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EFFECT OF GARLIC AND ATORVASTATIN ON LEPTIN AND LIPID PROFILE IN TRITON X-100 INDUCED HYPERLIPIDEMIA IN RATS

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ABSTRACT

Hyperlipidemia is widely known to be the major risk factor for the development of cardiovascular diseases. This study was conducted to determine the effect of Garlic on Leptin hormone and Lipid Profile in Triton x-100 induced hyperlipidemic rats compared with standard hypolipidemic Atorvastatin. A total number of 60 albino rats were allocated into six equal groups, 10 rats each. The first group was kept on Standard pellet diet (served as normal control), the second group was kept on 1% garlic added to standard pellet diet, the third group was administered with standard Atorvastatin 10 mg/kg p.o. daily (served as standard), the forth group was given a single dose of triton at a dose 100 mg/kg and repeated every two weeks, i.p. (served as Triton control), fifth and sixth groups were given a single dose of triton at a dose 100 mg/kg and repeated every two weeks, i.p. and the fifth group was given garlic 1% added to standard pellet diet while sixth group was administered with standard Atorvastatin 10 mg/kg

p.o. (served as treated groups), after inducing hyperlipidemia (for 6 weeks). Results showed that, garlic significantly decreased cholesterol, LDL-C, VLDL-C and TG and significantly increased HDL compared with triton x-100 -treated group. On the other hand, there were a non significant differences in total lipid, phospholipids and leptin levels compared with triton x-100 -treated group. We concluded that, Garlic has definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemia model which is equipotent activity when compared with Atorvastatin. This might returned in addition to its own hypolipidemc activity to increased leptin sensitivity in hypothalamus. Further studies on Garlic needed to identify the possible mechanism of action on leptin hormone.

Keywords: Atorvastatin, garlic, hyperlipidemia, leptin, Triton x-100.

INTRODUCTION

Current predictions estimate that by the year 2020 cardiovascular diseases, notably atherosclerosis, will become the leading global cause of total disease burden ***(Kaur, 2006)***. The same study speculated that, an important factor for atherosclerosis is hyperlipidemia. Hyperlipidemia is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood ***(Frederickson and Lee, 2006)***. It is also synonymously known as dyslipidemia ***(Chen, 2005)***. During the past decade, a vast amount of evidence has confirmed the critical role played by the dyslipidemias in the pathogenesis of atherosclerosis and coronary artery disease ***(Vega and Sirtori, 1996)***. The advantages of lowering lipid levels to satisfactory levels have been confirmed by several experimental and interventional studies indicating lower morbidity and mortality in coronary heart disease which commensurate with reduction of serum

cholesterol ***(Jackson and Beagle, 1995)***. Garlic (Allium sativum L.) possesses many healthful properties that are related to its bioactive compounds ***(Leelarungrayub et al., 2006, Arnault and Auger, 2006, Okada et al., 2005 and Lee et al., 2005)***. It was reported that, consumption of garlic is very helpful in regulating plasma lipid levels ***(Lau, 2006)*** as well as plasma anticoagulant activity ***(Pierre et al., 2005, Lawson et al., 1992)*** and in prevention of the atherosclerosis process ***(Rahman and Lowe, 2006)*** and even cancer ***(Velmurugan and Nagini, 2005)***. It was shown that, garlic also provides protection against ethanol induced gastric injury ***(Khosla et al., 2004)***. The most studied and reported health-promoting effect of garlic is cardioprotection ***(Lau, 2006, Rahman and Lowe, 2006)***. However, a pilot study of garlic consumption shows no significant effect on markers of oxidation or subfraction composition of low-density lipoprotein (LDL-C) ***(Byrne et al., 1999)***. There is no doubt that, garlic and garlic preparations possess anticoagulant abilities ***(Pierre et al., 2005, Lawson et al., 1992)***. Garlic has been proposed to have direct anti-atherogenic (preventive) and anti-atherosclerotic (causing regression) effects at the artery wall ***(Sukandar et al., 2010)***. Adipocytes cells secrete a protein hormone called leptin in proportion to the amount of triglycerides they have stored ***(Isganaitis and Lustig, 2005)***. Thus, in a long term, excess ingestion of calories resulting in increased fat deposition triggers an increase in leptin secretion. Leptin impinges on its receptors in the hypothalamus to alter the set point at which satiety is reached, so that the individual eats less due to loss of appetite and, hence, assimilates fewer calories ***(Isganaitis and Lustig, 2005, Mcphee et al., 2006)***. Although leptin is a circulating signal that reduces appetite, obese people generally have an unusually

