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Aqueous garlic extract attenuates hypercholesterolemic and hyperglycemic perspectives; rabbit experimental modeling

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Functional and nutraceutical foods are gaining popularity worldwide due to the consumer trend towards natural cure by means of dietary modifications. In 20th Century, nutritionists of the Western world focused mainly on the identification, isolation and purification of nutrients essential for human growth and development. Garlic (Allium sativum), is one of the most essential vegetables providing health benefits due to array of bioactive compounds. These bioactive moieties are responsible for curing various lifestyle related disorders like hyperlipidemia, diabetes, obesity and cancer insurgence. In this context, aqueous garlic extraction was carried out followed by in vivo modeling. Efficacy study was conducted by providing aqueous garlic extract along with basal diet on rabbits for a period of 28 days. Accordingly, four groups were made providing different concentrations of aqueous garlic extract (control, 3, 6 and 9 mL/kg body weight). Functional garlic extract containing water soluble active compounds resulted in significant reduction in total cholesterol and LDL level indicating their effectiveness against hypercholesterolemic perspectives and allied discrepancies. Likewise, serum glucose level was also substantially reduced by aqueous garlic extract. From the present investigation, it was deduced that different concentrations of aqueous garlic extract were effective against hypercholesterolemia and hyperglycemia.

Key words: Functional food, nutraceutical foods, aqueous garlic extract, hypercholesterolemia, hyperglycemic perspectives.

INTRODUCTION

Functional foods are significant due to easy accessibility, low price and allied health promoting perspectives. In this context, various fruits and vegetables, cereals, nuts and pulses are important owing to array of phytochemicals. Phytochemicals are the bioactive molecules in plant foods that are mainly concerned with modulation of various metabolic pathways, like free radical scavenging, antimicrobial properties and by providing protection against difficult diseases. Vegetables are rich source of phytochemicals, such as carotenoids, chlorophylls, anthocyanins, and flavonoids (Andersen and Jordheim, 2006).
Among the functional foods, use of garlic is increasing daily because of its health promoting potential besides basic nutrition. Health promoting aspects of garlic are mainly accredited to its sulfur containing compounds, mainly allicin and S-allyl cysteine. Among organosulfur compounds, thiosulfinate, ajoenes and allicin (diallyl thiosulphate) are the chief bioactive components in garlic (Tapiero et al., 2004). Scientific investigations have depicted that it contains 65% water, 30% carbohydrates, along with 5% of other bioactive components mainly sulfur containing compounds (Milner, 2001).

Allicin (diallylthiosulfinate) and S-allyl cysteine are the main thiosulfonates out of which about 60-80% is allicin (Lawson et al., 1992). Considering the importance, majority of the garlic supplements are sold today in the form of garlic tablets standardized on amount of allicin concentration (Ali et al., 2000). The garlic clove contains non-protein amino acid alliin, active precursor of allicin but has no allicin contents. Alliin is converted to its metabolites; allicin, pyruvate and ammonia, by the action of enzyme allinase on crushed garlic bulb (Rabinkov et al., 1994). In garlic aqueous extract, there is abundance of S-allyl cysteine and S-allyl mercapto cysteine involved in lowering cholesterol (Sterling and Eagling, 2001).

Oxidation of cholesterol fractions, especially of low-density lipoproteins (LDL), is playing a cardinal role in atherosclerosis development (Liu et al., 1992). Lipid peroxidation by toxic radicals increases the concentration of free radicals and causes certain diseases including atherosclerosis in humans (Halliwell and Chirico, 1993). Management of plasma cholesterol level is a keystone element to protect cardiovascular disease (CVD) disorders (Mezzetti et al., 1992). Garlic being a traditional medicine is used to improve the lipid profile (Kleijnen et al., 1989); garlic and its different supplementation hold remarkable effect on cholesterol level, LDL-cholesterol, and HDL-cholesterol. Consumption of garlic and garlic preparations are very useful in regulating plasma lipid levels (Lau, 2006), plasma anticoagulant activity (Pierre et al., 2005; Lawson et al., 1992) and also contributed toward the prevention of the atherosclerosis (Rahman and Lowe, 2006).

Meta-analysis conducted by Warshafsky et al. (1993) depicted that about 9% reduction in total cholesterol was due to the consumption of garlic at 1.5-3.0 g per day. Reinhart et al. (2009) in their study estimated and calculated the effect of garlic on lipid profile and revealed that, it significantly reduced total cholesterol and triglycerides level. Aqueous garlic extracts has the ability to decrease cholesterol synthesis upto 75% without cellular toxicity mediated by sterol 4-alpha-methyl oxidase. It is also effective in reducing coronary calcium progression (Yeh and Liu, 2001; Lewin and Popov, 1994).

