ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 9, Issue, 03, pp.48270-48274, March, 2017*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

## RESEARCH ARTICLE

# POTENTIAL HYPOLIPIDEMIC EFFECTS OF PROPOLIS IN HYPERLIPIDEMIC INDUCED MALE ALBINO RABBITS

***Kumud Joshi, Bharat Kotru and Sushil Kotru**

[1]Department of Zoology, JNVU University, Jodhpur 342 001, India
[2]Department of Bio Chemistry, SeraCare Lab & Research Centre, Jodhpur 342 001, India

**ARTICLE INFO**

*Article History:*
Received 13th December, 2016
Received in revised form
10th January, 2017
Accepted 16th February, 2017
Published online 31st March, 2017

*Key words:*

Antiatherosclerotic potential,
Propolis,
Hyperlipidemia,
Statin,
Cholesterol.

**ABSTRACT**

High fat or cholesterol have been implicated in the pathogenesis of atherosclerosis. The present study was designed to investigate the possible hypolipidemic effects of propolis on cholesterol fed rabbits and to compare with standard drug available in the market (statin). The rabbits were first made exogeneously hyperlipidemic by giving them high fat diet and cholesterol powder (500mg/kg body weight) in 5ml of coconut oil orally for 15 days and then were administered with drugs like Propolis and statin. In the hyperlipidemic rabbits there were an apparent reduction in the animal body weight and a significant increase in serum total cholesterol (78.67 %), triglyceride (27.24 %), LDL-c (78.56 %) and VLDL-c (38.04 %) with a concomitant non significant decrease in serum HDL-c (25.45 %) . Whereas oral treatment of animals with propolis for about 45 days in a dose of 25mg/Kg body weight lead to significant reduction in the animal body weight as well as in serum total cholesterol (93.61 %), triglyceride (76.91 %), LDL-c (96.4 %) and VLDL-c (75.6 %) with a slightly nonsignificant increase in HDL-c. In conclusion, propolis was found nearly equal in efficacy like statin standard drug in comparison with hyperlipidemic animals on all serum lipid parameters. Thus propolis extract offers promising hypolipidemic effects with no drug related disturbances in safety medications that may be mainly attributed to its potent antiatherosclerotic potential. Further studies will be needed in future in order to determine which one (or more) of its active constituents has the main hypolipidemic effects.



**Citation: Kumud Joshi, Bharat Kotru and Sushil Kotru, 2017.** "Potential Hypolipidemic effects of Propolis in Hyperlipidemic induced male albino rabbits", *International Journal of Current Research*, 9, (03), 48270-48274.

## INTRODUCTION

Propolis is a complex resinous material collected by honeybees from buds and exudates of certain plant sources neighboring its hives. Propolis consisting of sap, bark and bee excreta, accumulates in bee hives. The chemical consistency of propolis is highly dependent on the flora of the region from where it is collected (Marcucci, 1995; Burdock, 1998 and Banskota *et al*., 2001). Propolis contains at least 200 compounds that have been identified in different samples of propolis, with more than 100 being present in any given sample. These include fatty and phenolic acids and esters, substituted phenolicesters, flavonoids (flavones, flavanones, flavonols, dihydroflavonols, chalcones), terpenes, β-steroids, aromatic aldehydes and alcohols, and derivatives of sesquiterpenes, naphthalene and stilbenes (Greenaway *et al*., 1991; Aga *et al*., 1994 and Marcucci *et al*., 1996). The main types of flavonoids are rutin, quercetin, galangin (Isla *et al*., 2001) and caffeic acid phenethyl ester (Natarajan *et al*., 1996). A number of plant derived compounds have been proved as potent hypocholesterolemic agents with lesser side effects. Propolis possesses a broad spectrum of biological activities and is extensively being used in health food, pharmaceutical preparations (Havsteen, 1983; Wleklik *et al*., 1997; Khalil, 2006; Orsolic and Basic, 2006) and beverages with the aim of maintaining or improving human health (Greenaway *et al*., 1991; Aga *et al*., 1994; Bankova *et al*., 1995 and Marcucci *et al*., 1996). Some medicinal use of propolis are – Enhancement of immune system activities (Wleklik *et al*., 1997 and Orsolic and Basic, 2003 & 2006), oxygen radical scavenging (Moreno *et al*., 2000; Chen *et al*., 2004), antimicrobial, anti-inflammatory (Mizoeva and Calder, 1996) and antitumor activities (Orsi *et al*., 2005; Silici and Kutluca, 2005 and Duarte *et al*., 2006). The present study was designed to investigate the probable hypolipidemic effects of propolis in relation to statin.

