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HYPOCHOLESTEROLEMIC PERSPECTIVES OF GARLIC SOUP CONTAINING BIOACTIVE INGREDIENTS

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ABSTRACT

The present study was an attempt to probe the health claims of locally grown garlic variety/line lehsan gulabi (garlic pink) for the management of serum cholesterol. Functional chicken garlic soups were prepared by adding whole garlic, garlic powder and garlic oil. The treatments and storage exerted non-significant differences in total soluble solids of soups. However, pH and acidity affected substantially with storage. Color tone showed non-significant decline in L* value whilst enhancement in a* and b* values was observed as function of treatments. A non-substantial declining pattern was observed in color tonality. T_1 (soup containing whole garlic) exhibited highest scores for all sensory characteristics. During the efficacy study, provision of garlic based diets i.e. whole garlic (G_1), garlic powder (G_2) and garlic oil (G_3) resulted in reduction in cholesterol, LDL, VLDL, serum triglyceride as compared to control (G_0). The highest decline was observed in G_1. However, HDL level was raised and the highest rise was also observed in G_1. Thus pink garlic in different forms proved beneficial to cope with metabolic threats. Conclusively, the detrimental consequences of altered serum lipid can be ameliorated by the supplementation of garlic preparations specially with whole garlic in the daily diet.

KEY WORDS: Garlic soup, cholesterol, LDL, HDL and triglyceride.

INTRODUCTION

Novel dietary guidelines have established a strong relationship between functional/nutraceutical foods and public health indicating the affirmative role of bioactive

molecules in diet based therapy. During the last few decades, array of scientific evidences have proven the worth of various food bioactive moieties to ameliorate life threatening ailments like hypercholesterolemia, hyperglycemia, obesity and cancer insurgence. Among the diet based interventional strategies, plants derived functional foods with rich phytochemistry are important that not only enhance wellness but also attenuate health risk factors (Tapsell et al., 2006; Shahidi, 2009).

The consumption of traditional plants has progressively been increased because of their effectiveness against various physiological threats with fewer side effects (Venkatesh et al., 2003). Garlic (Allium sativum L.) contains a range of phytochemicals that play an important role in the maintenance of human health and disease prevention (Butt and Sultan, 2009). WHO (1999) recommended the intake of 2-5 g of fresh garlic, 0.3-1.2 g of dried garlic powder, 2-5 mg of garlic oil, 300-1,000 mg of aqueous garlic extract or any other formulation that yields the equivalent of 2-5 mg of allicin per day.

Historical background indicated Central Asia as the origin of garlic that further extended to the other parts of the globe. The composition of garlic varies with geographical location, harvesting time, cultivating conditions etc. It has been observed that garlic contains approximately 65% water, 30% carbohydrates and 5% of other bioactive components mainly sulfur containing compounds (Milner, 2001). Among organosulfur group, cysteine sulfoxides and thiosulfinates are relatively more important (Tapiero et al., 2004). The garlic cloves contain non protein amino acid i.e. alliin, an active precursor of allicin. The alliin is converted to its metabolites allicin, pyruvate and ammonia by the action of enzyme allinase in crushed garlic bulb (Yutani et al., 2011).

During the past few years, garlic has gained immense attention of the nutritionists as functional/ nutraceutical foods to combat against various metabolic dysfunctions (Pedraza-Chaverri et al., 2000; Banerjee et al., 2003). Accordingly, garlic and its formulations like garlic juice, aqueous extract, powder and oil are incorporated in different food products including bread, mayonnaise and pastes (Etoh *et al.*, 2001).

It has been deduced that most of the phytochemicals present in garlic and allied extract are derived from allicin thus helpful to improve human health. In aqueous garlic extract there is abundance of S-allayl cysteine and S-allyl mercepto cysteine, involved in cholesterol lowering (Sterling and Eagling, 2001).

Efficacy trials have depicted garlic as therapeutic agent against hypercholesterolemia, hyperglycemia and atherosclerotic plaque formation (Jabbari *et al.*, 2005). In hypertensive rats, provision of fresh garlic juice/aqueous garlic extract was found effective in lowering blood pressure and garlic cloves @ 0.5-1 g/day showed marked reduction in total cholesterol *i.e.* 7% and LDL cholesterol by 10% in hypercholesterolemic individuals (Yeh and Liu, 2001).

Management of serum cholesterol is a cardinal issue with special reference to cardiovascular disease (CVD). Hypercholesterolemia coupled with oxidized low density lipoproteins and poor dietary habits are the leading causes for the onset of atherosclerosis. Owing to rich phytochemistry with special reference to organosulfur compounds, garlic is considered beneficial for lowering blood pressure, plasma cholesterol level and ultimately in reducing platelet aggregation (Sterling and Eagling, 2001). In meta-analysis randomized controlled trials, effect of garlic ingestion on cholesterol was estimated and observed 9-12% reduction in total cholesterol (Silagy and Neil, 1994). Keeping in view the health claims of garlic, present study was designed to develop functional garlic soup containing bioactive ingredients from various preparations like whole garlic, garlic powder and garlic oil. Finally, the therapeutic role of garlic soup preparations against lifestyle related disorders was determined using rats modeling.

MATERIALS AND METHODS

Procurement of raw material

Garlic variety/line *i.e* lehsan gulabi (garlic pink) was procured from Vegetable Research Section, Ayub Agriculture Research Institute (AARI), Faisalabad. Garlic was cleaned to remove the adhered dirt, dust and other foreign debris. The garlic cloves were peeled. Various analytical and HPLC grade reagents and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). Male Sprague Dawley rats were housed in the Animal Room of NIFSAT for bioevaluation study. Diagnostic kits were purchased from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia) for efficacy trial.

