Effects of Orlistat and Its Relationship with Nitric Oxide in the Small Intestinal Mucosa

Metin Caner[1], Husniye Dogruman[2], Elif Taşkın[2], Aslı Kandil[3], and Cihan Demirci[3]

[1]*Cerrahpasa Medical Faculty, Department of General Surgery*
Istanbul Universiry
[2]*Veterinary Faculty, Department of Medical Biology*
Istanbul Universiry
and
[3]*Science Faculty, Department of Biology*
Istanbul University
Istanbul, Turkey

Abstract

Nitric oxide (NO) is known to be a messenger molecule that plays an important role in physiological and pathological conditions. It is synthesized by an enzyme called nitric oxide synthase (NOS). Inducible NOS (iNOS), one of the three isomers of NOS, has both protective and toxic properties. In this study, the role of NO has been evaluated by gastrointestinal symptoms induced by orlistat which is used in obesity treatment. Orlistat was given to Wistar rats with and without iNOS inhibition. The effects of orlistat and inhibition of NOS were studied. Glucose, urea, alanine transaminase (ALT), and gamma glutamil transpeptidase (GGT) were descreased after short- and long- term orlistat applications. Dexamethasone increased level of these enzymes. Cholesterol and triglyceride were increased in all experimental groups than the controls. This increment was more severe in animals received orlistat and dexamethasone together. Small intestinal tissue also were researched histologically and NADPH-diaphorase (NADPH-d) histochemistrically. Orlistat caused histological damages in brush border membranes, connective tissues of villi, and lymphocyte migration also increased. Dexamethasone treatment prevented these damages partially while orlistat increased the NOS distribution in the tissue sections. Dexamethasone, which is an iNOS inhibitor, decreased NADPH-d histochemistry. There was a similiar NOS distribution both in the control and orlistat+dexamethasone group. Hence, we concluded that long- term trials with orlistat and similar drugs are needed.

Key Words: orlistat, dexamethasone, nitric oxide

Introduction

Obesity is becoming a fast growing public health problem, and a high percentage of the population is facing this problem, especially in the developed countries. It can be successfully controlled by weight loss to reach the recommended ideal weight. Most obese patients are under cardiovascular risk factors (12, 8). Different medications have been employed in the pharmacological treatment of obesity. Orlistat (tetrahydrolipstatin: THL) is a lipophilic drug which has been commonly used in obesity treatment. It is a hydrogenated analogue of lipstatin isolated from *Streptomyces toxyticini*, and is a potential inhibitor of gastrointestinal lipase when it covalently binds to its active site (15). Orlistat helps to lose weight by inhibiting and subsequently preventing the digestion and absorption of dietary lipids. One of the side effects of orlistat is gastrointestinal symptoms. It caused increment of defecation, liquid and lipoid feces in 10-30% of obese patients who took 360 mg/day dose orlistat (6).

Nitric oxide (NO) is a gas molecule which can be quickly diffused (3). NO is synthesized by an enzyme called nitric oxide synthase (NOS) (2). NO plays endogenously secreted molecule and produced

Corresponding author: Dr. Cihan Demirci, Istanbul University, Science Faculty, Department of Biology, Vezneciler 34459 Istanbul/Turkey. Tel: 0090 (212) 455 58 42, Fax: 0090 (212) 528 05 27, E-mail: cihan_demirci@hotmail.com
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by endothelial cells, macrophages and neurons. NO plays a significant role in physiological conditions such as regulates of vascular tone, prevents platelet adhesion, aggregation and leukocyte adhesion to the endothelium and regulates myocardial contractility. This physiological production of NO is important for blood pressure regulation, blood flow distribution and tissue perfusion. NO is produced in small amount in physiological conditions, but following injury or certain inflammatory stimuli, NO is secreted in excessive amount in a great variety of cells (2). In the last decade, research on NO has suggested new treatment strategies for several diseases (1). iNOS, one of the known three isomers of NOS, produces iNO in many cells including macrophages, leukocytes and epithelial cells, which has both protective and toxic effects. iNO plays a role in many pathological conditions such as severe infections and autoimmune diseases (2, 7). Therefore, specific inducible NOS inhibitors are used to determine the role of NO in many experimental studies. One of iNOS inhibitors is dexamethasone. As a strong glucocorticoid, dexamethasone exhibits its impact stopping the mRNA expression during iNOS inhibition (7, 10, 14).