high circulating concentration of leptin. Obese individuals are therefore resistant to the effect of leptin due to low sensitivity of the hypothalamus to leptin ***(Considine et al., 1996)***. Although, some researchers have proved that regular addition of garlic to diet can help reduce blood cholesterol and glucose levels ***(Drobiova et al., 2009, Mahesaret al, 2010, Liuet al., 2007)***, there is still a controversy regarding the plasma lipid regulating and antioxidant increasing properties of garlic. Therefore, it was decided to study the possible changes in the plasma lipid levels and leptin hormone through an experiment on Triton x-100 hyperlipidemic rats in comparison with standard hypolipidemic atorvastatin.

MATERIAL AND METHODS

Chemicals:

Atorvastatin was obtained from market (LIPINORM®) Batch number: 11227. Triton X-100(a non-ionic detergent, iso octyl polyoxy ethylene phenol, formaldehyde polymer) was obtained from Sigma Aldrich. Cholesterol kit (Colorimetric Method), HDL-C kit, Triglycerides, LDL-C and Phospholipids kits were purchased from Biodiagnostic and Bilirubin, ALT activity and AST activity kits used were of analytical grade.

Plant material:

The fresh garlic-*Allium sativum*- was obtained from the local market. Dried and ground bulbs. It was mixed with basal diet as 1% mixture.

Animals:

This study was carried out at the faculty of Veterinary Medicine, Damanhur University using 60 male albino rats weighing 130-200g and 3.5-4 months old. Rats were housed in clean disinfected floor in wooden cages. Food and water were available *ad libitum*. They were housed in a room where the congenial temperature was 27°C ±2°C and nearly 12 hr light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 10 days and supplied with a standard pellet diet and water *ad libitum*.

Induction of hyperlipidemia:

Hyperlipidemia was induced in albino rats by intraperitoneal injections of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline after overnight fasting for 18 hours every 2 weeks 3 times from beginning of experiment ***(Mohale et al., 2008, Ansarullah et al., 2009)***.

Experimental design:

A total number of 60 albino rats were allocated into six equal groups, 10 rats per group as follow:

The first group was given Standard pellet diet (Served as normal control) [G1]. The second group was given 1% garlic added to standard pellet diet[G2], Third group was administered with Standard Atorvastatin 10 mg/kg p.o. daily (served as standard) [G3]. The forth group was given a single dose of triton at a dose 100 mg/kg and repeated every two weeks, i.p. (Served as Triton control) [G4]. Fifth and sixth group was

given a single dose of triton at a dose 100 mg/kg and repeated every two weeks, i.p. and the fifth group was given garlic 1% added to standard pellet diet[G5] while sixth group was administered with Standard Atorvastatin 10 mg/kg p.o. [G6] (served as treatment groups), after inducing hyperlipidemia.

Collection of blood samples:

After six weeks of treatment, the blood was collected by retro orbital sinus puncture, under mild ether anesthesia in plane tubes. Serum obtained by immediate centrifugation of blood samples at 4000 rpm for 10 minutes at room temperature and was directly used for estimating serum lipid profiles (serum TC, TG, LDL-C and HDL-C). All samples were stored at -80°C until analysis.

Biochemical analysis:

Serum lipid levels include TC, TG and HDL-C, LDL-C, were carried out using respective diagnostic commercial kits from Biodiagnostics, Egypt and VLDL was calculated as friedewald estimation ***(Friedewald et al., 1972)***, LDL-C= (TC-(TG/5+HDL)-C) mg/dl, VLDL=(TG/5).

- Leptin was determined using commercial ELISA kits method
- **Leptin Kit**
- **RayBio® Rat Leptin ELISA Kit Protocol;** (Cat#: ELR-Leptin-001); RayBio® ELISA kits: www.raybiotech.com, It was imported by Sigma Scientific Services Co., Egypt.

