ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Issue 2, 2014

ISSN - 0974-2441

Research Article

ANTI-OBESITY POTENTIAL OF *CYPERUS ROTUNDUS* L. AQUEOUS TUBER EXTRACT IN RATS FED ON HIGH FAT CAFETERIA DIET

ATHESH K[1*], DIVAKAR M[2], BRINDHA P[3]

[1]Dept. of Biochemistry, Srimad Andavan Arts & Science College, Trichy-5, India. [2]Dept. of Biotechnology, Srimad Andavan Arts & Science College, Trichy-5, India. [3] Carism Sastra University, Thanjavur, India.Email: athesh_rahul@yahoo.com

Received: 28 January 2014, Revised and Accepted: 26 February 2014

ABSTRACT

Objective: This study was undertaken to evaluate the anti-obesity potential of the aqueous tuber extract of *Cyperus rotundus* L. (ATECR) in high fat cafeteria diet (HFCD) fed obese rats.

Methods: Wistar strain of albino rats were divided into six groups comprising of six rats each. Group I served as normal control fed with normal pellet chow, group II served as disease control fed with high fat cafeteria diet, group III, IV and V animals, received ATECR at a dose level of 100, 200 and 300mg/kg bw along with HFCD for 40 days, while, group VI served as standard drug control, which received Orlistat at a dosage of 50mg/kg bw along with HFCD.

Results: Administration of HFCD for 40 successive days to experimental rats significantly increased the body weight, organ and fat pad weights, serum total cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides and glucose levels; and decreased HDL cholesterol as compared to normal control. While treatment with ATECR showed a significant reduction in the body weight gain, organ weight of the liver, kidney, spleen, weight of fat pads and the levels of serum triglycerides, total cholesterol, LDL cholesterol, VLDL cholesterol, glucose and increase in HDL cholesterol in a dose dependent manner. Further, the levels of liver markers such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), which were found to be elevated in the serum of obese rats, also resumed to normal on treatment with different concentrations of ATECR. Moreover, the consumption of ATECR reduced oxidative stress by enhancing the levels of glutathione (GSH), glutathione peroxidase (GPx), super oxide dismutase (SOD) and catalase in the hepatic tissue of rats with HFCD induced obesity.

Conclusion: These results demonstrate clearly that repeated oral administration of tubers of *Cyperus rotundus* L. aqueous extract can evoke a potent anti-obesity activity.

Keywords: High Fat Cafeteria diet, Obesity, *Cyperus rotundus* L., Orlistat.

INTRODUCTION

Overweight and obesity, the most common nutritional problems resulting mainly from an energy imbalance caused by an increased ratio of caloric intake to energy expenditure represent rapidly growing threats to the health of population worldwide. The International Obesity Task Force estimates that more than 300 million individuals worldwide are obese with body mass index (BMI) ≥ 30 kg/m^2 and 800 million are overweight (BMI between 25 and 29.9 kg/m^2) [1]. Due to the rising trend in obesity prevalence, this figure could double by the year 2025, if no action is taken against this threat. Obesity decreases the quality of life and life expectancy and is also a strong risk factor for diseases such as type 2 diabetes, heart disease, stroke, certain types of cancers, osteoarthritis, liver disease, urinary incontinence, sleep apnea, and depression [2]. Left unabated, the increasing rates of obesity in the world will place a severe burden on national healthcare systems.

Despite advances in understanding its pathogenesis, current pharmacotherapy for obesity remains limited both in the degree of achievable weight loss and the safety/tolerability of the drugs. Thus, discovery of new targets and therapeutic agents is a focal point for combating this epidemic. A large section of world's population relies on traditional remedies to treat various diseases. Medicinal herb is an indispensable part of traditional medicine practiced all over the world due to its efficacy, low costs, easy access, ancestral experience and lack of side effects [3]. Hence, the present study was carried out with an objective to investigate the anti-obesity effect of aqueous tubers extract of *Cyperus rotundus* L. (ATECR) in rats fed with high fat cafeteria diet.

