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ROLE OF PHYTOLACCA AMERICANA AND PHYTOLACCA BERRY IN LIPID PROFILE ALLERATION IN HYPERCHOLESTROLEMIA INDUCED RABBITS *ORYCTOLAGUS CUNICULUS*

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ABSTRACT

Present study is concerned to find out the induced effects of two herbal weight reducing drugs Phytolaccca Americana (PA) and Phytolacca Berry (PB) in hypercholestrolemia on lipid profile of Rabbits (*Oryctolagus cuniculus*). Pokeweed was the single constituent of PB, the other additional ingredients of PA are bladder wrack, garlic and grape fruit. Both herbal drugs were administered orally to the respective test animals in the doses of 33.3mg PA/day and 1.15mg PB/day, for 27 days and blood samples drawn on day 0,3,9,14,21 and 27 were used to measure mean plasma cholesterol, triglycerides (TG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) concentrations. After one week of administration of drugs although both drugs reduced the cholesterol and TG concentrations to lower levels but PB effectively decreased them below their normal concentrations when given approximately for four weeks. Similarly, both drugs lowered plasma LDL-C concentration after two weeks of treatment again PB reduced concentration below normal. However plasma HDL-C levels declined to normal levels following the one week of treatment, started to elevate along the passage of treatment and decreased to normal in the later part of experiment. These findings suggested that pokeweed comparatively, more effectively maintained the lipid profile at normal concentrations probably inhibiting the HMG-CoA reductase activity and affecting the lipoprotein metabolism. In case of PA interactions of ingredients may interfered the enzymes of lipoprotein metabolism thus constant elevated levels of LDL- C and TG were observed.

Keywords: Poke weed, phytolacca berry, phytolacca Americana, herbal drugs, lipid profile.

INTRODUCTION

Obesity is a chronic disease and should be treated with reasonable expectations (Scheen and Lefébvre, 1999) because of its related complications, an immediate safe and effective treatment is required (Sidhaye and Cheskin, 2006). Unfortunately, medication-induced weight loss is often associated with rebound weight gain after the cessation of drug use, side effects from the medications, and the potential for drug abuse. In the present study, two herbal weight reducing drugs Phytolacca Americana (PA) with a single constituent of Poke weed and Phytolacca Berry (PB) in addition to Poke weed, containing Bladder wrack, garlic and grape fruit, are used to assess the efficacy in the management of weight reduction through their effects on lipid profile in common rabbits.

The extract of various parts of poke weed plant are used in homeopathic medicines for the treatment of many diseases specially the obesity (Ravikiran *et al*., 2011). Weight loss in response to poke weed was noticed in molting hens (Willis *et al*., 2008) and in mice (Lazarus *et al*., 1998). Bladder wrack has three major active components; iodine and two types of dietary fibers, alginic acid and fucoidan (Chevrel, 1980). Iodine is used to treat obesity through its thyroid gland stimulating effect (Björvell and Rössner, 1986; Teas *et al*., 2007) while animal and in vitro studies had proposed the LDL- C lowering effects of both alginic acid and fucoidan (Vázquez *et al*., 1996). Garlic was known to be effective in decreasing plasma lipids and a number of studies have shown that garlic significantly decreased the total cholesterol and LDL – C in human (Milner, 1996 ; Steiner *et al*.,1996; Stevinson *et al*., 2000) and moderately raised HDL-C (Ashraf *et al*., 2005, 2011). Experimental studies by Gorinstein *et al*. (2006) have shown the plasma lipid and cholesterol lowering effects of garlic in rats.

Grapefruits are a good food to include in a sensible weight-loss diet. Grapefruit seed extracts with high concentrations of vitamin C and E claimed to have antimicrobial and antioxidant properties (Armando, 1998; von Woedtke *et al*., 1999; Dembinski *et al*., 2004). Experimental studies in rats have shown that naringenin, a flavonoid present in grapefruit might reduce the risk of coronary heart disease and other chronic diseases through its hypocholesterolemic effects (Daher *et al*., 2005; Cho *et al*., 2011). Reduction of serum cholesterol levels were also observed in laying hens with supplemented flavonoids (Lien *et al*., 2008).