**Corresponding author:* **Kumud Joshi,**
Department of Zoology, JNVU University, Jodhpur 342 001, India.

## MATERIALS AND METHODS

### *Collection of propolis*

Indian brown propolis (Gwalior, Madhya Pradesh) was obtained from Apiculture centre, Department of Zoology,

Jiwaji University, Gwalior, that was stored at $20^0$C prior to experimentation. It was suspended in a distilled water and administered orally at 9 AM in a dose of 25mg/kg body weight (Bhaduria *et al*., 2007) for 45 days. All the other used chemicals were of the highest analytical grades commercially available.

**Animals**

Healthy adult male New Zealand rabbits were procured from Forest Department, Jodhpur (Rajasthan). Weights and age of animals were 1.25-1.75 kg and 10-12 month respectively. Animals were housed in well-lighted air-conditioned room in metallic wire gauge cages, under controlled environmental conditions with 12 hours illumination and 12 hours darkness cycle. Animals were fed on standard rabbit chow supplied by Hindustan lever ltd., India. The food was supplemented with green leafy and seasonal vegetables and water *ad libitum.*

**Induction of hyperlipidemia**

The hyperlipidemic condition was induced by cholesterol feeding to rabbits. The cholesterol powder (500 mg/kg body weight) was mixed in 5ml of oil mixture and administered to the animals orally. In addition animals were fed with atherogenic diet. The atherogenic diet comprised wheat flour base with addition of milk powder, dried egg yolk, hydrogenated fat, butter, dried yeast, salt, sugar and vitamin mixture to produce the following nutrients in the given proportion. The average consumption of diet was 200g/rabbit per day.

| Nutrients | High fat diet | Normal diet |
|---|---|---|
| Protein | 15 % | 20 % |
| Carbohydrate | 60 % | 65 % |
| Sugar | 3 % | 3 % |
| Fats | 15 % | 5 % |
| Salt | 4 % | 4 % |
| Vitamin | 1 % | 1 % |
| Fiber | 2 % | 2 % |

**Standard drugs**

Atorvastatin was used as standard hypolipidemic drug, and it was given to the animals at the dose of 0.25mg/kg body weight dissolved in 5ml distilled water.

**Feeding of propolis**

For administration to the animals, the propolis (25mg/kg body weight) was suspended in 5ml of distilled water. The dose of the drug was determined by $LD_{50}$ test.

**Experimental groups**

Twenty four male albino rabbits were divided into four groups the control and experimental groups, usually consisted of six animals each.

Group 1 – Vehicle treated control or intact control (60 days)
Group 2 – Atherodiet + cholesterol feeding (500mg/kg body weight) for 60 days
Group 3 – Cholesterol feeding (500mg/kg body weight) for 15 days + propolis extract (25mg/kg body weight) for 45 days
Group 4 – Cholesterol feeding (500mg/kg body weight) for 15 days + statin (0.25mg/kg body weight) for 45 days

***Criteria of observation***

At the end of experimental period, all animals of the group were sacrificed under prolonged ether anesthesia. Blood was collected through cardiac puncture, and serum was separated by centrifugation for 10 minutes at 3000 RPM and was divided into 4 to 5 portions for different determinations.

1. **Total Cholesterol -** Total Cholesterol concentration was determined by an enzymatic method by using commercial total cholesterol test kit (CHOD-PAP; Centronic GmbH GERMANY).
2. **Triglyceride -** Triglyceride was determined by an enzymatic method by using commercial triglyceride test kit (GPO-POD; Centronic GmbH GERMANY).
3. **DL-Cholesterol - HDL**-Cholesterol was measured by using commercial Direct HDL test kit (Anamol pvt. Ltd. India.)
4. **LDL-Cholesterol -** LDL-Cholesterol and VLDL-Cholesterol was Calculated by the Friedewald Equation.