Cyperus rotundus L. belonging to the family Cyperaceae is a well-known plant in Indian traditional medicine prescribed for exerting analgesic [4], antimalarial [5], anti-inflammatory [6], antidiarrheal [7], antidiabetic [8], wound healing [9] and antioxidant [10] effects. In ancient ayurvedic literature (*Caraka Samhita* – 3000 B.C.), *C. rotundus* tubers were listed together with nine other plant species as *lekhania* drugs, means a drug capable of 'de-fatting' adipose or muscular tissues [11]. But there are no scientific reports regarding anti-obesity potentials of the aqueous extract of *C. rotundus* tubers. In the present study attempt was made to evaluate the anti-obesity activity of ATECR in high fat cafeteria diet induced obese rats, so as to provide a scientific evidence for this '*lekhania*' drug.

MATERIALS AND METHODS

Preparation of Aqueous Tubers Extract of *C. rotundus* (ATECR)

Sun dried *Cyperus rotundus* L. tubers were purchased from local market and authenticated with the help of the voucher specimen deposited at the RAPINAT Herbarium, St.Joseph's College, Trichy and were stored at ambient temperature. Tubers were ground continually in a mechanical grinder and sieved until all raw materials had a granulometry of ≤0.5mm. The granulated powder was mixed thoroughly with six times the volume of water, then boiled and stirred continuously until the volume reduced to 1/3rd. The extract was filtered with muslin cloth and the filterate was evaporated in a water bath till it reaches a thick consistency. The extract was stored in refrigerator till further use.

Experimental Animals

Healthy Wistar strain of Albino rats (6-7 weeks old) of both sexes weighing 100-150g obtained from Tamil Nadu Veterinary College, Chennai, India, and used for the present study. They were housed in polypropylene cages and maintained under controlled environment (24°±1°C temperature, 55-65% relative humidity, 12hr light/dark cycle) with free access to standard animal pellet diet (Sai Durga

feeds, Bangalore, India) and water. They were acclimatized to the laboratory conditions for a week before experiments.

The animal experiments were conducted as per the ethical guidelines of CPCSEA after obtaining necessary clearance from the Institutional Animals Ethics Committee (Approval No: 790/03/ac/CPCSEA).

Induction of Obesity by Feeding High Fat Cafeteria Diet

The cafeteria diet [12] consisted of 3 diets (condensed milk, 40g + bread, 40g), (chocolate, 15g + biscuits, 30g + dried coconut, 30g), (cheese, 40g + boiled potatoes, 50g). The three diets were presented to group of 6 rats on day 1, 2 and 3 respectively and then repeated in same succession for 40 days. These diets were provided in addition to normal pellet chow.

Experimental Design

Following one week of acclimatization, the rats were randomly divided into 6 groups with six rats each:

Group I: Normal control rats fed on Standard Chow Diet

Group II: Obesity control rats fed on High Fat Cafeteria Diet (HFCD) for 40 days

Group III: Rats fed with HFCD and treated with ATECR at a dose level of 100mg/kg b.w./day orally for 40 days

Group IV: Rats fed with HFCD and treated with ATECR at a dose level of 200mg/kg b.w./day orally for 40 days

Group V: Rats fed with HFCD and treated with ATECR at a dose level of 300mg/kg b.w./day orally for 40 days

Group VI: Rats fed with HFCD and treated with Standard drug orlistat at a dose level of 50mg/kg b.w./day orally for 40 days

Sample collection and Biochemical Analysis

At the end of experimental period of 40days, the animals were sacrificed by cervical decapitation. The blood was collected and the serum was separated and used for various biochemical analyses. The liver, kidney, spleen and fat pads (mesenteric and perirenal fat pads) were dissected out, washed in ice cold saline, blotted dry and weighed. Liver tissues were homogenized in 0.1M phosphate buffer (pH 7.4) and the homogenate was used to analyze the antioxidant status.