Diabetes is a metabolic syndrome that affects different physiological systems of the human body. It is one of the leading causes of mortality worldwide, if uncontrolled, can threaten multi-organ systems (Zakir et al., 2008). Uncontrolled blood glucose is believed to be the cardinal feature in the onset of diabetic mellitus of both Type 1 and Type 2 (American Association of Diabetes Educators, 2002). Most common type is Type 2 category, while Type-1 diabetes develops mostly in early childhood. Kidney plays a significant role and becomes the target organ for investigation of diabetic complications. Many other factors involved in the development of diabetic nephropathy are same as in common diabetic complications like retinopathy and microvascular diseases.

There is an estimate that in 2030, 376 million people in the world will be affected with diabetes (Wild et al., 2004). According to Nogichi (2007) 33% of all the diabetic patients take various medications amongst them garlic and garlic products was one of the most frequent remedy. Moreover, the effect of garlic oil on diabetic animals was studied by Anwar and Meki (2003), in order to verify the hypoglycemic perspectives of garlic. Lately, garlic juice was observed to reverse hyperglycemia in diabetic rats and to modulate oxidative stress (El-Demerdash et al., 2005). In addition to garlic extract and garlic oil, allyl sulfide, S-allyl-cysteine sulfoxide (alliin), were also verified to have the same hypoglycemic results in different experimental modeling (Sheela and Augusti, 1992).

In conclusion, different garlic products have become popular in the market owing to number of pharmaceutical preparations (Velisek et al., 1997). World Health Organization (1999) recommended 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 equal to the dose of 2-5 mg of allicin per day. Conclusively, the present research project was designed to explore the hypcholesterolemic and hypoglycemic perspectives of aqueous garlic extract using rabbit experimental modeling.

MATERIALS AND METHODS

Preparation of aqueous garlic extract

The weighted peeled garlic bulbs were meshed to obtain a fine garlic juice. Afterwards, it was homogenized in 100 ml of 0.9% cold and sterile saline solution in a blender at high speed for 15 min. Filtration of homogenized mixture was carried out with muslin cloth. Resultant aqueous extract of garlic was stored at −20°C. Garlic extract of different concentrations were prepared with 0.9% saline solution for further analysis.

Efficacy studies

In rabbit experimental modeling, twenty four New Zealand red eyes male rabbits were procured from National Institute of Health (NIH), Islamabad and housed in the Animal Room of National Institute of Food Science and Technology, University of Agriculture Faisalabad,
Table 1. Different groups conducted in efficacy study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₀</td>
<td>Control (Without garlic extract)</td>
</tr>
<tr>
<td>G₁</td>
<td>Garlic extract, 3 ml/kg body weight</td>
</tr>
<tr>
<td>G₂</td>
<td>Garlic extract, 6 ml/kg body weight</td>
</tr>
<tr>
<td>G₃</td>
<td>Garlic extract, 9 ml/kg body weight</td>
</tr>
</tbody>
</table>

Pakistan. During experiment, temperature (23±2°C) and relative humidity (55±5%) were controlled for 12 h light and dark period. For efficacy trials, four groups of rabbits were made five in each group (Table 1). At the initiation of study, some rabbits were sacrificed to develop baseline values. Different concentrations of aqueous garlic extract were given orally to the respective group for a period of twenty eight days regularly to determine the effect of functional garlic juice (aqueous garlic extract). Feed and water intake were recorded daily whilst body weight was measured after three days in whole experiment. Left diet and feces were also collected. In the end of study, the overnight fasted rabbits were sacrificed to evaluate the effect of respective treatment on the selected parameters including serum lipid profile and glucose levels. Blood samples of rabbits were collected through main jugular vein and serum was collected in heparin coated tubes for further assays through Microlab-300, Germany.

Water intake
Feed intake of each group was measured daily by subtracting split diet from the total feed (Wolf and Weisbrode, 2003). Likewise, intake of garlic extract of each group was also recorded on daily basis.

Body weight gain
Gain in body weight of all rabbit groups was measured after three days throughout the study to elucidate the effect of garlic extract on body weight.

Serum lipid profile
Serum lipid profile including cholesterol, low density lipoproteins, high density lipoproteins and triglycerides were estimated using their standard protocols as mentioned as follows:

Cholesterol
Serum cholesterol level was estimated by CHOD–PAP following the method of Stockbridge et al. (1989).

High density lipoprotein
High density lipoprotein (HDL) in blood serum was calculated by HDL Cholesterol Precipitant method as mentioned by Assmann (1979).