Statistical analysis:

The data of Triglycerides, Cholesterol, HDL, LDL, VLDL, Total Lipids, AST activity, ALT activity, Leptin, Bilirubin and Phospholipids at 6 weeks were analyzed by Statistical Package for the Social Sciences ***(SPSS Inc., 2011)***. One way analysis of variance ANOVA with the following model:

$$Y_{qj} = \mu + \alpha_q + e_{qj}$$

Where:

Yqj = an observational data q= 1, 2, 3, 4, 5 and 6 (1 =G1, 2 =G2, 3=G3, 4=G4, 5= G4 and 6=G6).

Aq = group effects.

e_{qj} = random error.

RESULTS AND DISCUSSION

Coronary heart diseases, stroke, atherosclerosis and hyperlipidemia are the primary cause of death ***(Grundy, 1986, Davey, 1993)***. Therefore, prime consideration in the therapy for hyperlipidemia and arteriosclerosis is to attenuate the elevated blood serum/plasma levels of lipids. The currently available antihyperlipidemic therapy includes mainly HMG-CoA reductase inhibitors (Statins), Bile acid sequesterants (Resins), Activate lipoprotein lipase (Fibric acid derivatives), Inhibit triglyceride synthesis (Nicotinic acid) and others (Gugulipid, Ezetimibe, Policosanol) (***Brown et al., 1993)***. Though there are a large number of antihyperlipidemic agents used in the treatment, none of the existing one available worldwide are fully effective, absolutely safe and free from side effects ***(Ghatak and Asthana, 1995)***. So efforts are being made to

find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. Many herbs and plant products have been shown to have antihyperglycaemic and antihyperlipidemic properties ***(Brown et al., 1993)***. The use of herbs and other natural products has gained popularity, and the increase in their consumption is backed by solid scientific evidence ***(Pierre et al., 2005, Ramaa et al., 2006, Slekovec and Goessler, 2005)***. One of these natural products is garlic, which has been used throughout the history of civilization for treatment of a wide variety of ailments ***(Leelarungrayub et al., 2006, Arnault and Auger, 2006, Okada et al., 2005, Lee et al., 2005)***. The wide range of ailments in which this vegetable is used ***(Lawson et al., 1992, Velmurugan and Nagini, 2005, Khosla et al., 2004)*** could create an impression that garlic is a panacea, but it is not ***(Byrne et al., 1999, Kerckhoffs et al., 2002)***. The most studied and reported health promoting effect of garlic is cardioprotection ***(Lau, 2006, Rahman and Lowe, 2006)***. Therefore, this investigation was to study the influence of garlic on lipid profile and leptin on Triton x-100 induced hyperlipidemic rats. The results of present study are given in Table (1). The rats treated with triton showed significant increase in serum cholesterol level from 146.80 mg/dl in normal rats to 197.50 mg/dl, triglyceride level from 67.37±4.63 mg/dl in normal rats to 100.72±2.90 mg/dl and LDL-C from 110.40±3.20 mg/dl in normal rats to 158.10±3.12 mg/dl. An increased risk of coronary heart disease is associated with a high serum concentration of total cholesterol, LDL and triglyceride ***(Sheehan and Jensen, 2000)***. Treatment with Garlic and Atorvastatin reduced the serum TC, TG & LDL-C levels when compared to the

hyperlipidemic control group. Garlic and Atorvastatin showed a decrease in the levels of cholesterol, Triglyceride, and LDL-C level. Administration of Garlic in triton induced hyperlipidemic rats decrease the cholesterol level to 170.70±4.23 mg/dl, triglyceride level is 80.26±2.06 mg/dl and HDL-C level is 22.87±1.91 mg/dl as compare to standard drug Atorvastatin where decrease of cholesterol level is by 158.40±5.57 mg/dl triglyceride level is by 76.51±1.91 mg/dl and HDL-C level is 21.13±1.48 mg/dl in triton induced hyperlipidemic rats. Diets supplemented with garlic not significantly decreased the total lipid levels and not significantly increased serum leptin and phospholipids levels ($P > .05$) compared with triton x-100 treated group. The increase in HDL-C level may be due to the activity of LCAT and inhibition of the action of hepatic TG-lipase on HDL, which may contribute for rapid catabolism of blood lipids through extra hepatic tissues ***(Gordon et al., 1989)***. The same authors revealed that, it is widely accepted that elevation in plasma HDL-C level decreased the risk factor for developing atherosclerosis, by increasing the clearance of cholesterol from the arterial wall. Garlic may have served other systemic and cardiovascular functions such as antithrombotic, antibiotic, antiglycative, anti-inflammatory, hypolipidemic, hypo-cholesteremic, anti-atherosclerotic, hypoglycemic and hypotensive activities, rather than reducing weight significantly in the normal rats and mice (***Drobiova et al., 2009, Mahesaret al., 2010, Liuet al., 2007)***. The possible mechanisms of garlic as lipid lowering agent are its inhibitory effect on hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl-CoA (HMG CoA)