In serum, the levels of glucose [13], total cholesterol [14], triglycerides [15], HDL cholesterol [16], LDL cholesterol [16], VLDL cholesterol [16] and biochemical markers of hepatic injury viz. aspartate transaminase (AST) [17], alanine transaminase (ALT) [17] and alkaline phosphatase (ALP) [17] were analyzed. Tissue lipid peroxides (LPO) [18], superoxide dismutase (SOD) [19], glutathione peroxidase (GPX) [20], reduced glutathione [21] and catalase [22] were determined in liver homogenate.

Statistical Analysis

All the data reported are expressed as mean ± S.E.M. Statistical analysis was performed using the Student's t-test. The values were considered to be significantly different when the P-value was less than 0.05 compared to baseline or control values.

RESULTS

Effect on Body Weight Gain, Organ Weight and Fat Pad Weight

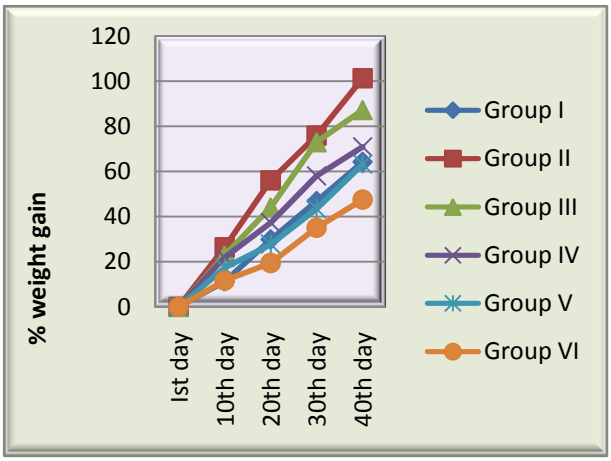

Fig.1: Effect of ATECR on Body Weight Gain in HFCD Induced Obese Rats

After 40 days of HFCD administration, body weight, organ weight and fat pad (mesenteric, perirenal) weights increased significantly in high fat cafeteria diet fed obesity control group (Group II) animals as compared with the normal control group (Group I) animals which were fed with normal pellet chow. However, rats fed on HFCD when treated with ATECR (100mg, 200mg, 300mg/kg b.w.) showed a significant decrease in body weight gain, organ weight and fat pad weights in a dose dependent manner when compared with the group II animals [Figure 1 & Table 1].

Table 1: Effect of ATECR on Organ and Fat Pad Weights in HFCD Induced Obese Rats

Groups	Liver (g)	Kidney (g)	Spleen (g)	Mesenteric Fat Pad (g)	Perirenal Fat Pad (g)
Group I	5.37±1.08*	1.19±0.07*	0.57±0.02*	4.53±0.49*	0.82 ± 0.02*
Group II	8.46±0.69*,**	1.49±0.06*,**	0.81±0.06*,**	14.31±0.09*,**	3.96±0.05*,**
Group III	7.49±1.06	1.43±0.09	0.79±0.04	12.86±0.83	3.17±0.06
Group IV	7.32±1.16	1.39±0.08	0.72±0.04	9.16±0.71	2.08±0.06
Group V	6.59±1.02**	1.37±0.08**	0.68±0.07**	8.47±0.62**	1.83±0.04**
Group VI	5.29±0.66	1.17±0.02	0.55±0.02	4.46±0.27	0.86±0.01

Values are mean ± S.E.M., (n = 6)
*** - Compared between Normal and Disease Control (p<0.05).**
**** - Compared between Disease Control and High Dose Drug Treated Group (p<0.05).**

Effect on Serum Lipid Profile

Serum lipids, such as total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol were significantly elevated and HDL-cholesterol was significantly decreased in the HFCD fed obese group animals (Group II), when compared to the normal controls animals (Group I). However, rats fed with HFCD when treated with ATECR (100mg, 200mg, 300mg/kg body weight) showed a significant improvement in these changes in a dose dependent manner [Table 2].