In this study, we aimed to microscopically investigate the small intestinal mucosal manifestations of the symptomatic gastrointestinal effects of Orlistat and to examine the relatioship between the symptomatic effects of orlistat and iNO. In addition, we also aimed to changes in the levels of some enzymes and biochemical parameters.

Materials and Methods

Sixty adult female Wistar albino rats (180-200g) were used in the present study in which they were arranged as 5 experimental and one control groups, each including 10 individuals. The groups were treated as follows:

Group 1 (n=10): 5 mg/kg/day orlistat (Xenical-Roche)
Group 2 (n=10): 10 mg/kg/day orlistat
Group 3 (n=10): 5 mg/kg/day orlistat + 2 mg/kg/day dexamethasone
Group 4 (n=10): 10 mg/kg/day orlistat + 2 mg/kg/day dexamethasone
Group 5 (n=10): 2 mg/kg/day dexamethasone
Control (n=10): saline (serum physiologic) solution

During the experiment, the rats were fed on pellets containing 18% protein and 4% lipid once a day and tap water *ad libitum*. Orlistat was given just before feeding. The rats of the groups 3, 4 and 5 were injected dexamethasone intraperitoneally. The rats were sacrificed under ether anaesthesia at the end of the 7 days experimental period. Serum glucose, urea, aspartate amino transferase (AST), alanine transaminase (ALT), gamma glutamil transpeptidase (GGT), total bilirubin, total cholesterol and triglyceride levels were determined in the blood samples by commercial kits. Statistical means among the groups with the controls were evaluated with Tukey test (13). Tissue samples were taken from the small intestine for histological and histochemical examinations. Tissue sections were stained with hematoxylin and eosin (H+E) for histology. Tissue samples were kept in 15% sucrose at +4°C overnight, following fixation in 4% paraformaldehyde at +4°C for 2 hours to detect the NADPH-diaphorase (NADPH-d) histochemistry (5). Sections in 8 μm were taken on the glass slides and NADPH-d histochemistry was applied to these cryostat sections. The catalytic activity of NOS was demonstrated by enzymatic reduction of nitroblue tetrazolium (NBT) in the presence of NADPH. Sections were incubated in phosphate buffer (pH 7.4) containing 0.3% Triton X-100, 0.01% NBT, 0.1% β-NADPH-d and 0.1 M Tris-HCl (pH 7.6) at 37°C for 15-20 min and rinsed with the same buffer (5). They were then mounted with glycerol gelatine and examined under an illuminated microscope.

Results

Glucose levels of the rats receiving 5 mg/kg orlistat in the Group 1 and 10 mg/kg orlistat in the Group 2 appeared to be substantially lower than those of the other groups. Serum AST levels of all the experimental groups were higher than the control, while ALT levels were lower in Groups 1 and 2 when compared to the others. However, those levels were higher in Groups 3, 4 and 5, and in the control group. Serum GGT levels were surprisingly lower in all the experimental groups compared to the control. Bilirubin levels did not show any difference among the experimental and the control groups. In the Group 2 given 10 mg/kg orlistat, cholesterol levels were lower than those of all the groups. Serum triglyceride levels were highest in all the experimental groups in comparison with the control (Table 1).

The appearance of the stained intestinal sections that were applied NADPH-d histochemistry is provided in Fig. 1. Compared with the control group (Fig. 1A). NADPH-d histochemistry reaction increased in the intestinal sections of the Group 1 which were given 5 mg/kg orlistat and Group 2 which were administered 10 mg/kg orlistat (Fig. 1B). However, decreased NADPH-d distribution was noted in Group 3 and Group 4 which were given orlistat and dexamethasone (Fig. 1C). Furthermore, NADPH-d reaction also decreased in the rats given only

Table 1. The mean biochemical results in the serum of the experimental groups.