LDL = TC – HDL – VLDL
Where VLDL = TG/5

**Statistical analysis of data**

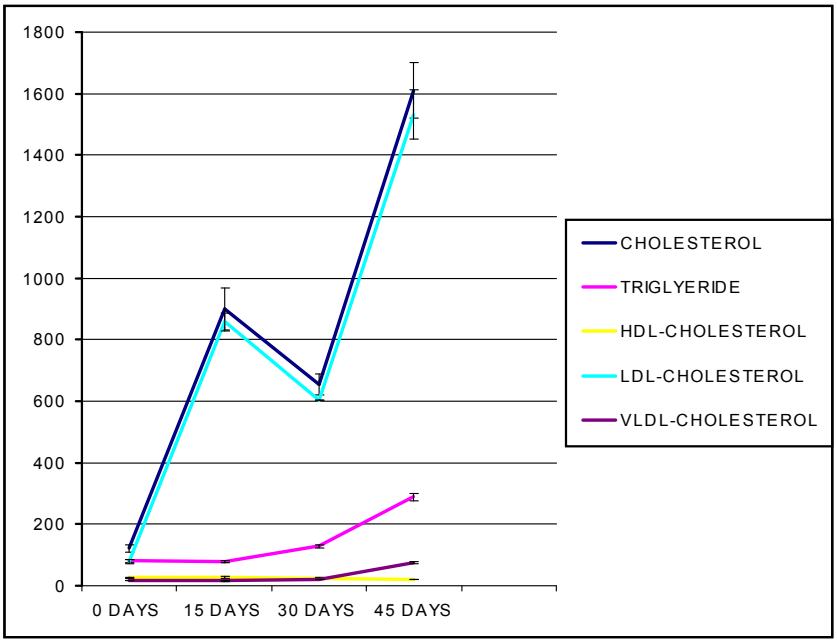
All the values of body / organ weights and Biochemical estimation were expressed in terms of mean value ± standard error by using SPSS- statistical data analysis software. The different groups were compared among each other using *post hoc* Sheffe's test. The level of significance was set at $p < 0.05$.

## RESULTS

The animal model which were fed with atherodiet for complete 60 days there serum cholesterol level was raised to its maximum limits. Simultaneously there was a highly significant increase in triglyceride, LDL-c, VLDL-c and non significant change was observed in HDL-c when compared with control group. Marginal decrease in the final body weight after the administration of atherodiet and drugs was observed. The weight of aorta and liver was increased in atherodiet fed rabbits, This could be due to lipid deposition after continuous atherodiet administration. The weight of heart and kidney in all the groups remain unaltered. Propolis or statin feeding did not change the weight of liver and aorta. A nine fold increase in serum cholesterol was noticed after 60 days of cholesterol feeding, while propolis lowered the serum cholesterol (93.61%), triglyceride (76.91%), LDL-c (96.4%) and VLDL-c (75.6%) with a non significant increase in HDL-c. Statin treated rabbits also showed reduction in serum cholesterol (94.78%) triglycerides (74.05%), LDL-c (97.1%) and VLDL-c (74.0%).

## DISCUSSION

Atherogenicity with subsequent cardiovascular manifestations is one of the important causes of high mortality and morbidity. Various agents like statin which affect hyperlipidemia are still not used for prevention of atherosclerosis because of their




**Graph 1. Percentage devation in concentration of lipid parameters of Atherodiet Treated Rabbits**

**Table 1. Body and organ weight of propolis treated intact rabbits (mean of 5 values ± SEM)**

| Treatment groups | Body Weight (Kg) | | Liver | Heart | Kidney | Aorta |
|---|---|---|---|---|---|---|
| | Initial | Final | ----------gm /Kg body weight ---------- | | | |
| Hyperlipidemic (Gr. 2) | 1.64±0.14 | 1.36±0.02 | 38.71±1.61$^{c}$ | 2.53±0.16$^{d}$ | 6.64±0.34$^{d}$ | 0.36±0.12$^{c}$ |
| Propolis (Gr. 3) | 1.15±0.10 | 1.10±0.01 | 23.71±1.63$^{d,g}$ | 2.47±0.12$^{d,h}$ | 7.10±0.37$^{d,h}$ | 0.33±0.12$^{d,h}$ |
| Statin (Gr. 4) | 1.54±0.14 | 1.36±0.02 | 27.71±1.61$^{d,g}$ | 2.53±0.16$^{d,h}$ | 6.64±0.34$^{d,h}$ | 0.35±0.12$^{d,h}$ |