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MATERIALS AND METHODS

Animals
Rabbits (*Oryctolagous cuniculus*) used in the experiment were one to one and half years old; 12 in number with their average weight 1160 to 1510gm were obtained from local supplier. They were kept in barred cages, placed in well ventilated environment. Four *O. cuniculus* were kept as control (Lab standard) and the remaining eight in equally divided two groups were used as test animals.

Induction of hypercholesterolemia
All rabbits were made hypercholesterolemic by feeding a modified diet of 5g of butter fat per kg of daily diet (Moghadasian *et al*., 1999) for 15 days and blood samples were collected at day 0 and 15.

Drug Therapy
The herbal weight reducing drugs used for experimental purpose include PA and PB, purchased from local chemist shop in tablet form. A dose of PA calculated for daily oral administration was 33.3mg. While an oral daily dose of PB calculated was 1.15mg.

Blood Sampling
Blood samples were obtained with the help of 3cc disposable syringes from the marginal vein of ear. Sampling was done from day 0,3,9,14,21 and 27. In order to obtain plasma; heparinized blood samples were centrifuged at 3600rpm for 5minutes and the supernatants were stored at 4^0C to be read on spectrophotometer (Model No. NV 201, China).

Biochemical and Statistical Analysis
Blood lipid profile i.e. cholesterol, TG, LDL–C and HDL-C were measured by using commercial biochemical kits (Randox, Cat. No. CH200, TR1696, CH203, CH1350). The absorbance of samples was read on spectrophotometer. The data was statistically analyzed by t-test and two-way ANOVA.

RESULTS

A consideration of table1 indicates that control and test rabbits fed on the diet with additional 5g butter fat per kg of diet for 15 days resulted in a significant rise ($P<0.05$) in mean plasma concentrations of cholesterol, TG, LDL-C and HDL-C.

Control
Control rabbits after 15 days when reverted to their normal diet showed a reduction in mean plasma concentration of cholesterol and HDL-C from day 3 and reached to normal levels. However mean plasma TG and LDL-C remained significantly higher ($P<0.05$) than normal concentrations (Figs. 1-4).

Cholesterol
Test 1 (T1) rabbits with initial mean plasma cholesterol level of 227.44±49.40 mg%, when administered with a dose of 33.3 mg/day of PA for 27 days showed an immediate fall in mean cholesterol concentration on day 3 then a rise on day 9, followed by a gradual significant ($P<0.05$) reduction of 138.17±8.87 mg% on day 27 (Fig. 1).

Test 2 (T2) rabbits with mean plasma cholesterol concentration of 185.2± 26.39 mg% on day 0, administered a dose of 1.15 mg/day of PB for 27 days showed a fall and rise of mean cholesterol concentration in similar pattern on day 3 and day 9 respectively. However, the reduction in cholesterol concentration continues to fall non-significantly below normal level i.e. 36.958±10.77mg% on day 27 (Fig. 1).

TG
T1 animals with increased mean plasma TG concentration of 354.63±36.47mg% following the treatment showed a reduction and elevation of TG level on day 3 and day 9 respectively. Afterward the mean triglyceride

Table 1. Effect of Butter Fat (5g/kg diet) on Mean Plasma lipid profile (mg%) of rabbits *Oryctolagus cuniculus*.

	Cholesterol			Triglyceride		
Days	C	T1	T2	C	T1	T2
0	67.65±7.52	70.97±6.70	68.24±3.21	149.99±6.82	157.97±4.89	149.67±7.34
15	202.01±14.43	227.441±49.40	185.26±26.39	326.13±14.44	354.63±36.47	443.98±69.94

LDL			HDL		
C	T1	T2	C	T1	T2
72.08± 8.557	52.16 ± 6.03	59.76 ± 6.68	101.20 ±1.59	118.29 ± 31.19	102.41 ±22.08
326.67 ±6.08	402.82±137.12	275.32±47.19	242.86 ±14.88	256.56 ± 10.69	246.01 ±4.92

Each figures is the Mean ± SD of four values
C = Control, T1 = Phytolacca Americana (33.3 mg/day), T2 = Phytolacca Berry (1.15 mg/day)

concentration continued to reduce up to day 27, but remained significantly higher than normal concentration. Animals treated with PB also followed the similar pattern of changes in mean plasma TG concentration from day 3 to day 9, but the reduction of TG concentration from day 9 on-wards was significantly below the normal levels (Fig. 2).