reductase ***(Sukandar et al., 2010)***. It was also suggested that garlic lowers serum lipids by delaying lipid absorption from gastrointestinal tract and diminishing LDL cholesterol synthesis in the liver. Garlic also increases the body's metabolic rate by stimulating the adrenal gland to release adrenaline which increases the rate of fat metabolism in the body and in turn helps burn more calories to decrease weight ***(Noaki et al., 2007)***. Garlic actions related to lipid metabolism is probably due to the action of allicin, a potent compound in crushed garlic that increases the body's metabolic rate by stimulating the adrenal gland to release adrenaline which increases the rate of fat metabolism in the body and in turn helps burn more calories and decrease weight ***(Noaki et al., 2007)***. The significant hypolipidemic activity observed in group 5 may also be due to the fact that, sulphur-containing compounds in garlic acted on the hypothalamus of the rat, increasing the sensitivity of the hypo-thalamus to leptin, a hormone secreted by fat cells in the body, which functions by impinging its receptors in the hypothalamus altering the set point at which satiety is reached, so that the organism eats less ***(Mahesar et al., 2010)***. The hypoglycemic effect may be due to the presence of flavonoids and sulphur-containing compounds in the garlic extract ***(Mahesar et al., 2010)***. Garlic may potentiate insulin effect on plasma by increasing secretion of insulin from beta cells of the pancreas ***(Sukandar et al., 2010)***. This elevated insulin level causes a decrease in blood glucose. Allicin, the principal bioactive compound in garlic extract also has a scavenging effect on excess glucose stored as fat. Some of the excess glucose is also converted to ATP ***(Sukandar et al., 201)]***.This might increase the sensitivity of leptin. Concerning the effect of garlic in

combination with triton x-100 on liver functions. Table (2) revealed that, the administration of garlic was resulted in decrease in activity of transaminases in serum and Bilirubin level compared with triton x-100 group. These come in agreement with those obtained by ***Hattori et al., (2001)*** and *Sumioka et al., (2001)* who reported that, Ajoene (a garlic-derived sulfur-containing compound) and S-allylmercaptocysteine suppressed the rise in serum ALT activity in mice. In the contrary, the present findings disagree with those obtained by ***Joseph et al., (1989)*** who reported significant rise in serum AST in rats fed garlic extract. Moreover, ***Ilker et al., (2004)*** found that, there were no differences in ALT activity and AST activity when a total of 23 volunteer kept on high cholesterol diet and ingested garlic extract at the dose (10 g garlic/day) for 4 months.

Table (1): Means ± standard error of Cholesterol, Triglycerides, HDL-C, LDL-C, VLDL-C and Total Lipids levels at 6 weeks.

Criteria Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Total Lipids (mg/dl)
1	67.37 ± 4.63^{cd}	146.80 ± 6.73^{c}	22.83 ± 0.62^{b}	110.40 ± 3.20^{c}	13.47 ± 0.92^{cd}	0.97 ± 0.14^{ab}
2	69.84 ± 4.42^{cd}	128.70 ± 5.01^{d}	31.07 ± 1.30^{a}	83.60 ± 1.72^{d}	13.97 ± 0.89^{cd}	0.90 ± 0.14^{b}
3	64.86 ± 1.45^{d}	114.40 ± 5.13^{d}	28.73 ± 1.09^{a}	72.60 ± 2.88^{d}	12.97 ± 0.29^{d}	0.99 ± 0.15^{ab}
4	100.72 ± 2.90^{a}	197.50 ± 4.41^{a}	19.23 ± 2.07^{b}	158.10 ± 3.12^{a}	20.15 ± 0.58^{a}	1.40 ± 0.17^{a}
5	80.26 ± 2.06^{b}	170.70 ± 4.23^{b}	27.87 ± 1.91^{a}	131.80 ± 5.11^{b}	16.05 ± 0.41^{b}	1.16 ± 0.07^{ab}
6	76.51 ± 1.91^{bc}	158.40 ± 5.57^{bc}	28.13 ± 1.48^{a}	121.90 ± 6.06^{b}	15.30 ± 0.38^{bc}	1.27 ± 0.13^{ab}

Number of samples = 10

Means carry different superscripts are significantly different at $P\leq 0.05$.

