| AST activity (U/L) | 41. 80 ± 18.3 | 44.40 ± 14.41 | 35.61 ± 9.3 | 31.44* ± 6.4 | 27.36** ± 3.3 |
| ALT activity (U/L) | 40.44 ± 3.95 | 50.07 ± 12.02 | 39.84 ± 6.44 | 35.85* ± 3.35 | 31.51** ± 4.01 |

*** Statistical significant differences (P < 0.05)** **** Statistical significant differences (P < 0.01)**
***** Statistical significant differences (P < 0.001)** **[a] Normal control group** **[b] High fat fed group**
**[c] Atherogenic index: (Total cholesterol - HDL-cholesterol)/HDL-cholesterol.**

**Histopathological findings of liver and heart tissues of tested rats treated with oral administration of red yeast rice (MK) extract at different concentration.**

**Normal control rat group (NC):**

**Liver slide showed that, there was no histopathological finding observed and the normal histological structure of the central vein and surrounding hepatocytes were recorded in Fig.3 (G1a). Heart slide explained that, there was no histopathological finding observed and the normal histological structure of the cardiac muscle bundles were recorded in Fig. 3 (G1b).**

**High fat fed group (HF):**

**Liver slide showed that, there was a Sever dilatation and congestion was observed in the central and portal veins as well as hepatic sinusoids Fig. 3 (G2a). Heart slide showed oedema in the subendocardial tissue Fig.3 (G2b) while the myocardium showed focal mononuclear leucocytes inflammatory cells infiltration degeneration and hyalinization. There was focal haemorrhage in the subpericardium tissue associated with sever dilatation and congestion in the myocardial blood vessels.**

**Treated rat group with 0.2 g Monacolin k/ml extract/day:**

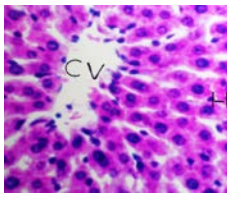
**Liver slide showed degenerative changes in the hepatocytes associated with sever congestion in the central veins and sinusoids Fig.3 (G3a). Heart slide showed that the myocardium had congestion in the sclerotic blood vessels Fig.3 (G3b) as well as focal mononuclear leucocytes inflammatory cells infiltration.**

**Treated rat group with 0.4 % MK/ml extract/day:**

**Liver slide showed that, there was congestion in the central veins and sinusoids with focal haemorrhages in the hepatic parenchyma and inflammatory cells infiltration in between the hepatocytes Fig. 3 (G4a). Heart slide explained that, the myocardium was degenerated Fig. 3 (G4b), associated with focal haemorrhages in the subepicardial tissue. Focal haemorrhages were noticed in the deep area of the myocardium.**

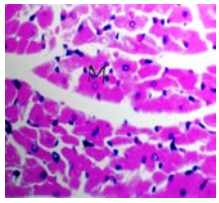
**Treated rat group with 0.8 % MK/ml extract/day:**

**Liver slide showed congestion in the central veins associated with fatty change in the hepatocytes surrounding the central veins as well as surrounding the portal area Fig. 3 (G5a). Heart slide explained that, the myocardium had focal inflammatory cells infiltration adjacent the congested blood vessels Fig. 3 (G5b), associated with sclerosis in the wall of other congested myocardial blood vessels. There was oedema with inflammatory cells infiltration in the pericardium, while the underlying myocardium showed degenerative changes.**



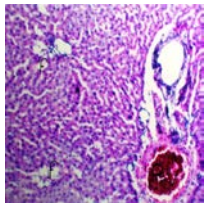

**Liver of rat in normal control group (NC) showing the magnification of the central vein (cv) and surrounding hepatocytes (H). (HPE x 160)**

**(G1a)**



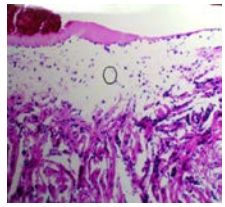

Heart of rat in normal control group (NC) showing the magnification of identify the myocardium (M).(HPE x 160)

**(G1b)**



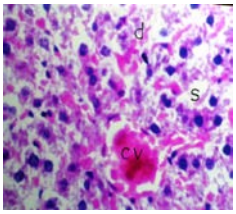
**Liver of rat in high fat fed group (HF) showing sever dilation and congestion of the portal vein (pv). (HPE x 40)**

**(G2a)**



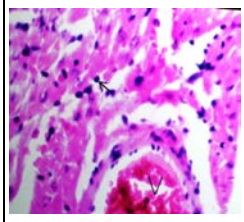

**Heart of rat in high fat fed group (HF) showing oedema in thesubendocardial area (O). (HPE x 64)**

**(G2b)**



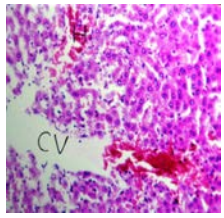

**Liver of rat treated by 0.2 % Monacolin k/ml extract/day showing congestion of the central vein (cv) and sinusoids (s). (HPE x 160)**