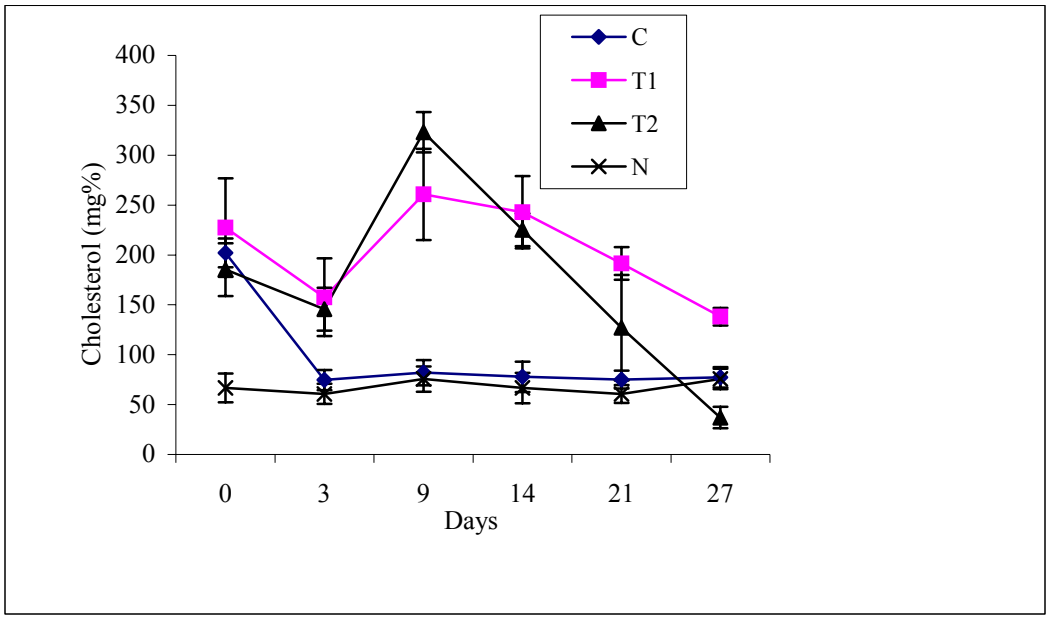

Fig. 1. Comparison of mean plasma cholesterol concentration in control and test rabbits *Oryctolagus cuniculus* following the administration of weight reducing drugs.
Each figures is the Mean ± SD of four values.
C = Control, T1 = Phytolacca Americana (33.3 mg/day), T2 = Phytolacca Berry (1.15 mg/day), N= Normal