Table 2: Effect of ATECR on Serum Lipid Profile in HFCD Induced Obese Rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	VLDL-Cholesterol (mg/dl)
Group I	87.16±0.74*	69.12±1.25*	53.46±0.54*	21.14±0.39*	13.49±0.37*
Group II	181.16±1.17*,**	120.96±1.05*,**	26.14±1.08*,**	128.39±1.25*,**	24.05±0.84*,**

Group III	165.83±1.62	103.21±1.92	30.83±1.14	113.58±0.97	20.15±1.27
Group IV	131.16±1.17	90.10±1.79	44.71±1.03	75.26±1.28	18.11±1.59
Group V	101.16±1.10**	81.16±1.31**	46.16±1.10**	41.55±1.38**	16.33±1.59**
Group VI	84.23±0.67	67.93±0.91	51.62±0.72	20.78±0.76	13.63±0.62

Values are mean ± S.E.M., (n = 6)
*** - Compared between Normal and Disease Control (p<0.05).**
**** - Compared between Disease Control and High Dose Drug Treated Group (p<0.05).**

Effect on Blood Glucose and Serum Hepatic Marker Enzymes

The concentration of blood glucose was higher in HFCD fed obese control rats (Group II) than in rats fed with normal diet (Group I). Whereas, ATECR (100mg, 200mg, 300mg/kg b.w.) supplementation normalized the blood glucose level [Table 3] significantly. Also, the data of the present study showed significant increase in the activities of hepatic enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in HFCD fed obese control rats (Group II) when compared to group I animals fed with normal rat chow pellet whereas, the levels of AST, ALT and ALP significantly reduced in association with ATECR treatment [Table 3].

Table 3: Effect of ATECR on Blood Glucose and Serum Hepatic Markers (AST, ALT and ALP) in HFCD Induced Obese Rats

Groups	Glucose (mg/dl)	AST activity (U/L)	ALT activity (U/L)	ALP activity (U/L)
Group I	87.57±1.77*	64.86 ± 0.75*	37.45 ± 0.91*	77.66 ± 0.74*
Group II	191.83±1.84*,**	158.06 ± 0.91*,**	109.46 ± 1.27*,**	145.10 ± 1.34*,**
Group III	179.16±1.62	143.76 ± 1.66	87.29 ± 1.17	127.33 ± 1.18
Group IV	154.83±1.74	109.83 ± 1.53	69.51 ± 1.23	102.21 ± 1.46
Group V	108.58±1.48**	91.60 ± 1.65**	52.35 ± 0.81**	85.33 ± 0.89**
Group VI	89.63±0.62	69.43 ± 1.87	36.71 ± 0.91	80.82 ± 1.07

Values are mean ± S.E.M., (n = 6)
*** - Compared between Normal and Disease Control (p<0.05).**
**** - Compared between Disease Control and High Dose Drug Treated Group (p<0.05).**

Effects on Hepatic Antioxidant Enzymes and Lipid Peroxidation

The effects of ATECR on hepatic antioxidant enzyme activities are presented in Table 4. The Group-II animals administered with HFCD resulted in the decreased level of reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase and increased rate of lipid peroxidation when compared to rats fed normal pellet chow (Group I). Treatment with different dose levels (100, 200, 300mg/kg b.w.) of ATECR resulted in increased levels of reduced glutathione glutathione peroxidase, Superoxide dismutase and catalase. In the respect of lipid peroxidation in liver, treatment with ATECR resulted in significant reduction in the rate of lipid peroxidation [Table 4].

Table: 4 Effect of Aqueous Tubers Extract of *Cyperus rotundus* L. on antioxidants of Experimental Rats

Groups	LPO (n mol of MDA/mg protein)	Reduced Glutathione (mg/g tissue)	GPx activity (U/mg protein)	SOD activity (U/mg protein)	Catalase activity (U/mg protein)
Group I	1.23±0.03*	2.67±0.07*	3.04±0.32*	5.24±0.04*	32.41±0.93*
Group II	2.86±0.08*,**	1.39±0.06*,**	1.15±0.77*,**	2.36±0.07*,**	14.25±0.86*,**
Group III	2.55±0.13	1.72±0.31	1.83±0.82	2.95±0.32	19.30±1.04
Group IV	2.24±0.23	1.93±0.13	2.25±0.93	3.45±0.33	24.40±1.50
Group V	1.86±0.21**	2.16±0.11**	2.64±0.83**	4.21±0.21**	27.57±1.35**
Group VI	1.45±0.19	2.57±0.09	2.92±0.42	5.34±0.63	30.11±0.96