Garlic preparations

The following garlic preparations were made for the development of functional/nutraceutical chicken garlic soup. Whole garlic: The whole garlic bulbs were peeled and crushed. Garlic powder: The bulbs were mechanically peeled in a four-step process *i.e.* heating, cracking,

blowing and fine cutting with high pressure air blow. The dehydrated garlic was ground to powder. Garlic oil: Distilled water was added to the garlic cloves followed by blending. The resultant mixture was steam-distilled for 3 hr using n-hexane as a solvent and subjected to rotary evaporator for oil collection.

Product development

The chicken garlic soup was prepared after conducting some preliminary trials to assess the best suitable formulation. For the preparation of functional/nutraceutical chicken garlic soup, four treatments were prepared. The treatment T_1 contained crushed garlic in the recipe, whilst T_2 and T_3 comprised of garlic powder and garlic oil, respectively. However, chicken soup without garlic *i.e.* T_0 worked as control.

The prepare control treatment (T_0), chopped chicken pieces were fried in corn oil over medium heat till light brown color followed by the addition of finely ground onion. Afterwards, chicken broth containing condiments salt and pepper was poured in the fried material followed by simmering for 25 min on reduced flame. Later, corn starch and vinegar were added to stabilize the recipe. Further, three separate soup preparations were made considering the treatments *i.e.* T_1, T_2 and T_3 by adding whole garlic (2 g), garlic powder (2 g) and garlic oil (1 mL), respectively in the recipe (100 mL/ serving). Each soup sample was served in tureen, garnished with chopped green herbs.

Physicochemical assay of chicken garlic soup

Physicochemical analysis of chicken garlic soups was carried out at 0, 3 and 6 days during storage according to their respective protocols as mentioned herein.

Color

The color of chicken garlic soups was estimated through CIE-Lab Color Meter (CIELAB SPACE, Color Tech-PCM, USA). Soup (5 mL) was taken and color values like *a** (–a greenness; +a redness), *b** (–b blueness; +b yellowness) and *L** (lightness) were recorded. The data obtained was used to compute chroma (C*) and hue angle following the method of Duangmal *et al.* (2008).

$$\text{Chroma } (C^*) = [(a^*)^2 + (b^*)^2]^{1/2} \quad \text{..... (v)}$$

$$\text{Hue angle } (h) = \tan^{-1} (b^*/a^*) \quad \text{..... (vi)}$$

Total soluble solids

Total soluble solids of chicken garlic soups were estimated by Hand Refractometer (TAMCO, Model No. 90021, Japan) and interpreted as percent soluble solids (°Brix).

Acidity and pH

Acidity of garlic soup was determined by adopting the guidelines of AOAC (2006). Soup was taken in 50 mL beaker and pH was recorded by pH meter (Ino Lab 720, Germany) following the method of AOAC (2006).

Sensory evaluation

Sensory evaluation of chicken garlic soup was carried out by a trained taste panel, employing 9-point hedonic scale following the guidelines of Meilgaard *et al.* (2007). On evaluation day, warm chicken garlic soup samples were served in respective tureens with random codes. Unsalted crackers and mineral water were provided for neutralizing and rinsing the taste receptors for rational assessment.

Bioevaluation studies

Therapeutic potential of the nutraceutical constituents of garlic were probed against various lifestyles linked maladies. Garlic preparations were probed for their hypocholesterolemic perspectives in 8 week model feeding trial. Accordingly, 60 male Sprague Dawley rats were housed in the Animal Room of NIFSAT, University of Agriculture, Faisalabad. Initially, the rats were acclimatized by feeding basal diet for one week period. The environmental conditions were maintained *i.e.* temperature 23±2ºC and relative humidity 55±5% with 12 hr light-dark period. At the commencement of trial, some rats were sacrificed to get the baseline values for the selected traits. At the mid of study (28th day) half of the overnight fasted rats in each group were scarified, while remaining decapitated at the termination of trial (56th day) and blood samples were collected in EDTA coated tubes. Serum was separated after centrifuging the blood through centrifuge machine (Centrifugal Machine, China) for 6 min @ 4000 rpm. The collected sera samples were kept for biochemical evaluation through Microlab 300 (Merck, Germany). Various biochemical traits like total cholesterol, LDL, HDL & triglycerides were assessed using respective commercial kits. The whole efficacy trial was repeated for results verification regarding the impact of garlic preparations against the selected biochemical traits.

Feed plans for experimental rats

During the efficacy study, rats were divided in to four homogenous groups (G_0, G_1, G_2 and G_3) with ten rats in each. For control group (G_0), experimental diet was prepared by using corn oil (10%), corn starch (66%), protein (10%), cellulose (10%), mineral (3%) and vitamin mixture (1%). Whereas, for G_1, G_2 and G_3 whole garlic (250 mg/kg body weight), garlic powder (250 mg/kg body weight) and garlic oil (100 mLs/kg body weight) were added, respectively in the aforementioned diet formulation.

Feed and water intake

The gross feed intake of each group was calculated every day, excluding the spilled diet throughout the study period. The net water intake was also recorded on daily basis by measuring the difference in graduated bottles.