	Group 1	Group 2	Group 3	Group 4	Group 5	Control
Glucose	87.333	91.111	140.714	167.666	147.571	118.571
(mg/dl)	±4.512**	±4.064*	±4.734	±7.770***	±7.371*	±5.740
Urea	29.125	20.500	30.750	38.285	26.666	31.142
(mg/dl)	±1.903	±1.254***	±1.645	±2.101	±1.626	±1.565
AST	143.000	115.285	168.857	155.666	156.666	64.666
(U/L)	±2.059***	±2.008***	±3.991***	±3.283***	±2.801***	±2.824
ALT	56.337	50.275	132.516	103.200	202.185	75.350
(U/L)	±1.448**	±1.401***	±3.305***	±3.973***	±4.175***	±3.199
GGT	2.714	3.000	6.714	5.285*	7.285	10.000
(U/L)	±0.420***	±0.723***	±0.993	±0.865	±0.473	±1.732
Total Bilirubin	0.500	0.433	0.444	0.450	0.442	0.60
Cholesterol	72.111	51.375	103.625	145.142	108.833	55.571
(mg/dl)	±3.871	±5.979	±6.884***	±7.747***	±3.092***	±4.439
Triglyceride	74.875	62.285	172.600	169.800	230.142	51.875
	±4.223**	±5.219	±3.970***	±9.124***	±3.863***	±3.876

Group 1: 5 mg/kg/day orlistat, Group 2: 10 mg/kg/day orlistat, Group 3: 5 mg/kg/day Orlistat+2 mg/kg/day dexamethasone, Group 4: 10 mg/kg/day orlistat+2 mg/kg/day dexamethasone, Group 5: 2 mg/kg/day dexamethasone, Control: Saline (serum physiologic) solution. Tukey test was used for comporison of the groups (±SEM)

* Significant *vs*. control ($P < 0.05$)

** Significant *vs*. control ($P < 0.01$)

*** Significant *vs*. control ($P < 0.001$)

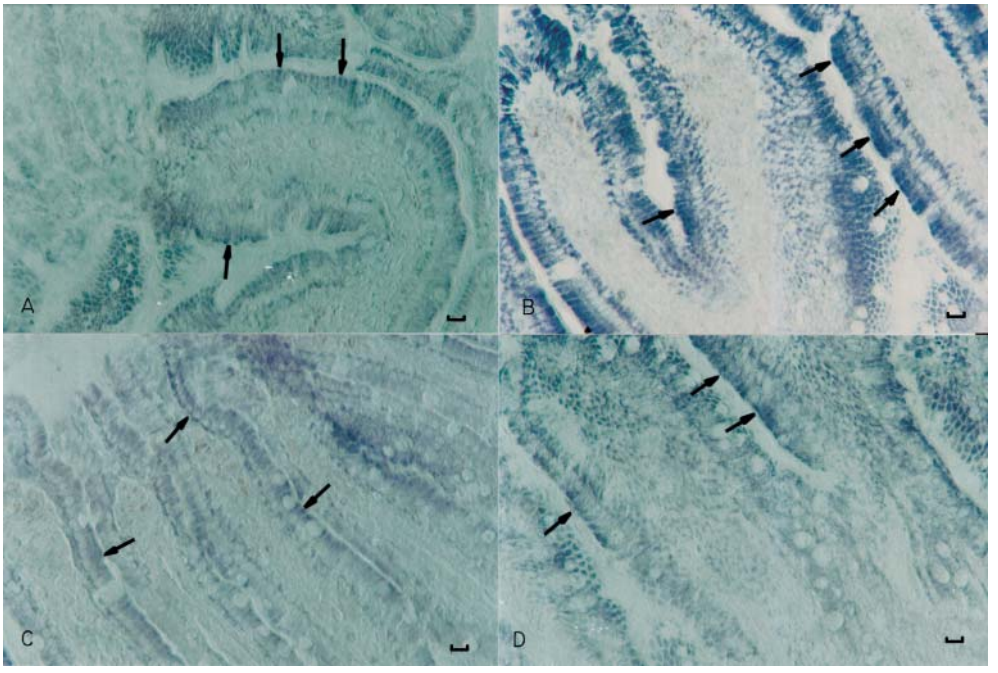

Fig. 1. NADPH-d histochemistry in small intestinal section of experimental groups. A) Control, B) Orlistat treated, C) Orlistat and dexamethasone-treated and D) Dexamethasone-treated groups. Arrows show NADPH-d reaction. Scale bar: 10 µm.

dexamethasone when compared with other experimental groups (Fig. 1D).

Hematoxylin and eosin (H+E) staining exhibited that intestinal epithelium was not regular in Groups 1 and 2 (Fig. 2B and 3B). The brush border of the epithelium also lost its continuity in these groups.