Values are mean ± S.E.M., (n = 6)
*** - Compared between Normal and Disease Control (p<0.05).**
**** - Compared between Disease Control and High Dose Drug Treated Group (p<0.05).**

DISCUSSION

Obesity is a medical condition in which excess body fat has been accumulated mainly due to sedentary life styles, lack of exercise and intake of energy rich high fat diet. The global prevalence of obesity is increasing rapidly among adults as well as among children and is associated with serious mortalities including a high incidence of type 2 diabetes, hyperlipidemia, hypercholesterolemia, fatty liver, cardiovascular diseases, osteroarthritis as well as an increased risk of many forms of cancer [23]. The currently available treatment options are not potent enough to control obesity permanently besides they produce side effects. Hence, there is a great demand for safer and long term effective drugs to treat this global epidemic problem. Medicinal herbs are indispensable parts of traditional medicines and there is a big renaissance of the herbal medicines globally as these drugs are effective and safe without any side effects. Hence, in the present work the anti-obesity potential of ATECR in rats fed a high fat cafeteria diet (HFCD) was investigated by analyzing the body weight, organ and fat pad weight and blood and tissue biochemical profiles.

Body weight gain, organ and fat pad weights were significantly increased in HFCD fed rats when compared with the normal diet fed rats. This increase in weight might be due to increased energy intake leading to increased fat deposition in tissues and organs. While, HFCD fed rats when treated with ATECR, the gain in body weight,

organ and fat pad weights significantly decreased. It was also observed that administration of ATECR did not alter food intake in treated rats, indicating that the prevention of weight gain induced by this extract was not due to a reduction of energy intake. ATECR might have increased the catabolism of lipids in adipose tissue resulting in a decrease in mean body weight.

Elevated serum concentrations of Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), Triglycerides (TG) along with decreased concentration of High Density Lipoprotein Cholesterol (HDL-C) observed in HFCD fed disease control rats are the major risk factors for the development of coronary heart disease and atherosclerosis. The significant increase in TC and TG level in cafeteria diet fed animals can be attributed to increase in both *de novo* synthesis and intestinal absorption of cholesterol [24]. Also, increased oxidative stress produces reactive oxygen species (ROS) which react with lipoproteins to produce oxidation states, thus diminishing the cellular uptake of lipids from the blood [25, 26]. Data of this study suggested that a 40 days administration of ATECR exerts a positive effect on lipid profile ie., ATECR administration

decreased the serum concentration of TC, LDL-C, VLDL-C, TG and increased the concentration HDL-C in treated group animals when compared with untreated disease control group. The results may be partly caused by the decreased absorption of cholesterol from the diet or by the antioxidants present in the plant drug might have contributed to elevated cellular lipid uptake [27]. Also, a substantial reduction of total cholesterol in serum in the plant drug treated groups could be attributed to a reduction in the activities of the liver enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, which is a rate-limiting enzyme in cholesterol biosynthesis. A higher content of HDL-C is correlated with a reduced risk of coronary heart disease. The increased level of HDL-C facilitates the transport of cholesterol from the serum to the liver, where it is catabolized and excreted. The decrease of TG in plant drug treated groups may be attributed to an increase in the activity of endothelium bound lipoprotein lipase that hydrolyses the triglycerides.

The significant increase in glucose in cafeteria diet fed animal can be due to defective insulin synthesis and decreased insulin efficiency [28]. Treatment with ATECR almost normalized the glucose levels in dose dependent manner. The test drug might have enhanced the secretion of insulin from the β-cells of the islets of pancreas or increased the efficiency of insulin which facilitates the delivery of glucose from blood to target tissues.