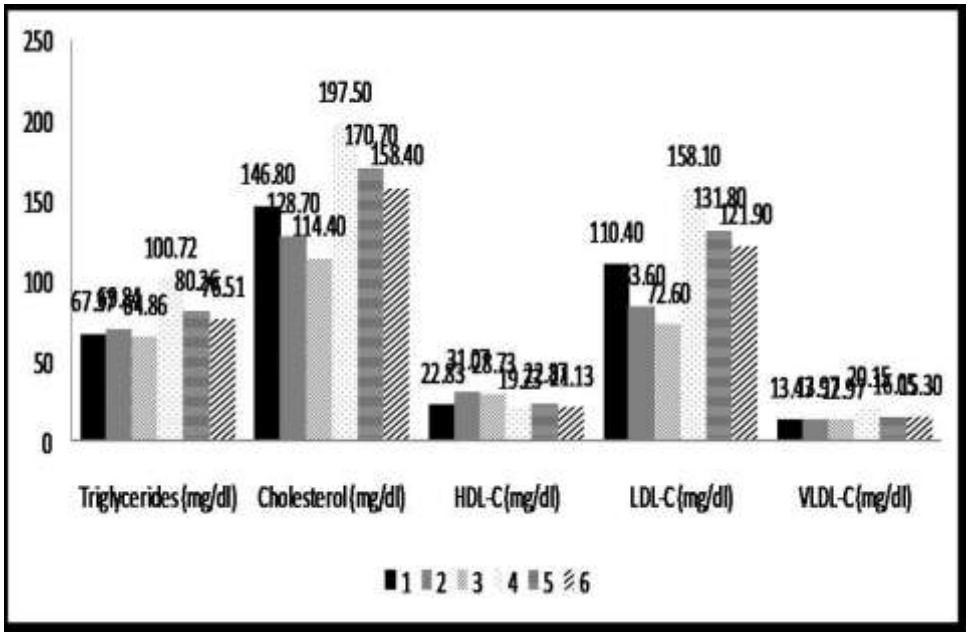

Fig. (1a): Means of Triglycerides, Cholesterol, HDL, LDL and VLDL at 6 weeks.

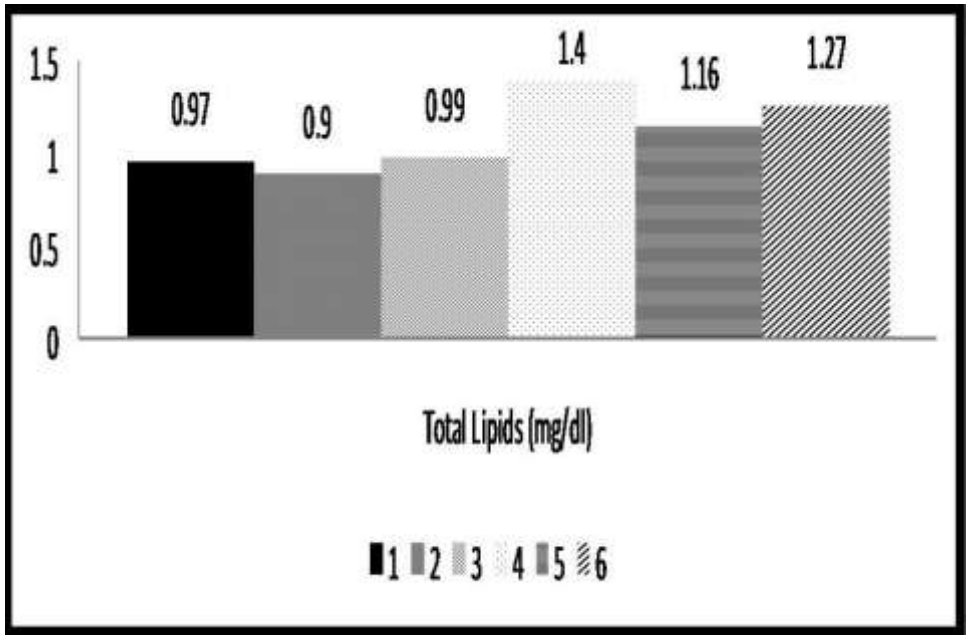

Fig. (1b): Means of Total lipids at 6 weeks.