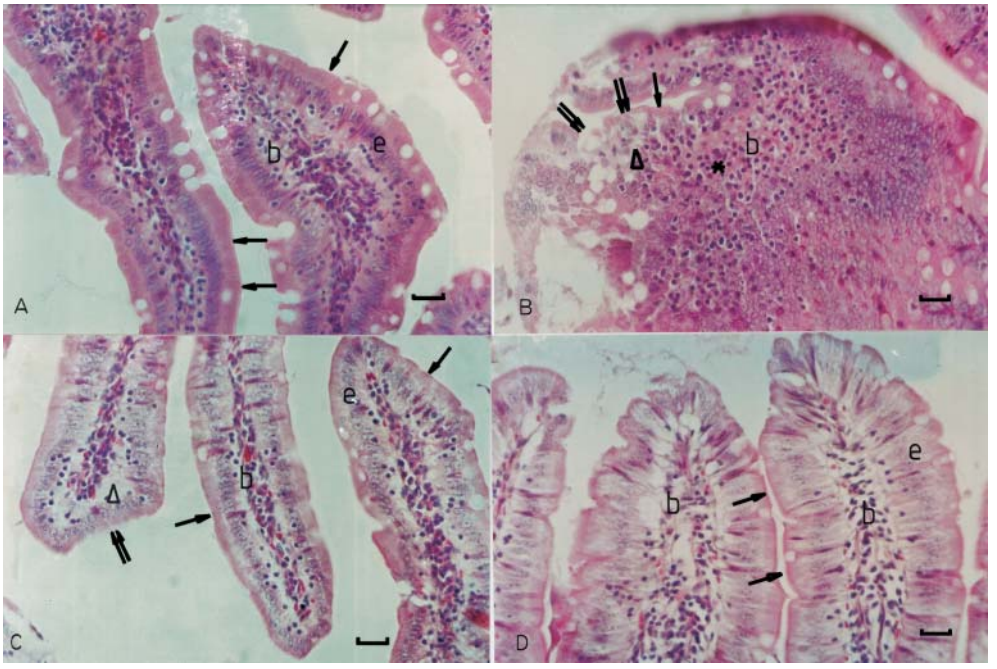

Fig. 2. A) Control, B) Orlistat treated, C) Orlistat and dexamethasone-treated and D) Dexamethasone-treated groups. (e) Epithelial Cells, (b) Connective Tissue, (↑) Brush Border, (↑↑) Destroyed Brush Border, (Δ) Destroyed Connective Tissue and (*) Lymphocyte Filtration. H+E, Scale bar: 5 µm.

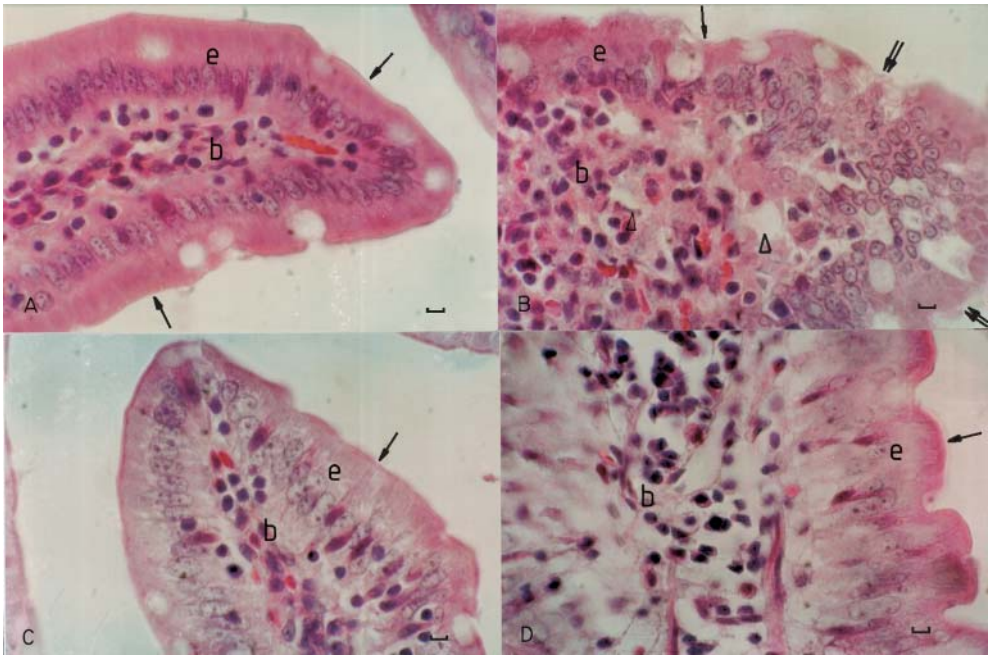

Fig. 3. A) Control, B) Orlistat treated, C) Orlistat and Dexamethasone-treated and D) Dexamethasone-treated groups. (e) Epithelial Cells, (b) Connective Tissue, (↑) Brush Border, (↑↑) Destroyed Brush Border and (Δ) Destroyed Connective Tissue. H+E, Scale bar: 1 µm.