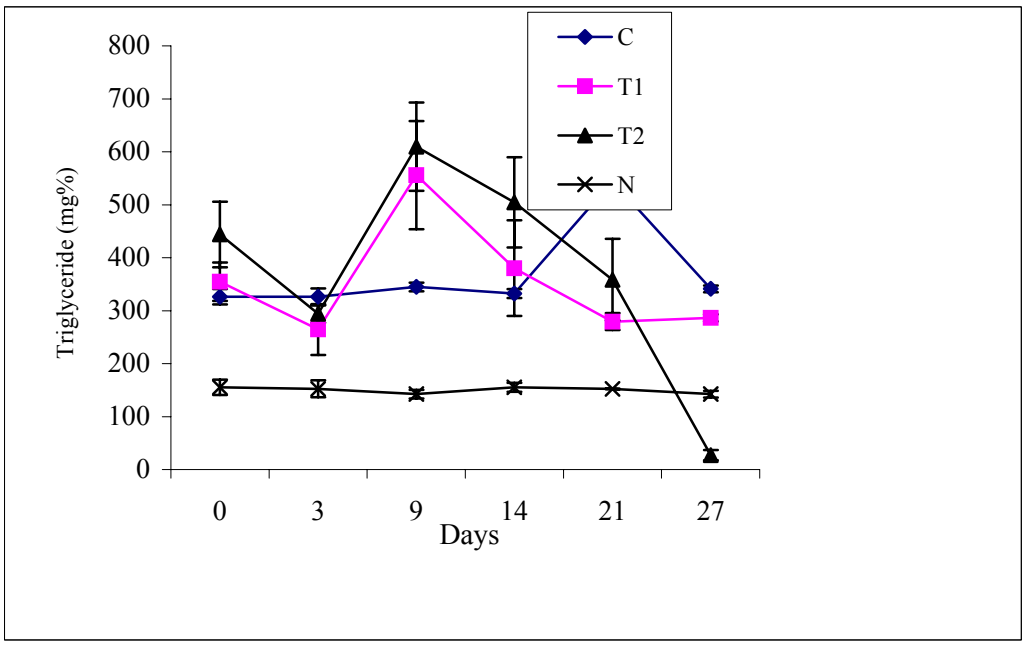

Fig. 2. Comparison of mean plasma TG concentration in control and test rabbits *Oryctolagus cuniculus* following the administration of weight reducing drugs.
Each figures is the Mean ± SD of four values
C = Control, T1 = Phytolacca Americana (33.3 mg/day), T2 = Phytolacca Berry (1.15 mg/day), N= Normal

LDL-C

Figure 3 shows the animals of T1 group have a reduction in mean plasma LDL-C concentration on day 3 followed by a rise and fall on day 9 and day 14 respectively along the treatment. However the mean LDL-C concentration remained higher i.e. 243.80± 21.93 mg% on day 27 and did not reach to normal value.

T2 animals following the treatment with PB have a maximum mean LDL-C concentration of 473.30±94.53 mg% on day 9 and reduced to normal level on day 27 (Fig. 3).

HDL-C

T1 rabbits when treated with PA for 9 days showed a reduction in mean plasma HDL-C concentration of 115.87±9.89 mg%. Further administration of drug increased the mean HDL-C level followed by a reduction to near normal concentration (Fig. 4).

In T2 rabbits, administration of drug also resulted in a fall of mean HDL-C concentration on day 9, which gradually elevated up to day 21 and returned to approximately normal levels.

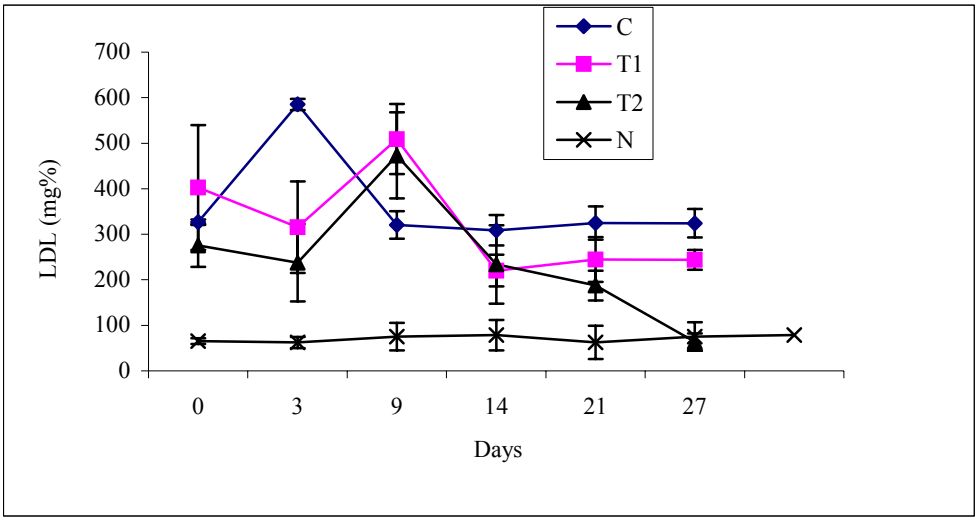

Fig. 3. Comparison of mean plasma LDL-C concentration in control and test rabbits *Oryctolagus cuniculus* following the administration of weight reducing drugs.
Each figures is the Mean ± SD of four values
C = Control, T1 = Phytolacca Americana (33.3 mg/day), T2 = Phytolacca Berry (1.15 mg/day), N= Normal