Table (2): Means ± standard error of AST, ALT, Leptin, Bilirubin and Phospholipids at 6weeks.

Criteria Groups	AST (u/ml)	ALT (u/ml)	Leptin (pg/ml)	Bilirubin (mg/dl)	Phospholipids (mg/dl)	Criteria Groups
1	16.80±1.27^{b}	9.80±0.61^{b}	6308.40±407.71^{a}	0.18±0.05^{c}	82.22±2.32^{c}	16.80±1.27^{b}
2	13.60±1.17^{b}	7.80±0.70^{b}	6656.60±270.41^{a}	0.19±0.04^{c}	83.89±1.80^{c}	13.60±1.17^{b}
3	13.00±0.89^{b}	7.70±0.40^{b}	5060.10±539.92^{b}	0.22±0.04^{bc}	78.89±1.28^{c}	13.00±0.89^{b}
4	26.30±2.72^{a}	13.00±2.00^{a}	4136.90±342.76^{bc}	0.50±0.06^{a}	139.00±2.88^{a}	26.30±2.72^{a}
5	14.80±1.02^{b}	8.20±0.61^{b}	4013.50±189.80^{bc}	0.32±0.02^{b}	132.00±2.10^{a}	14.80±1.02^{b}
6	14.20±1.36^{b}	8.40±0.93^{b}	3365.50±325.69^{c}	0.26±0.03^{bc}	117.56±3.90^{b}	14.20±1.36^{b}

Number of samples = 10

Means carry different superscripts are significantly different (P≤ 0.05)

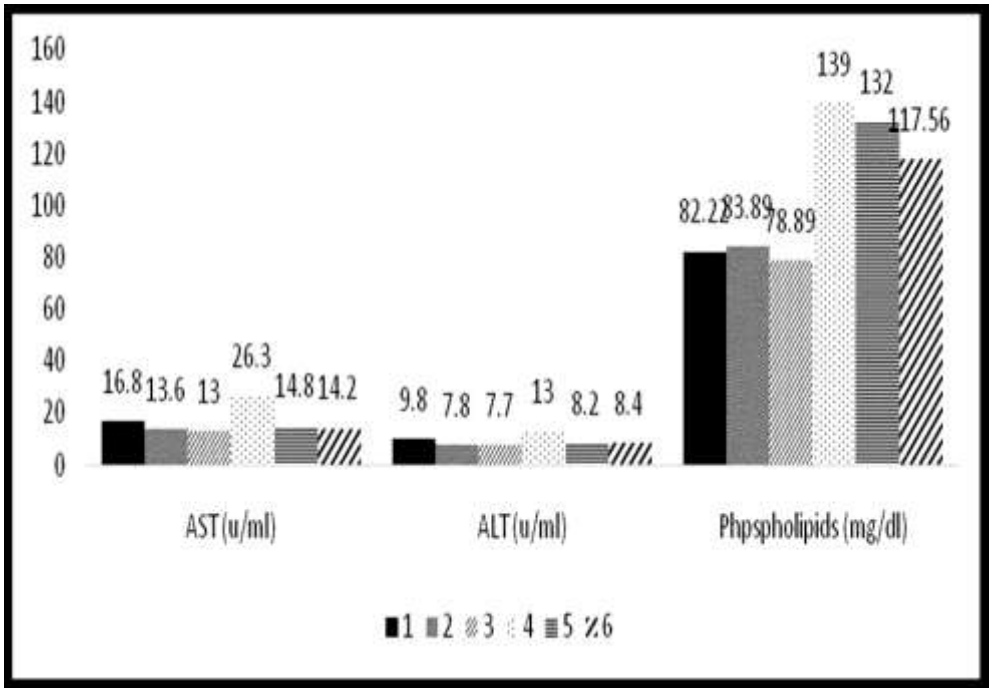

Fig. (2a): Means of AST activity, ALT activity and Phospholipids at 6 weeks.