Statistical Analysis

The results were inferred statistically to establish a conclusive approach. The data were obtained by applying completely randomized design (CRD) and further subjected to statistical analysis using Statistical Package (Costat-2003, Co-Hort, v 6.1). Level of significance was determined (ANOVA) using 2-factor factorial CRD where applicable following the principles outlined by Steel *et al.* (1997).

RESULTS AND DISCUSSION

Analysis of chicken garlic soup

The effect of treatments on pH and acidity was found to be non-momentous (Table 1). Moreover, storage affected significantly for pH and acidity, and non-significantly for total soluble solids (TSS) (Table 2). Maximum pH was found for T_1 (chicken garlic soup with garlic extract) *i.e.* 5.39±0.08. Moreover, pH value decreased with the passage of time. A progressive increase in acidity was observed in all garlic soups, higher acidity value *i.e.* 0.31±0.04 was estimated in T_1. Maximum TSS 26.4±0.46 was observed in T_1. However, minimum pH, acidity and TSS was noted in T_0.

The instant findings are in accordance with the research work of Ahmed and Shivhare (2001) for pH, acidity and TSS for chicken garlic soup. They recorded pH and acidity of garlic paste 4.10 and 0.35%, respectively, whilst total solids, TSS and water activity 27%, 33 °brix and 0.86%, respectively. Sallam *et al.* (2004) observed that initial pH value ranged from 6.65 (in control samples) to 6.78 (in fresh samples) for chicken garlic sausages. In all sausage

formulations, storage had a significant effect on pH values that increased with the passage of time. The result regarding acidity and pH are comparable with the study of Ahmed *et al.* (2001), narrated variations in pH from 2 to 10 and titratable acidity 0.35% of garlic puree. The dependency of alliinase activity on pH is indicated when allicin and other thiosulfinates are released during incubation of garlic powder in buffer solutions adjusted from pH 2 to 10 (Lawson *et al.*, 1992). They recorded 33% TSS, 9.6% NaCl and 0.35% titratable acidity while 0.86 and 4.10 water activity and pH, respectively. The result of Jung *et al.* (2010) are also correlated to instant investigations for vegetable soups containing garlic with pH 3.85±0.03, titratable acidity 0.64±0.03, TSS 4.48±0.17◦Brix and total solids 6.87±0.25 g/100g.

Color

Color is one of the desirable attributes for any product to be accepted by the consumer. Color tonality includes L*, a* and b* where L* value represents lightness, b* gives indication for yellowness whilst a* shows greenness and redness. The color of chicken garlic soup indicated non-momentous effect of treatments, storage and their interaction on L* and b* except for a* value that showed significant differences with storage (Table 1 and 2). The storage resulted non-momentous decline in L* value from 69.05±0.14 to 65.87±0.06. The a* value significantly reduced from -3.89±0.14 at 0 day to -3.16±0.06 at 6th day. It is evident that b* values increased non-significantly as function of different garlic forms.

Ahmed *et al.* (2001) had reported L*, a* and b* values of garlic puree by 69.37, -3.25 and 15.95, respectively. They observed that when garlic puree was subjected to thermal processing; a* value shifted towards more greenish with non-substantial change in L* and b* values. Ahmed (2004) monitored the color changes in garlic paste and noticed enhancement in a*and b* values with a decline in L*. The recorded values for a*, b* and L* were 59.93, 2.01 and 22.95, respectively. The effect of thermal treatment on garlic color kinetics was evident from the work of Ahmed and Shivhare (2001), observed degreening of garlic paste during storage at 25^{o}C. They were in the view that heat treatment initiated the enzymatic and chemical reaction that resulted shift in a*, b* and L* values.

The work of Ahmed *et al.* (2001) elucidated that amino acid S-(l-propenyl) cysteine sulfoxide is one of the possible causes for the development of green color. The current results regarding color values are also supported by Constenla and Lozano (2005), reported L* and b* value of garlic puree as 66 and 25, respectively. Jeong *et al.* (2008) examined the wet noodles after

supplementation of garlic and observed a non-substantial decrease in L* and increase in a* and b* values as function of garlic dose. In a similar study, the addition of garlic in cheese showed a non-significant rise in a* and b* values with a decline in L* (Tarakci *et al.*, 2011). In current investigation, addition of garlic did not impart any undesirable change in the color attributes of the resultant soup. Thus garlic can successfully be used for the development of functional soup without causing any physical and structural damage.

Sensory evaluation

Sensory profiling of chicken garlic soup showed significant differences due to treatments on color and overall acceptability. However, storage depicted momentous impact on all quality attributes except texture. T_1 showed highest rating for all sensory parameters (Table 3). Storage depicted substantial decline from 0 to 6^{th} day for all sensory characteristics (Table 4).

The current findings are strengthened by the results of Jung *et al.* (2010) regarding sensory evaluation of chicken garlic soup. The combination of garlic and meat showed synergistic effect for the acceptance and overall scoring of the soup. Similarly Michon *et al.* (2010) estimated the consumer acceptance for newly developed vegetable soup. Recently, functional soup with cholesterol lowering capacity and less salt in recipe was assessed with healthy choice logo. The results showed higher acceptance of soup with healthy logo as compared to control however, there was not any perceivable difference in sensory attributes between the healthy and control samples (Liem *et al.* 2012). In current case, chicken garlic soup showed reasonably close results as that of control additionally, chicken garlic soup has health enhancing properties.