**(G3a)**



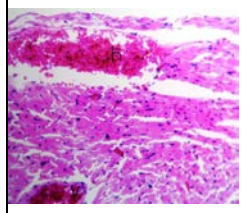
**Heart of rat treated by 0.2 % Monacolin k /ml extract/day showing mononuclear leucocytes inflammatory cells in myocardium (arrow). (HPE x 160)**

**(G3b)**



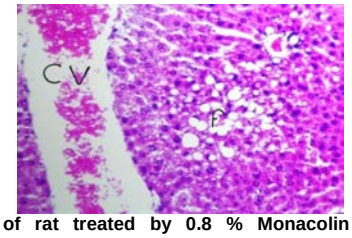

**liver of rat treated by 0.4 % Monacolin k /ml extract/day showing congestion in central vein (cv) with focal haemorrhage (H) in the hepatic parenchyma. (HPE x 64)**

**(G4a)**



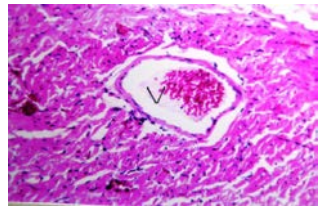
**Heart of rat treated by 0.4 % Monacolin k /ml extract/day showing subpericardial haemorrhages (h). (HPE x 64)**

**(G4b)**




**Liver of rat treated by 0.8 % Monacolin k /ml extract/day showing congestion and dilatation of central vein (cv) with fatty change in the adjacent hepatocytes (f). (HPE x 64)**

**(G5a)**




**Heart of rat treated by 0.8 % Monacolin k /ml extract/day showing congestion sclerotic wall of the blood vessel of the myocardium (v). (HPE x 64)**

**(G5b)**

**Figure 3: Histopathological findings of tested rats groups, Liver and heart of negative normal control group (G1a&G1b), Liver and heart of positive control group (G2a&G2b), Liver and heart of 0.2% MK treated group (G3a&G3b), Liver and heart of 0.4% MK treated group (G4a&G4b) and Liver and heart of 0.8% MK treated group (G5a&G5b).**

## CONCLUSION

**From the above results it could be concluded that Monacolin k extract improved lipid profiles by lowering plasma total cholesterol and triglyceride concentrations compared with the HF and NC groups. The plasma HDL-cholesterol concentration was higher in the Monacolin k extract treated groups than in the HF group, however, the ratio of HDL- cholesterol /Total-cholesterol was significantly increased by 37.58%, 61.67% and 79.95% of treated samples with 0.2, 0.4 and 0.8% MK/ml extract/day, respectively when compared with the HF group.**

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# دراسات بيولوجية على مستخلص الأرز الأحمر المنتج بواسطة فطر مونـاسكس بروبوريوس

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## الملخص العربي

لدراسة انتاج مادة المونـاكولين ك تمت التنمية بالطريقة الصلبة بواسطة فطر ***Monascus purpureus*** على كسر الأرز بنظام التخمر الصلب عند مستوى رطوبة ٣٥% ودرجة حرارة تحضين ٢٨ °م لمدة ١٧ يوم. تمت عمليات التحليل الكيميائي للنواتج بعد عملية التخمر فأعطت انتاجية من مادة المونـاكولين ك وقدرها ٣٦ ,٠ % وقد تم تقدير نسبة المونكولين ك المنتجة بواسطة وسائل التقدير الكروماتوجرافية (HPLC). كذلك أعطت نسب البروتين والدهن والرماد القيم التالية ٢٠,٤٣ % و٦,٨٤ % و٠,٩٣٣ % على التوالي. كما تم استخدام الصبغة الخام في صورة أرز أحمرفي التغذية البيولوجية لثلاثة مجموعات من فئران التجارب بنسب من المونـاكولين ك قدرها ٠,٢ ، ٠,٤ و٠,٨ % مونـاكولين ك/ملليتر مستخلص/يوم على التوالي وذلك بالاضافة الى مجموعتين من فئران التجارب القياسية فأعطت المعاملات الثلاثة نسب خفض معنوية عند دراسة نسب الكوليسترول ووظائف الكبد لحيوانات التجارب قبل وبعد المعاملة حيث كانت نسب الكوليسترول مرتفع الكثافة في مجموعات الفئران المعاملة بالمونـاكولين اعلى من المجموعتين القياسيتين. بلغت الزيادة في نسب الكوليسترول مرتفع الكثافة ٣٧,٥٨ % ،٦١,٦٧ % و٧٩,٩٥ % وذلك للمجموعات ٠,٢ ، ٠,٤ و٠,٨ % مونـاكولين ك/ملليتر مستخلص/يوم على التوالي. أوضحت الدراسة الهستولوجية لانسجة الكبد والقلب لمجموعات الفئران محل الدراسة تحسنا ملحوظا وخصوصا مجموعتان الفئران المعاملة بـ ٠,٢ و ٠,٤ % مونـاكولين ك/ملليتر مستخلص/يوم.