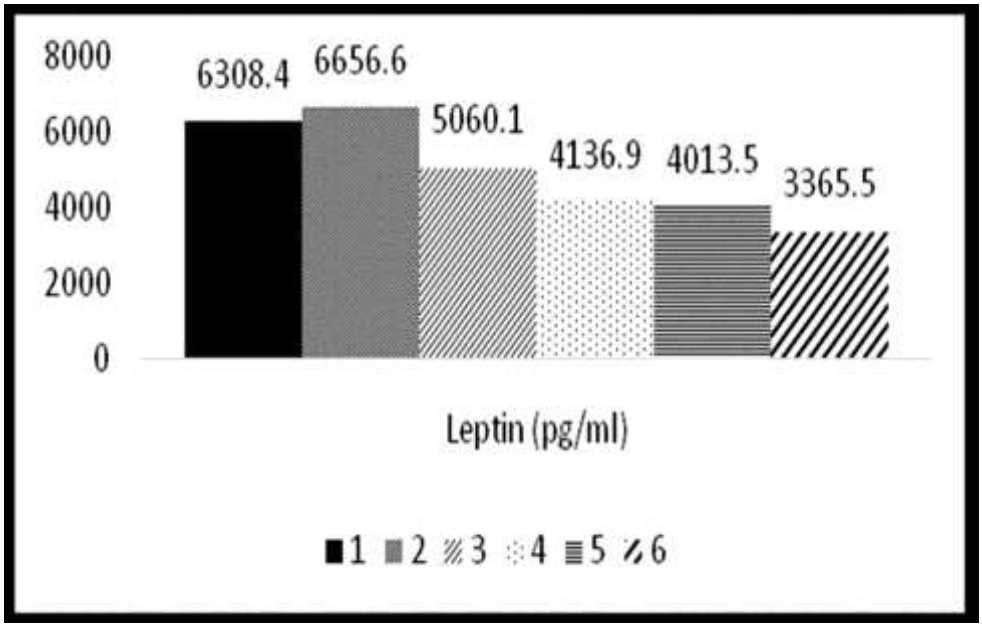

Fig. (2b): Means of Leptin6 weeks.

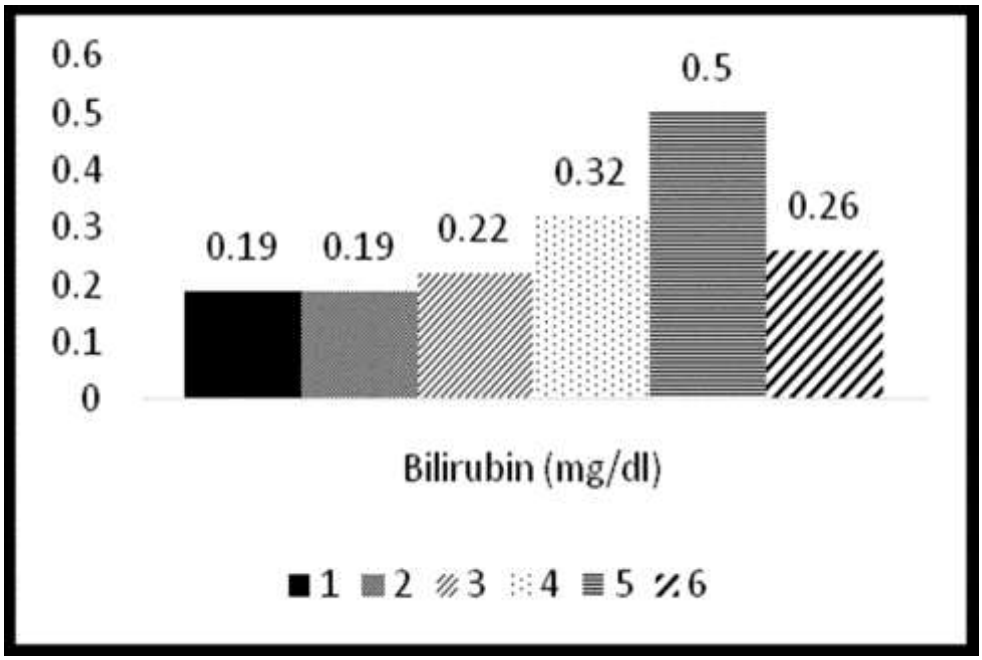

Fig. (2c): Means of Bilirubin6 weeks.

CONCLUSION

The results concluded that Garlic has definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemia model which is equipotent activity when compared with Atorvastatin. This might returned in addition to its own hypolipidemc activity to increased leptin sensitivity in hypothalamus. Further studies on Garlic needed to identify the possible mechanism of action on leptin.

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