Moreover, we observed that connective tissue in the villi was destroyed, and intercellular spaces were dilated. Lymphocytic infiltrations were also found in the lamina propria of the intestinal in these groups (Fig. 2B and 3B). Epithelial damage was partially recovered in the groups which dexamethasone was applied together with orlistat (Fig. 2C and 3C). Intestinal epithelium of Group 5 which were injected only dexamethasone was similar to that of the control, along with the presence of connective tissue damage

(Fig. 2D and 4D). We scored these results as Table 2.

Table 2. The injury of small intestinal tissue in experimental rats.

	Groups			
	Orlistat	Orlistat+Dex	Dex	Control
Brush border	++	+	–	–
Connective tissue	+++	+	–	–
Lymphocyte infiltration	+++	–	–	–

Discussion

Clinical and experimental studies on obese patients have reported positive effects of orlistat on serum lipid, cholesterol and glucose levels in addition to its promoting effect on weight loss (12, 8). A lot of studies have been done with orlistat in humans who have long been hospitalized (6). In this study we wished to demonstrate the acute side effects of orlistat by using normal female rats.

Because some people who do not want to be obese use drugs such as orlistat, we thought that it would be beneficial to know the effects of this drug on intestine healthy people. According to the biochemical results obtained at the end of our trial, the total cholesterol levels of serum were lower than those of the control group only in the rats that received 10 mg/kg/day orlistat (Group 2). On the other hand, 5 mg/kg orlistat increased the total cholesterol level. Triglyceride levels are higher than the control in all the experimental groups. This increment was significant in group 1 given 5 mg/kg orlistat. Hsieh *et al*., found that cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) increased, and triyglyceride increased in obese patients (4). Our results showed that cholesterol and triglyceride could increase and decrease the early application of orlistat. These results agreed with the observations of Nishioka *et al*. (11). They have also shown that this effect is dependent on orlistat doses. Fasting blood glucose levels in Groups 1 and 2 which were given orlistat were lower than those of the other experimental groups and the control. Some authors reported that glucose levels decrease during orlistat treatment (8). Orlistat prevents fat digestion and absorbtion (5); consequently, the rats used other enegy sources such as glucose. In addition, serum AST and ALT values were found to be different from the control. AST increased in two groups in which orlistat was given in different doses, whereas ALT and GGT decreased. Montero *et al*., showed that these three enzymes increased 3 weeks later with 120 mg/kg/day orlistat treatment in an obese patient (9). However, our results were different in that only AST increased; other enzymes decreased. We also aimed to examine the drug effect on liver. We have seen that orlistat did not cause a liver damage. On the other hand, the increase of AST may be due to muscle activities. Unfortunately, we could not compare these results with those of the other studies because they are about persons who are obese for a long period of time. Since we conducted only one week's trial, and our rats were not obese, our results showed the acute effects of orlistat. Bilirubin levels decreased but insignificantly. Nishioka *et al*. have also found that biluribin levels were less in orlistat treated Gunn rats (11).

Gastrointestinal symptoms have been commonly seen in 75% of the patients who had clinical treatment for obesity (6). Diarrhea was observed in the female rats during the 7 days of orlistat administration. Intestinal lumens of the animals were observed to be empty after they were sacrificed at the end of the 7th day. Formation of high volume liquid and lipoid feces reported as a side effect of orlistat was regarded to be a negative effect on the intestinal mucosa. Therefore, intestinal mucosa was examined by NADPH-d histochemistry to evaluate a possible change of NOS (5). The intestinal epithelium of Group 1 (5 mg/kg/day) and Group 2 (10 mg/kg/day) which were given only orlistat clearly indicated increased NOS. NADPH-d histochemistry results were obtained in the intestinal sections of Groups 3, 4 and 5 which were injected dexamethasone as a specific iNOS inhibitor. These results implied that the symptoms of orlistat might be associated with NO. These symptoms may be parallel to an increase in iNO because diarrhea occurred. If iNO production can be inhibited with specific iNOS inhibitors such as dexamethasone and amioguanidine (7, 14), one would expect some of these symptoms, to decrease. Our results seemed to support this idea, because they showed that dexamethasone treatment did protect the intestinal epithelium from damage. We have also seen some histological differences among our experimental groups by H+E staining. Destructions were observed in the intestinal epithelial cells in Groups 1 and 2 which received only orlistat. Loss of continuity and destruction in the brush border were observed. In addition to lamina propria, lymphocytic infiltrations also occurred in Groups 1 and 2 while the small intestinal tissue of the group which were injected specific iNOS inhibitor dexamethasone with orlistat application was similar to that of the control group, this indicated that iNOS inhibition can protect the tissue from lymphocytic infiltration.