Earlier, Tarakci *et al.* (2011) indicated that using garlic in herby brined cheese enhanced the sensory profile without any evident difference in texture and appearance. Gundogdu *et al.* (2009) illuminated the sensory parameters of set and stirred yogurt with 0.5 and 1% of garlic during 28 days storage. The incorporation of garlic in yogurt showed appealing results for flavor with better consistency. The overall acceptability for garlic yogurt was also increased owing to its safe consumption and nutritional profile.

Conclusively, the addition of garlic in chicken soup was found appropriate during storage and sensory assessment without any undesirable effect on the quality. All samples attained satisfactory scores thereby confirmed that garlic preparations are suitable for the development of therapeutic product.

Bioevaluation studies

Biological evaluation was carried out through rodent modeling to explore the functional/nutraceutical worth of garlic preparations with special reference to serum lipids. The Sprague Dawley rats were used rather than human for *in vivo* assessment due to convenient management, controlled diet, close observation and similar environmental conditions.

Feed intake

Statistical results elucidated that treatments imparted non-substantial effect on feed intake. However, this trait affected significantly with time intervals (weeks) in both trials. Means (Figure 1) showed that feed intake (g/rat/day) in G_0, G_1, G_2 and G_3 groups increased substantially in trial I & II. The non-substantial effect of treatments on feed intake is in harmony with the findings of Ali *et al.* (2000), they observed that garlic provision did not impart any significant differences in the diet intake of the rats. Likewise, the research outcomes of Itoh and Furuichi (2009) attributed non-significant variations in the feed consumption after ingesting bioactive moieties. Further, Kang *et al.* (2008) assessed non-significant variations for feed efficiency in rats fed on fresh garlic powder, steamed garlic powder and black garlic powder for four weeks. Conclusively, the intake of garlic based diets did not show noticeable differences thus suitable for consumption.

Drink intake

The drink intake showed non-significant effect of treatments however, weeks imparted significant differences on this trait in both trials. The drink intake (mL/rat/day) for G_0, G_1, G_2 and G_3 groups increased substantially in trial I & II (Figure 2). The results of instant exploration for drink consumption with concomitant intake of garlic based functional diets are in accordance with the work of Kang *et al.* (2008) that garlic and its preparation did not impart any significant change in the drink intake. Previously, Kempaiah and Srinivasan (2004) probed the influence of culinary spices on the feed and drink consumption of experimental rats and recorded non-significant impact of garlic on both parameters. Likewise, Aouadi *et al.* (2000) demonstrated non-significant variations in water consumption among all garlic fed groups.

Cholesterol

Cholesterol revealed significant differences due to treatments and study intervals however, their interaction showed non-significant variations during trial I & II. The maximum

cholesterol level for control G_0 (78.37±4.71 mg/dL) reduced in G_1 (74.83±4.47 mg/dL) groups in trial I (Table 5). During the study (0, 28th & 56th day) highest decline from 79.43±5.53 to 74.73±4.20 & 70.34±4.08 mg/dL was noticed for whole garlic (G_1) (Table 6). Figure 3 expounded substantial declining trend for serum cholesterol level of rats after provision of therapeutic diets containing whole garlic, garlic powder and garlic oil. Trial I presented a diminishing tendency 11.05% in G_1.

Berthelod *et al.* (1998) explicated the role of garlic in the cholesterol management by exploring the preventive route of action. They designed double-blind placebo study and administrated garlic @ 5 g/day to 25 moderately hypercholesterolemic patients for 12 weeks. They found an inverse association of serum cholesterol and amount of garlic consumed thus designated garlic as a therapeutic herb against cholesterol synthesis. Mukherjee *et al.* (2009) observed that garlic preparations provided cholesterol lowering and cardioprotective cover in rats however, freshly crushed garlic showed better performance than processed garlic.

Agarwal (1996) observed significant reduction in serum cholesterol level after consuming raw garlic for 2 to 3 months in healthy subjects and patients with ischemic heart disease. Kwon *et al.* (2003) noticed a decline in liver cholesterol level 9.89% by garlic consumption. Chetty *et al.* (2003) suggested that garlic contributes cholesterol synthesis inhibition owing to the presence of high levels of tellurium and selenium. The provision of garlic proved protective in lowering the total cholesterol and managed LDL to HDL ratio due to the presence of S-allyl cysteine and diallyl disulfide.

Yeh and Liu (2001) noticed effective role of garlic in hypercholesterolemic human subjects by 7% reduction in serum cholesterol. They observed 44 to 87% inhibition in cholesterol synthesis by the provision of fresh garlic in cultured rat hepatocytes. The significant reduction in cholesterol synthesis is due to the action of garlic derived bioactive components on the activities of cholesterogenic and lipogenic enzymes such as fatty acid synthase, malic enzyme, 3-hydroxy-3methyl-glutary CoA (HMG CoA) reductase and glucose-6-phosphate dehydrogenase (Ologhobo *et al.*, 2008).

The findings of Durak *et al.* (2004) related to the lipid lowering potential of garlic validate the present data set. They reported 6.97±1.56 and 5.23±1.40 mmol/L reduction in total cholesterol level before and after garlic consumption, respectively with 24.96% gross decline. Further, in a clinical trial garlic was compared with commercially available cholesterol

lowering drug (bezafibrate) and found equally effective in decreasing plasma lipid to a statistically significant extent (Siegel and Klussendorf, 2002). Another study by Rahman (2001) expressed that consumption of 3 g fresh garlic per day for 16 weeks imparted 21% reduction in serum cholesterol level.