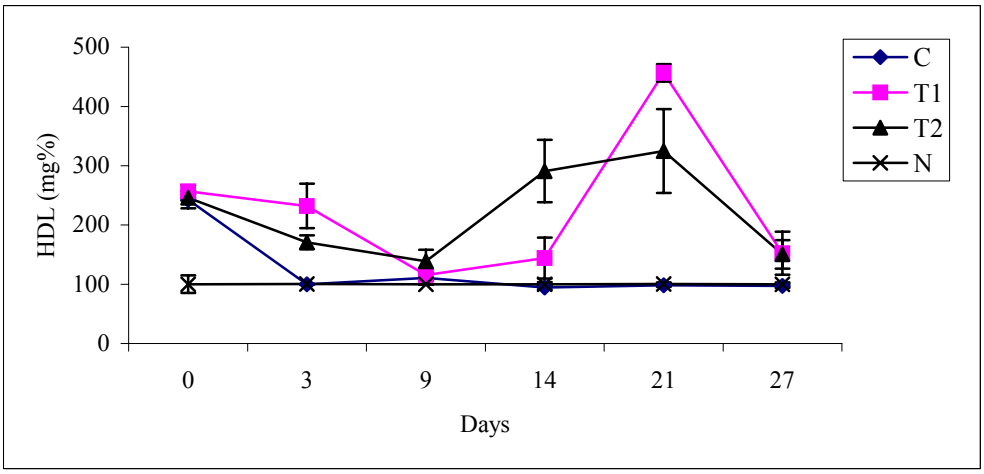

Fig. 4. Comparison of mean plasma HDL-C concentration in control and test rabbits *Oryctolagus cuniculus* following the administration of weight reducing drugs.
Each figures is the Mean ± SD of four values
C = Control, T1 = Phytolacca Americana (33.3 mg/day), T2 = Phytolacca Berry (1.15 mg/day), N= Normal

DISCUSSION

The mechanism of reduction of body fat and ultimate reduction in body weight by herbal drugs is achieved either by suppression of appetite and decreasing the intestinal absorption (Fanghanel *et al*., 2000; Smith and Goulder, 2001) or stimulating the satiety centers with increased metabolic rates (Girola *et al*., 1996; Allison *et al*., 2001). The ingredients of both drugs i.e. PA and PB used in this study are laxative (Väzquez *et al*., 1996), thus limiting the intestinal absorption and increasing the loss of fluid.

A diet with added butter fat given to rabbits cause a significant rise in lipid profile concentrations, although removal of fat from the diet in control animals show the reversal plasma mean concentrations of cholesterol and HDL-C to normal level while TG and LDL-C remained elevated.

The experimental animals of T1 and T2 administrated with PA and PB for 27 days respectively show the lipid profile of both treatments returning to normal values. Comparatively PB more effectively decreases the mean cholesterol, TG and LDL-C concentrations to very low values.

One of the major constituents of PA is bladder wrack and the alginic acid present in it is a good source of iodine that affects the thyroid function and lipid metabolism (Katamine *et al*., 1985; Zhao *et al*., 2011) while other constituent, fucoidan, which is a type of polysaccharide, significantly lowers blood cholesterol, TG, LDL-C with an increase in HDL-C in hypercholesterolemic mice (Li *et al*., 2008; Huang *et al*., 2010) as well as hyperlipidemic patients without any adverse effects on liver and kidney (Wang and Bi, 1994).

The garlic present in PA is known to reduce total cholesterol moderately without a significant reduction and elevation in LDL-C and HDL-C respectively (Reinhart *et al*., 2009) actually produces this effect by interfering the cholesterol metabolism through the inhibition of HMG-CoA reductase activity.

Grape fruit present in PA can reduce the LDL-C and TG levels (Cerda, 1987; Carper, 1988; Daher *et al*., 2005). One of the grapefruit flavonoids, naringenin is found to affect lipoprotein metabolism resulting in hypolipidemia (Anthony *et al*., 1997) and the other constituent nootkatone, greatly increases the energy metabolism in skeletal muscle and liver cells, as a result diet induced reduction in body weight is achieved through the increased activity of enzyme adenosine monophosphate kinase (Murase *et al*., 2010).