As a result, histological and histochemical examination of the gastrointestinal symptoms indicated

that the use of orlistat could be associated with NO. Some of the small intestinal mucosal damages that might be related with the use of orlistat were found in this short- term trial. During the time of the use of orlistat, the presence of increased iNOS, which had played a role in the synthesis of NO which had both protective and toxic effects, has been a substantially salient result for us. For this reason, long- term trials with orlistat and similar drugs are needed.

References

1. Aktan, F. iNOS-mediated nitric oxide production and its regulation. *Life Sci*. 75: 639-653, 2004.
2. Bogdan, C. Nitric oxide and immune response. *Nat. Immunol*. 2: 907-916, 2001.
3. Furchgott, R.F. and Vanhoutte, P.M. Endothelium-derived relaxing and contracting factors. *FASEB J*. 3: 2007-2018, 1989.
4. Hsieh, C.J., Wang, P.W., Liu, R.T., Tung, S.C., Chien, W.Y., Chen, J.F., Chen, C.H., Kuo, M.C. and Hu, Y.H. Orlistat for obesity: benefits beyond weight loss. *Diabetes Res. Clin. Pract*. 67: 78-83, 2005.
5. Hope. B.T., Micheal, G.J., Knigge, K.M. and Vincent, S.R. Neuronal NADPH diaphorase is a nitric oxide synthase. *Proc. Natl. Acad. Sci. USA*. 88: 2811-2814, 1991.
6. Joyce, B. Harp. An assessment of the efficacy and safety of orlistat for long-term management of obesity. *J. Nutr. Biochem*. 9: 516-521, 1998.
7. Kirkeboan, K.A. and Strand, O.A. The role of nitric oxide in sepsis-an overview. *Acta Anaesthesiol. Scand.* 43: 275-288, 1999.
8. Lucas, C.P., Boldrin, M.N. and Reaven, G.M. Effect of orlistat added to diet (30% of calories from fat) on plasma lipids, glucose and insulin in obese patients with hypercholesterolemia. *Am. J. Cardiol*. 91: 961-964, 2003.
9. Montero, J.L., Muntane, J., Fraga, E., Delgado, M., Costan, G., Serrano, M., Padillo, J., de la Mata, M., and Mino, G. Orlistat associated subacute hepatic failure. *J. Hepatol*., 34: 173, 2001.
10. Nakashima, M.N., Ajiki, K., Nakashima, K. and Takahasmi, M. Possible role of nitric oxide in anxiety following transient cerebral ischemia in mice. *J. Pharmacol. Sci*. 91: 47-52, 2003.
11. Nishioka, T., Hafkamp, A.M., Havinga, R., vn Lierop, P.P., Velvis, H., and Verkade, H.J. Orlistat treatment increases fecal bilirubin excretion and decreases plasma bilirubin concentrations in hyperbilirubinemic Gunn rats. *J. Pediatr*., 143: 327-334, 2003.
12. Sjostrom, L., Rissanen, A., Andersen, T., Boldrin, M., Golay, A., Koppeschaar H.P.F. and Krempf, M. Randomized placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. *Lancet* 352: 167-173, 1998.
13. Snedecor, G.W. and Cochran, W.G. Statistical Methods. 7 th. Ed. The Iowa State Univ. Press. Ames, Iowa 1980.
14. Spanier, A.J. and Mc Donough, K.H. Dexamethasone blocks sepsis-induced protection of heart from ischemia reperfusion injury. *Proc. Soc. Exp. Biol. Med*. 223: 82-87, 2000.
15. Tiss, A., Lengsfeld, H., Hardvary, P., Cagna, A. and Verger, R. Transfer of orlistat through oil-water interfaces. *Chem. Physic. Lipids* 119: 41-49, 2002.