Cholesterol biosynthesis inhibition by garlic is associated with elevated catecholamine level through increased fat catabolism. Alongside, consumption of allyl groups, allicin and diallyl present in crushed raw garlic has been reported to increase hepatic fatty acid oxidation (Zhang *et al.*, 2001). In the nutshell, garlic preparations especially whole garlic proved beneficial in managing the serum cholesterol level thereby has potential to address lipids related abnormalities.

Low density lipoprotein (LDL)

Serum LDL was significantly affected by treatments and study intervals (Table 5 and 6). In trial I, maximum LDL reduction was noticed in G_1 with 17.21% decline. The trial II behaved alike (Fig 4).

Garlic has been reported beneficial in lowering the plasma lipoproteins and postprandial lipemia (Superko *et al.*, 2000). Garlic derived organosulfur compounds *i.e.* diallyl sulphide (DAS), diallyl disulphide (DADS), S-ethylcysteine and N-acetylcysteine (NAC) are protective against human LDL oxidation and glycation (Yin *et al.*, 2002), and reduce LDL (Ou *et al.* 2003). Antioxidation properties of garlic exerted inhibition of LDL peroxidation by organosulfur components and Cu^{+2} induced oxidative modifications (Durak *et al.*, 2004). In an animal modeling, hypercholesterolemic rats fed on raw and frozen garlic, showed a decrease in serum LDL cholesterol with relative greater impact due to raw garlic. Likewise, Lau (2006) studied *in vitro* modeling and observed concentration dependent inhibition of LDL oxidation with garlic derived bioactive compounds. Besides, numerous studies have demonstrated that garlic significantly reduces serum lipids especially cholesterol and low density lipoprotein (LDL) in humans and reduces blood pressure. In a single-blind, placebo controlled study, 150 hyperlipidemic subjects were evaluated for lipid lowering capacity of garlic. They were administrated with enteric-coated garlic powder tablet (equal to 400 mg garlic, 1 mg allicin), anethum tablet (650 mg) and placebo tablet twice a day. The garlic group showed reduction in total cholesterol by 12.1% and LDL 17.3% (Kojuri *et al.*, 2007). The instant findings are in accordance with the observations of Adler and Holub (1997) with 11.5% reduction in total cholesterol and 14.2% in LDL. Other scientists noticed decline in

cholesterol and LDL by 9 and 15%, respectively (Tohidi and Rahbani 2000), 16.1 and 24.1% (Jabbari *et al.* 2005). The lipid profile modulation by garlic in combination with fish oil has also been proved effective through a single-blind placebo controlled crossover study with 40 participants. A decrease in cholesterol, LDL and triglycerides were noticed by 11, 10 and 34%, respectively (Rahman, 2001).

The LDL lowering ability of garlic is due to its effect on hydroxymethylglutaryl-CoA reductase inhibition through active component allicin. The ruling mechanism behind its activity is the reaction between alliin and alliinase that occurs in garlic aqueous solutions and garlic powder. It is documented that active alliin-alliinase system only exists in fresh garlic. The amino acid L-alliin and the enzyme alliinase are considered as the markers for fresh and powdered garlic preparations. In fresh garlic, they are present in isolated segments thus no enzymatic conversion has been observed (Ashraf *et al.*, 2005). The current study suggested that garlic preparations especially whole garlic is a therapeutic food for the management of lipid metabolism with a significant reduction in LDL.

High density lipoprotein (HDL)

The treatments and study duration imparted significant impact on HDL whilst their interaction exhibited non-momentous differences. Maximum HDL 39.73±2.54 mg/dL was recorded in G_1 (diet containing whole garlic) during trial I (Table 5). Similar results were observed in trial II. Diets containing garlic preparations raised HDL level as compared to control (Table 5 and 6).

The present results are in accordance with the findings of Kwon *et al.* (2003). They provided garlic to rabbits and assessed HDL level at 6^{th} and 12^{th} day with 13.89 and 26.96% rise, respectively. Kannar *et al.* (2001) investigated the hypocholesterolemic effect of an enteric coated garlic supplement in 46 hypercholesterolemic human subjects with an amount of garlic capable to provide 9.60 mg of allicin. After three months, significant reduction in total cholesterol and LDL was observed with an evident rise in HDL *i.e.* 9.10%. The study demonstrated that the garlic supplements have potential to reduce hypercholesterolemia from mild to moderately affected patients. It is also reported that garlic consumption for four months by hypercholesterolemic patients with arterial hypertension had high HDL with decreased cholesterol level (Durak *et al.*, 2004). It has been observed that 11 weeks garlic phytotherapy significantly enhanced the HDL level of human subjects by 13% whilst, cholesterol and LDL decreased. Furthermore, powdered garlic showed 13% rise in HDL

cholesterol (Kerckhoffs *et al.*, 2002). Later, Afkhami-Ardekani *et al.* (2006) evaluated the effect of garlic in hyperlipidemic type 2 diabetic patients. Accordingly, 45 subjects were administrated garlic tablets containing 300 mg garlic extract, which showed a marked rise in HDL level with a decline in LDL and total cholesterol.

Very low density lipoprotein (VLDL)

The statistical analysis elucidated significant effect of treatments on VLDL, whilst time intervals and their interaction behaved non-differentially. The VLDL contents (Table 5) varied from 7.09±0.59 to 5.37±0.48 mg/dL and 7.13±0.69 to 6.13±0.77 mg/dL for trial I, respectively.