**Table 2. Serum biochemistry of propolis drug treated intact rabbits (mean of 5 values ± SEM)**

| Treatment groups | CHO. (mg/dl.) | TG. (mg/dl.) | HDL-C (mg/dl.) | LDL-C (mg/dl.) | VLDL (mg/dl.) | CHO/HDL | LDL/HDL |
|---|---|---|---|---|---|---|---|
| Control (Gr. 1) | 84.50±4.50 | 93.00±6.0 | 29.5±1.5 | 35.8±6.2 | 19.3±1.3 | 2.98±0.02 | 1.62±0.18 |
| Hyperlipidemic (Gr. 2) | 1609.50±90.50$^{c}$ | 377.50±12.50$^{c}$ | 30.5d±1.5$^{d}$ | 1503.50±5.50$^{c}$ | 75.50±2.50$^{c}$ | 52.77±0.99$^{c}$ | 49.29±0.02$^{c}$ |
| Propolis (Gr. 3) | 102.80±5.4$^{d,g}$ | 87.15±11.65$^{d,g}$ | 31.35±2.35.$^{d,h}$ | 53.10±7.40$^{d,g}$ | 18.35±3.25$^{d,g}$ | 3.42±0.72$^{d,g}$ | 1.84±0.66$^{d,g}$ |
| Statin (Gr. 4) | 83.96±2.98$^{d,g}$ | 97.93±10.91$^{d,g}$ | 24.86±1.74$^{d,h}$ | 42.10±5.35$^{d,g}$ | 19.56±2.16$^{d,g}$ | 3.36±0.12 $^{d,g}$ | 1.18±0.68$^{d,g}$ |

potential toxicity and intolerance. It is well known fact that elevated total cholesterol and low density lipoprotein cholesterol (LDL-c) levels promote atherosclerosis and cardiovascular complications (Pedersen, 2001). Oxidative modification of low density lipoprotein cholesterol (LDL-c) appears to have an important role in initiation and progression of atherogenic changes in aorta (Esterbauer *et al.,* 1993). The agents which can lower serum cholesterol and scavenge or inhibit free radicals formation have gained wide therapeutic value. Cholesterol feeding in rabbits caused a significant increase in the circulating total cholesterol, LDL-cholesterol, VLDL cholesterol and also in the ratios of total cholesterol : HDL-cholesterol and LDL-cholesterol : HDL-cholesterol. These results are consistent with earlier reports (Prasad, 2005 and Vijaimohan *et al.,* 2006) which have clearly established a correlation between dietary lipids and serum lipid profile. Supplementation of cholesterol in diet rapidly results in a marked increase in the production of cholesteryl ester rich-VLDL by the liver and intestine (Demacker *et al.,* 1991) and a reduced number as well as rate of cholesterol removal by the hepatic LDL receptors (Goldstein *et al*., 1983). Consequently serum levels of LDL-cholesterol and VLDL-cholesterol is increased. A significant increase in the ratios of total cholesterol : HDL-cholesterol and LDL-cholesterol : HDL-cholesterol indicate increased risk of atherosclerosis and coronary heart disease (Ram, 1996). Simultaneous administration of propolis caused a significant decrease in serum total cholesterol, LDL-cholesterol and VLDL-cholesterol suggesting beneficial modulatory influence on cholesterol metabolism and turnover. Decline in the ratios of total cholesterol : HDL-cholesterol and LDL-cholesterol : HDL-cholesterol observed in the extract treated rabbits might be a consequence of higher proportion of HDL-cholesterol which reduced atherogenic risk by virtue of increased reverse cholesterol transport from peripheral organs to liver (Kinosian *et al.,* 1994 and Hermansen *et al.,* 2003). Elevated serum triglycerides is considered as independent risk factor for cardiovascular disease (Asia Pacific Cohort Studies Collaboration, 2004). Reduction in LDL-cholesterol and increase in HDL-cholesterol concentration are significantly related to lipid-lowering therapy (Singh et al., 1997). The investigation reveals that the propolis extract lowers the serum

total cholesterol and LDL-cholesterol levels significantly, which reduces the risk of coronary heart disease.