Finally, it may be concluded that herbal drugs might not be free of side effects specially when used in combination form, as the chances of adverse interactions of herbal ingredients increase. Also studies on human beings are insufficient thus herbal drugs should be used under the supervision of health care professionals.

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REFERENCES

Allison, DB., Fontaine, KR., Heshka, S., Mentore, JL. and Heymsfeild, SB. 2001. Alternative treatment for weight loss: A critical review. Food. Sci. Nutr. 41(1):1-28.

Anthony, MS., Clarkson, TB., Bullock, BC. and Wagner, JD.1997. Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. Arterioscler. Thromb. Vasc. Biol. 17:2524-2531.

Armando, C., Maythe, S. and Beatriz,NP. 1998. Antioxidant activity of grapefruit seed extract on vegetable oils. J. Sc. Food Agri. 77:463-467.

Ashraf, R., Aamir, K., Shaikh, AR. and Ahmed, T. 2005. Effects of garlic on dyslipidemia in patients with type-2 diabetes mellitus. J. Ayub Med. Coll. 17(3): 60-64.

Ashraf, R., Khan, RA. and Ashraf, I. 2011. Garlic (*Allium sativum*) supplementation with standard antidiabetic agent provides better diabetic control in type-2 diabetic patients. Pak. J. Pharm. Sci. 24(4):565-570.

Björvell, H. and Rössner, S. 1986. Long term effects of commonly available weight reducing programmes in Sweden. Int. J. Obes. 11:67-71.

Carper, J. 1988. Grapefruit In: The Food Pharmacy. Bantam Books, New York, USA. 213-215.

Cerda, J. 1987. The role of grapefruit pectin in health and disease. Trans. Am. Clin. Climatol. Assoc. 99:203-213.

Chevrel, B. 1980. A comparative crossover study on the treatment of heartburn and epigastric pain: Liquid Gaviscon and a magnesium-aluminum antacid gel. J. Int. Med. Res. 8:300-303.

Cho, KW., Kim, YO., Andrade, JE., Burgess, JR. and Kim, YC. 2011. Dietary naringenin increases hepatic peroxisome proliferators – activated receptor α protein expression and decreases plasma triglyceride and adiposity in rats. Eur. J Nutr. 50 (2):81-88.

Daher, CF., Abou- Khalil, J. and Baroody, GM. 2005. Effect of acute and chronic grapefruit, orange and pineapple juice intake on blood lipid profile in normolipidemic rat. Med. Sci. Monit. 11(12):465-472.

Dembinski, A., Warzecha, Z., Konturek, SJ., Ceranowicz, P., Dembinski, M., Pawlik, WW., Kusnierz-Cabala, B. and Naskalski, JW. 2004. Extract of

Grapefruit-Seed Reduces Acute Pancreatitis Induced by Ischemia/Reperfusion in Rats; Possible Implication of Tissue Antioxidants. J. Physiol. Pharmacol. 55(4):811-821.

Franghanel, G., Cortinas, L., Sanchez, RL. and Berber, A. 2000. A clinical trial of the use of subutramine for the treatment of patint suffering essential obesity. Int. J.Obes. Relat. Metab. Disord. 24(2):144-150.

Girola, M., DeBernardi, M. and Contos, S. 1996. Dose effect in lipid lowering activity of new dietry integrator (Chitosan, Garcinia cambogia extract and Chrome).Toxicol. Ther. 17:25-40.

Gorinstein, S., Leontowicz, H., Leontowicz, M., Drzewiecki, J., Najman, K., Katrich, E., Barasch, D., Yamamoto, K. and Trakhtenberg, S. 2006. Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats. Life Sci. 655-663.

Huang, L., Wen, K., Gao, X. and Liu, Y. 2010. Hypolipidemic effect of fucoidan from Laminaria japonica in hyperlipidemic rats. Pharm. Biol. 48(4):422 - 426.

Katamine, S., Hoshino, N., Totsuka, K. and Suzuki, M. 1985. Effects of the long – term (17 – 19 months) feeding of high – iodine eggs on lipid metabolism and thyroid function in rats. J. Nutr. Sci. Vitaminol. 31 (3):339-353.