The instant findings are in accordance with the outcomes of Kwon *et al.* (2003). They studied the effect of garlic on VLDL in hypercholesterolemic subjects and recorded 43.05 and 75.84 % reduction at the 6^{th} and 12^{th} day of the trial. Kang *et al.* (2008) fed garlic to hyperlipidemic rats and observed an evident decline in VLDL, LDL, triglycerides and total cholesterol accompanied with a rise in HDL. Durak *et al.* (2004) provided garlic supplements to 23 volunteers with high blood cholesterol for 4 months and found a significant reduction in VLDL and triglycerides. VLDL served as a precursor of LDL cholesterol in blood circulatory system whilst dietary cholesterol particularly saturated fats cause plasma cholesterol concentration to be elevated by down regulating LDL receptor synthesis (Yeh and Liu, 2001). During cholesterol transportation between serum and tissues, the peripheral cells obtained cholesterol through native synthesis as well as uptake from VLDL and LDL. The serum cholesteryl ester transfer protein (CETP) participated as a key element in the reverse cholesterol transport system, moving cholesterol to liver from peripheral tissues. Alongside, it mediates the exchange of cholesteryl ester in HDL (Durak *et al.*, 2004; Seidel and Stocks, 2008).

Sadiya and Chaturvedi (2011) evaluated supplementation of garlic in type 2 diabatic patients. The results expressed a significant decline in VLDL (20.4%), LDL (6.7%) and rise in HDL (30%). It is suggested that supplementation of garlic is beneficial in the management of hypercholesterolemia in diabetic patients that delays the onset of cardiovascular complications. Lipid lowering potential of garlic was also explicated by Choudhary *et al.* (2011) in hyperlipidemic guinea pigs. They observed a momentous decline in VLDL, LDL and cholesterol with a significant rise in HDL as compared to control. Similar VLDL

lowering results after garlic consumption was narrated by Heidarian *et al.* (2011) with 30.51% reduction.

Triglycerides (TG)

Treatment and study intervals exerted significant variations on triglycerides whereas, their interaction exhibited non-significant differences. The recorded triglycerides values (trial I) in G_0, G_1, G_2 and G_3 groups were 68.52±4.65, 61.17±4.31, 65.96±4.22 and 67.95±4.98 mg/dL, respectively (Table 5). Hypertriglyceridemia is a risk factor associated with various heart complications hence reduction of triglyceride (TG) may attenuate the progression of cholesterol dependent diseases. The effect of garlic on lipid metabolism owing to the inhibitory effect of organosulfur compounds in cultured rat hepatocytes indicated a decline of TG in a dose dependent manner. It was deduced that garlic causes fatty acid impairment that in turn inhibits synthesis of TG which suppressed VLDL in liver, thus reduced TG in plasma (Liu and Yeh, 2001). In present investigation, garlic provision reduced the synthesis of triglyceride in rats (Table 6). Likewise, Kwon *et al.*, (2003) observed 23.26 and 33.81% reduction in serum TG levels during 1st and 2nd week of study in hypercholesterolemic subjects. According to them sulfur containing cysteine derivatives of garlic inhibit TG synthesis. Over production of TG rich VLDL in liver is known to induce hypertriglyceridemia however, consumption of organosulfur compounds of garlic have proven hypotriglyceridemic to cope with the menace. Ali *et al.* (2000) revealed significant diminishing tendency in the TG of hypercholesterolemic rats fed on fresh garlic extract. The instant findings are also supported by the work of Shin and Kim (2004) for the assuaging role of garlic against hyperlipidemia. According to them, garlic proved effective in reducing plasma triglycerides, total lipids and total cholesterol whilst raised HDL level significantly.

Table 1. Effect of treatments on pH, acidity, TSS and color tonality of chicken garlic soup.

Parameters	T_0	T_1	T_2	T_3
pH	5.33±0.06	5.39±0.08	5.35±0.04	5.34±0.06
Acidity	0.29±0.06	0.31±0.04	0.30±0.06	0.30±0.08
TSS	25.66±0.56	26.4±0.46	25.12±0.46	26.33±0.49
L* value	67.25±0.72	66.22±0.79	67.10±0.81	67.39±0.84
a* value	-3.49±0.84	-3.51±0.79	-3.52±0.81	-3.58±0.72
b* value	12.65±0.79	13.06±0.72	13.64±0.81	14.01±0.84

T_0 = Control (soup without garlic), T_1 = Soup containing whole garlic,

T_2 = Soup containing garlic powder, T_3 = Soup containing garlic oil

Table 2. Effect of storage on pH, acidity, TSS and color tonality of chicken garlic soup.

Storage (Days)	pH	Acidity	TSS	L* value	a* value	b* value
0	5.74±0.14[a]	0.28±0.14[b]	27.51±0.12	69.05±0.14	-3.89±0.14[a]	14.29±0.14
3	5.38±0.09[a]	0.30±0.09[a]	25.73±0.15	67.06±0.09	-3.52±0.09[a]	13.48±0.09
6	4.94±0.06[b]	0.32±0.06[a]	24.62±0.17	65.87±0.06	-3.16±0.06[b]	12.56±0.06

Table 3. Effect of treatments on sensory attributes of chicken garlic soup.