Regarding the mechanism of action, it is possible that the hypocholestrolemic effect is associated with a decrease in intestinal absorption of cholesterol and with an increase in fecal excretion of steroids and bile acids (Mehta *et al*., 2003). Hypolipidemic effect of propolis may be achieved due to its interference in the biosynthetic pathway of cholesterol. A significant reduction in serum total cholesterol, triglyceride and LDL-c may have been achieved by the alcoholic components and its derivatives which were said to be present in propolis mixture under various studies (Bankova *et al.*, 1996). Maybe these components put some effect on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate controlling enzyme in cholesterol biosynthesis. The inhibition of this enzyme depresses the *de novo* synthesis of HMG-CoA reductase or stimulates its degradation. This hypothesis not yet been conclusively proven, studies supporting it had done by some researchers (Menendez *et al*., 1997) and some recent studies had cast doubt on this hypothesis (Wang *et al*., 2003). A significant fall in the total cholesterol/HDL-cholesterol ratio and LDL-c/HDL-c ratio is observed in drug treated groups. A low level of HDL is associated with high risk of coronary artery disease (Boden and Pearson, 2000). HDL-c participates in reverse cholesterol transport transferring cholesterol from tissues back to the liver. This action aids the efflux of cholesterol from the arterial wall. HDL-c may also influence atherosclerosis by carrying enzymes that are antioxidants which may block early steps in atherogenses and slow down the progression of lesions. Phytochemical investigations of propolis have demonstrated the presence of flavonoids and polyphenolic components as main active ingredients having potent antioxidant activities (Moreno *et al*., 2000 and Hosnuter *et al*., 2004). HDL-c alters the balance of unesterfied cholesterol between plasma and cells by increasing its utilization in the lecithin cholesterol acyl transferase (LCAT) system to form cholesterol ester, which moves rapidly back into the cells (Libby, 2001). These results indicated the hypolipidemic and antiatherosclerotic nature of the propolis therefore it may be concluded that propolis possesses hypolipidemic and antiatherosclerotic potential.

## REFERENCES


Aga H, Shibuya T, Sugimoto T, Kurimoto M and Nakajima SH 1994. Isolation and identification of antimicrobial compounds in Brazilian propolis. *Biosci Biotechnol Biochem.,* 58: 945-946.

Bankova V, Christov R, Kujumgiev A, Marcucci MC and Popov S 1995. Chemical composition and antibacterial activity of Brazilian propolis. *Z Naturforsch [C]*, 50: 167-72.

Banskota AH, Tezuka Y and Kadota S 2001. Recent progress in pharmacological research of propolis. *Phytother Res.,* 15(7): 561-571.

Bhadauria M, Nirala SK and Shukla S 2007. Propolis protects CYP2E1enzymatic activity and oxidative stress induced by carbon tetrachloride. *Mol Cell Biochem.,* 302(1-2): 215-224.

Boden WE and Pearson TA 2000. Raising low levels of high-density lipoprotein cholesterol is an important target of therapy. *Am J Cardiol.,* 85: 645-650.

Burdock GA 1998. Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem Toxicol.,* 36(4): 347-363.

Chen CN, Weng MS, Wu CL and Lin JK 2004. Comparison of radical scavenging activity, cytotoxic effects and apoptosis induction in human melanoma cells by Taiwanese propolis from different sources. *Evid Based Complement Alternat Med.,* 1(2): 175-185.

Demacker P N M, Reijnen I G M, Katan M B, Stuyt P M J and Stalenhoef A F H 1991. Increased removal of remants of triglyceride rich lipoproteins on a diet rich in polyunsaturated fatty acids. *Eur J Clin Invest,* 21: 197-203.

Duarte S, Rosalen P L, Hayacibara MF, Cury JA, Bowen WH, Marquis RE, Rehder VL, Sartoratto A, Ike qaki M and Koo H 2006. The influence of a novel propolis on Potential antidiabetic and hypolipidemic effects of propolis extract in streptozotocin-induced diabetic rat smutans streptococci biofilms and carries development in rats. *Arc Oral Biol.,* 51(1): 15-22 .