The high levels of serum enzymes (AST, ALT and ALP) in group II animals are attributed to fatty liver induced by a HFCD. Fatty liver associated with obesity is an independent risk factor for liver peroxidation which result in cell damage and elevation of the levels of these enzymes in serum. AST, ALT and ALP were significantly lowered in rats administered with ATECR, suggesting the amelioration of fatty liver. This decrease may be due to the consequence of prevention of liver damage by the antioxidant potential of the the extract [29, 30].

Generally obesity is associated with oxidative stress which results from an imbalance between the production of free radicals and an effective antioxidant system. Reduced glutathione (GSH) constitutes the first line of defense against free radicals in the liver, and it is also responsible for the maintenance of protein thiols and act as a substrate for Glutathione peroxidase (GPx). The results indicated that GSH contents depleted in the rats with obesity induced by a high fat diet, and were restored after the treatment with ATECR. Enzymatic antioxidants, like superoxide dismutase (SOD), catalase (CAT) or GPx, can scavenge reactive oxygen species (ROS) and free radicals or prevent their formation. The present study suggested decreased activities of antioxidant enzymes SOD, CAT and GPx in the liver of rats fed with high fat cafeteria diet as compared to those on normal diet and these results are in agreement with reports of earlier workers which suggest that feeding a high fat diet to experimental animals depresses their antioxidant system due to increased lipid peroxidation and formation of free radicals [31]. On treatment with ATECR the activities of antioxidant enzymes (SOD, CAT and GPx) were significantly elevated in liver and this enhanced

antioxidant capacity might have been mediated through natural antioxidants like quercetin, kaemferol, catechin and myricetin present in the plant drug.

CONCLUSIONS

To conclude, the results of the present study depict that administration of aqueous tuber extract of *Cyperus rotundus*.L (ATECR) regulates serum lipid profiles, reduces the oxidative stress and decreases adipose tissue mass and body weight gain. The mechanism induced by aqueous tuber extract of *Cyperus rotundus*.L will be further researched.