Lazarus, DD., Trimble, LA. and Moldawer, LL. 1998. The metabolic effects of pokeweed mitogen in mice. Metab. 47 (1):75-82.

Li, B., Lu, F., Wei, X. and Zhao, R. 2008. Fucoidan: Structure and bioactivity. Molecules. 13(8):1671-1695.

Lien, TF., Yeh, HS. and Su, WT. 2008. Effect of adding extracted hespertin, naringenin and pectin on egg cholesterol, serum traits and antioxidant in laying hens. Arch. Anim. Nutr. 62(1):33-43.

Milner, JA. 1996. Garlic: Its anticarcinogenic and antitumorigenic properties. Nutr. Rev. 54:82-86.

Moghadasian, MH., Manus, BM., Godin, DV., Rodrigues, B. and Frohlich, JJ. 1999. Proatherogenic and antiatherogenic effect of probucol and physterols in apolipoprotein E deficient mice, possible mechanism of action. Circulation. 99:1733-1739.

Murase, T., Misawa, K., Haramizu, S., Minegishi, Y. and Hase, T. 2010. Nootkatone, a characteristic constituent of grapefruit, stmulates energy metabolism and prevents diet – induced obesity by activating AMPK. Am. J Physiol. Endocrinol. Metab. 299:266-275.

Ravikiran, G., Raju, AB. and Venugopal, Y. 2011. Phytolacca Americana: A Review. Int. J. Res. Pharma.Biomed. Sci. 2(3):942-946.

Reinhart, KM., Talati, R., White, CM. and Coleman, CI. 2009. The Impact of garlic on lipid parameters: A systematic rewiew and meta – analysis.Nutr. Res Rev. 22 (1): 39-48.

Scheen, AJ. and Lefébvre, PJ. 1999. Pharmacological treatment of obesity: present status. Int. J. Obes. Relt. Metab. Disord. 23(1):47-53.

Sidhaye, A. and Cheskin, LJ. 2006. Pharmacologic Treatment of Obesity. In: Health and Treatment Strategies in Obesity. Ed. Vaidya, V. Adv. Psychosom. Med. Basel, Karger, Switzerland. 27:42-52.

Smith, IJ. and Goulder, MA. 2001. Randomized placebo controlled trail of long term treatment with sibutramine in mild to moderate obesity. J. Fan. Pract. 50(6):505-512.

Steiner, M., Khan, AH., Holbert, D. and Lin, RI. 1996. A double-blind crossover study in moderately hypercholesterolemic men that compared the effect of aged garlic extract and placebo administration on blood lipids. Am. J. Clin. Nutr. 64:866-870.

Stevinson, C., Pittler, MH. and Ernst, E. 2000. Garlic for treating Hypercholesterolemia. Ann. Intern. Med. 133:420-428.

Teas, J., Braverman, LE., Kurzer, MS., Pino, S., Hurley, TG. and Hebert, JR. 2007. Seaweed and soy: companion foods in Asian cuisine and their effects on thyroid function in American women. J. Med. Food. 10(1):90-100.

Vázquez-Freire, MJ., Lamela, M. and Calleja, JM. 1996. Hypolipidaemic activity of a polysaccharide extract from Fucus vesiculosus L. Phytother. Res. 10:647-650.

von Woedtke, T., Schlüter, B., Pflegel, P., Lindequist, U. and Jülich, WD. 1999. Aspects of the antimicrobial efficacy of grapefruit seed extract and its relation to preservative substances contained. Pharmazie. 54:452-456.

Wang, SZ. and Bi, AF. 1994. Clinical observation of fucoidan on patients with hyperlipidemia. Med. J.Qilu. 173-174.

Willis, WL., Goktepe, I., Isekhuemhen, OS., Reed, M., King, K. and Murray, C. 2008. The effect of mushroom and pokeweed extract on salmonella, egg production and weight loss in molting hens. Poult. Sci. 87(12):2451-2457.

Zhao, SJ., Ye, Y., Tian, EJ. and Chen, ZP. 2011. The impact of dietary iodine intake on lipid metabolism in mice. Biol. Trace Elem. Res. 142(3):581-588.

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