Esterbauer H, Wag G and Puhl H 1993. Lipid peroxidation and its role in atherosclerosis. *Brit Med Bull.,* 49: 566-576.

Goldstein J L, Kita T and Brown M S 1983. Lipoprotein receptors in the liver. Control signals for plasma cholesterol traffic. *N Engl J Med.,* 309: 288-296.

Greenaway W, May J, Scaysbrook T and Whatley FR 1991. Identification by gas chromatography-mass spectrometry of 150 compounds in propolis. *Z Naturforsch [C],* 46: 111-121.

Havsteen B. 1983. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol.,* 32(7): 1141-1148.

Hermansen K, Dinesen B, Hole L H, Morgenstern E and Gruenwald J. 2003. Effect of soy and other natural products on LDL : HDL ratio and other lipid parameters. *A literature review Adv Therapeutics,* 20: 50-78.

Hosnuter M, Gurl A, Babuccu O, Armutcu F, Kargi E and Isikdemir A. 2004. The effect of CAPE on lipid peroxidation and nitric acid levels in the pasma of rats of rats following thermal injury. *Burns*, 30(2): 121-125.

Isla MI, Moreno MI, Sampietro AR and Vattuone MA 2001. Antioxidant activity of Argentine propolis extracts. *J Ethnopharmacol.,* 76: 165-170.

Khalil ML. 2006. Biological activity of bee propolis in health and disease. *Asian Pac J Cancer Prev.,* 7(1): 22-31.

Kinosian B, Glick H and Garland G 1994. Cholesterol and coronary heart disease: Predicting risk by levels and ratio. *Ann Int Med.,* 121: 641 -647.

Libby P 2001. Managing the risk of atherosclerosis: The role of high-density lipoprotein. *Am J Cardiol.,* 88 (S) : 3N-8N.

Marcucci MC 1995. Propolis: Chemical composition, biological properties and therapeutic activity. *Apidologie* 26: 83-99.

Marcucci MC, DeCamargo FA and Lopes CMA. 1996. Identification of amino acids in Brazilian propolis. *Z Naturforsch [C],* 51: 11-14.

Mehta K, Balaraman R, Amin AH, Bafna PA and Gulati OD 2003. Effects of fruits of Moringa oleifera on the lipid profile of normal and hypercholesterolaemic rabbits. *J Ethanopharmacol.,* 86: 191-195.

Menendez R, Arruzazabala L and Mas R. 1997. Cholesterol lowering effect of policosanol on rabbits with hypercholesterolaemia induced by a wheat starch-casein diet. *Br J Nutr.,* 77, 923–932.

Mizoeva OK and Calder PC 1996. The effect of propolis and its components on eicosanoid production during the

inflammatory response. *Prostagland Leuk Essent Fatty,* 55: 441-449.

Moreno M, Isla M, Sampietro A and Vattuone M 2000. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *J Ethnopharmacol.,* 71: 109-114.

Natarajan K, Singh S, Burker TR Jr, Grunberger D and Aggarwal BB 1996. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. *Proc Natl Acad Sci USA,* 93(17): 9090-9095.

Orsi RO, Sforcin J M, Funari S R and Bankova V. 2005. Effects of Brazilian and Bulgarian propolis on bactericidal activity of macrophages against Salmonella Typhimurium. *Int Immunopharmacol*., 5(2): 359-368.

Orsolic N and Basic I 2006. Cancer chemoprevention by propolis and its polyphenolic compounds inexperimental animals. *Recent Prog Med Plants,* 17:55-113.

Silici S and Kutluca S 2005. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *J Ethnopharmacol.,* 99(1): 69-73

Singh K, Chander R and Kapoor NK 1997. High density lipoproteins subclasses inhibit low-density lipoprotein oxidation. *Indian J Biochem Biophys.,* 34: 313-319.

Wang YW, Jones PJH, Pischel I and Fairow C 2003. Effects of policosanols and phytosterols on lipid levels and cholesterol biosynthesis in hamsters. *Lipids,* 38, 165–170.

Wleklik M, Zahorska R and Luczak V 1997. Interferoninducing activity of flavonoids. *Acta Microbiol Pol.,* 36: 151-154.

*******