REFERENCES

1. Vinay Kumar, Uma Bhandari, Chakra Dar Tripathi, Geetika Khanna. Evaluation of antiobesity and cardioprotective effect of *Gymnema sylvestre* extract in murine model. Indian J Pharmacol 2012; 44(5): 607-613.
2. Mokdad A, Ford E, Bowman B, Dietz W, Vinicor F, Bales V, Marks J. Prevalence of obesity, diabetes, and obesity related health risk factors. JAMA 2003; 289: 76–79.
3. Cragg GM, Newman DJ. Natural Product Drug Discovery in the Next Millennium. Pharm Biol 2001; 39(1): 8-17.
4. Gupta MB, Palit TK, Singh N, Bhargava KP. Pharmacological studies to isolate the active constituents from *Cyperus rotundus* possessing anti-inflammatory, anti-pyretic and analgesic activities. Indian J Med Res 1971; 59: 76–82.
5. Thebtaranonth C, Thebtaranonth Y, Wanauppathamkul S, Yuthavong Y. Antimalarial sesquiterpenes from tubers of *Cyperus rotundus*: structure of 10,12-peroxycalamenene, a sesquiterpene endoperoxide. Phytochemistry 1995; 40: 125–128.
6. Seo WG, Pae HO, Oh GS *et al*. Inhibitory effects of methanol extract of *Cyperus rotundus* rhizomes on nitric oxide and superoxide productions by murine macrophage cell line, RAW 264.7 cells. J Ethnopharmacol 2001; 76: 59–64.
7. Uddin SJ, Mondal K, Shilpi JA, Rahman MT. Antidiarrhoeal activity of *Cyperus rotundus*. Fitoterapia 2006; 77: 134–136.
8. Raut NA, Gaikwad NJ. Antidiabetic activity of hydroethanolic extract of *Cyperus rotundus* in alloxan induced diabetes in rats. Fitoterapia 2006; 77: 585–588.
9. Puratchikody A, Devi Nithya C, Nagalakshmi G. Wound healing activity of *cyperus rotundus* linn. Indian J Pharmaceutical Sciences 2006; 68: 97-101.
10. Nagulendran KR, Velavan S, Mahesh R. *In Vitro* Antioxidant Activity and Total Polyphenolic Content of *Cyperus rotundus* Rhizomes. E-Journal of Chemistry 2007; 4(3): 440-449.
11. Trivedi VP, Mann AS. Vegetable drugs regulating fat metabolism in Caraka (Lekhania Dravyas). J Crude Drug Res 1972; 12: 1988–1999.
12. Harris RB. The impact of high- or low-fat cafeteria foods on nutrient intake and growth of rats consuming a diet containing 30% energy as fat. Int J Obes 1993; 17: 307–315.
13. Folin O and Wu H. A system of blood analysis. J Biol Chem 1919; 38: 81-110.
14. Parekh AC, Jung DH. "Determination of Cholesterol with ferric acetate, Uranium Acetate and sulphuric acid-ferrous sulphate reagents". Anal Chem 1970; 42: 1523-1529.
15. Foster LB, Dunn RT. "Stable reagents for determination of Serum triglycerides by a colorimetric Hantzsch condensation method". Clin Chem 1973; 196: 338-340.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. J Clin Chem 1972; 18(6): 499-502.
17. King J. *In: Practical Clinical Enzymology*, Princeton MJ (Fol) Van D Nostrand Company, and London 1965: 363.
18. Ohkawa H, Ohishi N, Yagi K. "Assay of lipid peroxides in animal tissues for thiobarbituric acid reaction". Annual Biochem 1979; 95: 351-358.
19. Misra HP, Fridovich I. "The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD". J Biol Chem 1972; 247: 3170-3175.
20. Rotruck JT, Pope AC, Ganther H, Swanson AB, Hafeman DG, Hoeksirawa. "Selenium: Biochemical role as a component of Glutathione Peroxidase". Science 1973; 179(73), 588-590.
21. Moron MS, Depierre JW, Mannervik B. "Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver". Biochim and Biophys Acta 1979; 5820: 60-68.
22. Sinha AK. "Calorimetric assay of catalase". J Biochem 1972; 47: 389-394.
23. Yilmaz A, Suleyman H, Umudum Z, Sahin YN. The effect of adrenalectomy on leptin levels and some metabolic parameters in rats with diet-induced obesity. Biol Pharm Bull 2002; 25: 580—583.
24. Jiao S, Matsuzawa Y, Matsubara K, Kubo M, Tokunaga K. Abnormalities of plasma lipoproteins in a new genetically obese rat with non-insulin dependent diabetes mellitus (Wistar fatty rat). Int J Obes 1991; 15: 487-495.
25. Diniz YS, Rocha KKHR, Souza GA, *et al*. Effects of N-acetylcysteine on sucrose-rich diet-induced hyperglycaemia,

dyslipidemia and oxidative stress in rats. Eur J Pharmacol 2006; 543: 151-157.

26. Brizzi P, Tonolo G, Carusillo F, Malaguarnera M, Maioli M, Musumeci S. Plasma lipid composition and LDL oxidation. Clin Chem Lab Med 2003; 41: 56-60.
27. Irene PT, Ilias PD, Laskarina MK, George A, Ioannis SV, Alkisti P *et al*. Water Soluble Vitamin E Administration in Wistar Rats with Non-alcoholic Fatty Liver Disease. The Open Cardiovascular Medicine Journal 2012; 6: 88-97.
28. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000; 106: 473–481.
29. Zamora R, Hidalgo FJ, Tappel AL. Comparative antioxidant effectiveness of dietary b-carotene, vitamin E, selenium and coenzyme Q10 in rat erythrocytes and plasma. J Nutr 1991; 121: 50-56.
30. Nakano T, Kanmuri T, Sato M, Takeuchi M. Effect of astaxanthin rich red yeast (Phaffa rhodozyma) on oxidative stress in rainbow trout. Biochim Biophys Acta 1999; 1426: 119-125.
31. Moonkyu K, Jung WO, Hee KL, Hwan SC, Sang ML, Changsook K *et al*. Anti-obesity Effect of PM-F2-OB, an Anti-obesity Herbal Formulation, on Rats Fed a High-Fat Diet. Biol Pharm Bull 2004; 27(8): 1251-1256.