Treatments	Color	Flavor	Taste	Texture	Overall acceptability
T_0	6.95±0.58ab	6.83±0.59	6.81±0.41	6.87±0.51	6.73±0.64b
T_1	7.09±0.52a	6.99±0.51	7.22±0.37	7.31±0.60	7.20±0.42a
T_2	7.02±0.61b	6.91±0.42	7.04±0.29	6.10 ±0.43	7.02±0.49a
T_3	7.00±0.49b	6.85±0.49	7.05±0.41	6.66±0.47	7.12±0.51a

T_0 = Control (soup without garlic), T_1 = Soup containing whole garlic,

T_2 = Soup containing garlic powder, T_3 = Soup containing garlic oil

Table 4. Effect of storage on sensory attributes of chicken garlic soup.

Parameters	0 day	3 days	6 days
Color	7.53±0.51[a]	7.05±0.41[b]	6.47±0.49[b]
Flavor	7.29±0.59[a]	6.91±0.20[b]	6.47±0.52[b]
Taste	7.47±0.39[a]	7.13±0.41[a]	6.49±0.32[b]
Texture	7.15±0.67	6.90±0.51	6.15±0.49
Overall acceptability	7.45±0.69[a]	6.99±0.51[b]	6.61±0.49[b]

Table 5. Effect of study intervals on cholesterol, LDL, HDL, VLDL and triglyceride of rats.

Parameters	Trials	Storage (days)		
		0 day	28th day	56th day
Cholesterol (mg/dL)	Trial I	78.52±5.72[a]	76.51±5.68[b]	74.34±5.84[b]
	Trial II	78.34±4.79[a]	75.51±4.65[b]	73.97±4.21[c]
Low density lipoprotein (LDL) (mg/dL)	Trial I	28.74±2.13[a]	26.84±1.93[b]	24.83±1.75[b]
	Trial II	30.35±1.99[a]	28.12± 1.77[b]	25.70± 1.69[c]
High density lipoprotein (HDL) (mg/dL)	Trial I	35.80±2.48[b]	36.41±3.07[b]	37.94±2.86[a]
	Trial II	33.39±2.05[b]	35.43± 2.02[b]	36.34± 2.12[a]
Very low density lipoprotein (VLDL)	Trial I	5.98±0.49	6.11±0.42	5.73±0.54
	Trial II	6.56±0.65	6.68±0.63	6.35±0.66
Plasma Triglyceride (mg/dL)	Trial I	68.03±4.08[a]	65.32±3.92[b]	65.14±4.54[b]
	Trial II	67.92±3.51[a]	65.69±3.22[b]	64.28± 3.82[b]

Table 6. Effect of diets on cholesterol, LDL, HDL, VLDL and triglyceride of rats.

Parameters	Trials	Diet Groups			
		G_0	G_1	G_2	G_3
Cholesterol (mg/dL)	Trial I	78.37±4.71[a]	74.83±4.47[b]	76.19±5.36[b]	76.43±5.12[b]
	Trial II	76.23± 4.51[a]	74.57±4.25[b]	75.40± 4.28[a]	76.18±4.32[a]
Low density lipoprotein (LDL) (mg/dL)	Trial I	28.60±1.83[a]	25.11±1.85[c]	26.64±2.17[b]	26.81±2.08[b]
	Trial II	30.77±1.72[a]	26.18±1.70[b]	26.91±1.72[b]	28.49±1.77[ab]

High density lipoprotein (HDL) (mg/dL)	Trial I	35.36±2.37c	39.73±2.54a	36.82±3.16b	36.36±2.96b
	Trial II	33.25±2.11c	37.22±2.11a	35.96±2.02ab	34.45±2.10b
Very low density lipoprotein (VLDL)	Trial I	7.09±0.59[a]	5.37±0.48[c]	6.52±0.55[b]	6.88±0.44[b]
	Trial II	7.13±0.69[a]	6.13±0.77[c]	6.16±0.65[c]	6.69±0.75[b]
Plasma Triglyceride (mg/dL)	Trial I	68.52±4.65[a]	61.17±4.31[c]	65.96±4.22[b]	67.95±4.98[a]
	Trial II	68.61± 4.25[a]	63.42±4.13[c]	64.27±3.89[c]	67.55±3.98[b]

G_0 = Control (Diet without garlic), G_1 = Diet containing whole garlic, G_2 = Diet containing garlic powder, G_3 = Diet containing garlic oil

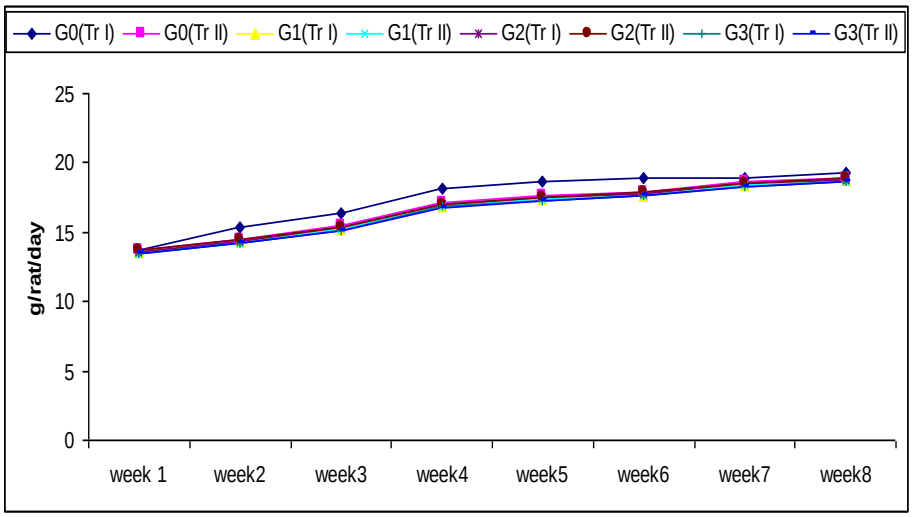

Figure 1. Feed intake of rats (g/rat/day).

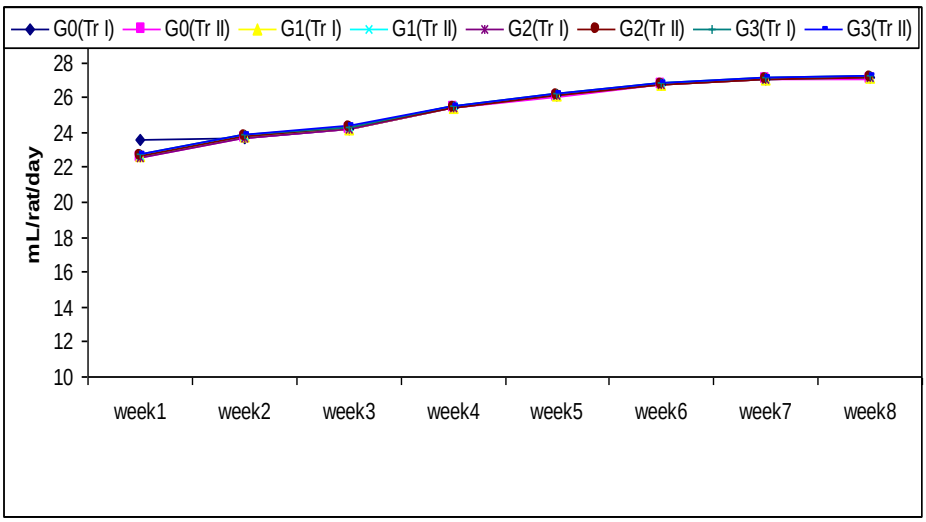

Figure 2. Water intake of rats (mL/rat/day).

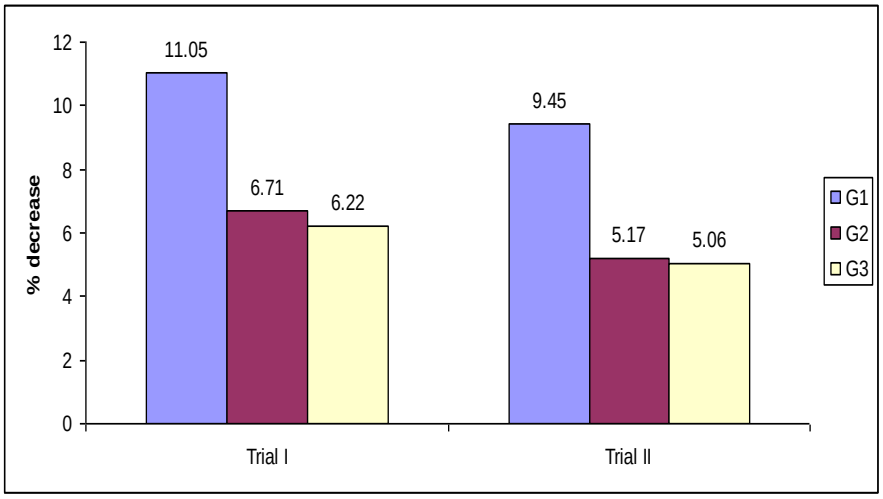

Figure 3. Percent decrease in cholesterol level.

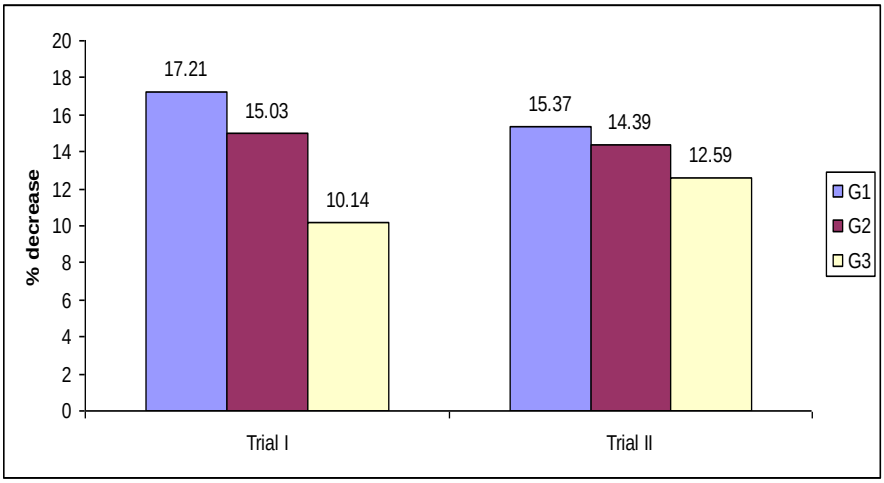

Figure 4. Percent decrease in LDL level.

CONCLUSION

The addition of garlic in chicken soup was found appropriate and without any undesirable effect on the quality and confirmed that garlic soup is suitable as therapeutic product. The garlic has tendency to lower the total cholesterol, LDL, VLDL and TG significantly with a rise in HDL thus attenuate the adverse health events. In the nutshell, garlic based diets have potential to curtail different physiological malfunctioning thus should be encouraged in diet based therapy against various metabolic syndromes in the developing economies.

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