Low density lipoprotein
Blood serum was also analyzed for low density lipoproteins (LDL) following the method of McNamara et al. (1990).

Triglycerides
Total triglycerides in blood serum were monitored by liquid triglycerides (GPO–PAP) method as mentioned by Annoni et al. (1982).

Serum glucose
In each group, blood serum was determined for glucose concentration by GOD-PAP protocol described by Thomas and Labor (1992).

Statistical analysis
The data obtained from entire study were subjected to statistical analysis to determine the level of significance as described by Steel et al. (1997). Analysis of variance was calculated by ANOVA test and means were interrupted by Duncan’s Multiple Range Test.

RESULT AND DISCUSSIONS
Garlic is used as food additive, spice and for medicinal purposes. Shahid and Bhanger (2005) reported the range of aqueous garlic extract yield 6.24 to 23.2% in methanol and its antioxidant activity was different in different solvents. Highest yield was obtained in methanol however, lowest was found in ethyl acetate.

Nasim et al. (2009) computed the therapeutic effect of garlic leaf extract obtained from control and sulphur treated plant group. It was concluded that yield of alliin, the bioactive compound of garlic leaf extract was 32% and more than 20 antioxidants have been found in methanolic extracts of garlic (Dwivedi et al., 1998). It revealed that the antioxidant potential of polar solvent extracts is greater than less/non-polar solvent extract. It has also been investigated that aqueous-soluble organosulfur compounds are localized in human body fluids longer time after ingestion for metabolic modification like hydrolysis or hydroxylation.

Therefore, most of the metabolic studies should be performed in future to investigate the action of aqueous garlic extract as potential modulators of various signaling pathways ultimately leading to the promotion of cell death.

Efficacy studies
Efficacy trial was conducted to evaluate the functional/
nutraceutical potential of garlic against lifestyle-related disorders. Experimental trial was carried out on rabbits rather than humans due to ease in handling, organized supervision, controlled diet and environmental conditions. Safety concern of the product was also one of the considered reasons to conduct this study on rabbits. Additionally, it is hard to find out volunteers who could restrict themselves on specific diet.

**Feed intake**

Means regarding feed intake in all treatments indicated (normal diet) maximum intake (51.34±0.03 g/rabbit/day) whilst minimum (49.18±0.59 g/rabbit/day). Feed intake also increased with time because during 1st week it was 49.18±0.59, 46.20±0.33, 47.66±0.41 and 45.30±0.46 g/rabbit/day in G_0, G_1, G_2 and G_3 groups that subsequently increased to 51.34±0.03, 48.21±0.03, 50.12±0.04 and 47.23±0.03 g/rabbit/day, respectively at 28th days (Table 2). It was observed statistically that feed intake was significant in both days and groups while their interaction was non significant at the level of significance P<0.05. In all treatments, there was a progressive increase in feed intake with passage of time however, the differences were more pronounced in group relying on the control drink (without active ingredients). Myung-Ja et al. (2003) reported that the average feed intake was about 100 g/day during the experimental period. Although the garlic supplementation was fed to rabbits but they adjusted well despite the spicy and odor taste and smell of garlic.

Yanni et al. (2003) reported that food intake of rabbits are strongly correlated with the time. According to their study including feed intake was 49.37±1.02 while after feeding the amino acids diet increased up to 50.59±0.95 g/day.

**Water intake**

Means to water intake of rabbits showed that at 1st week, it was 164.50±1.87, 167.17±2.63, 169.33±3.93 and 170.83±2.48 ml/rabbit/day in G_0, G_1, G_2 and G_3 groups that increased to 166.67±2.73, 170.83±2.31, 171.83±3.31 and 173.33±3.01 ml/rabbit/day, correspondingly at 28th days (Table 3). It was observed statistically that water intake was significant in both days and groups while their interaction was non significant at the level of significance P<0.05.

Collectively, there was an increase in drink consumption during the entire trial with non-significant differences among the treatments thus showing suitability of the product. Anderson et al. (1979) reported that the water intake was improved by taking the functional drinks like haloperidol from 86.3±15.0 to 151.7±8.3 ml/day. It was further assumed that the presence of various active ingredients may force the animal to consume more water.

**Body weight**

Body weight of different groups of rabbits was 2.44±0.02,
2.58±0.05, 2.50±0.06 and 2.30±0.12 kg/rabbit G₀, G₁, G₂ and G₃, respectively however, after 28 days resulted in increased body weight up to 3.12±0.11, 3.05±0.12, 3.24±0.08 and 2.92±0.08 kg/rabbit (Figure 1). It was recorded that body weight was significant in days, groups and their interaction at the level of significance \( P<0.05 \).

It was deduced from the data that there was gradual increase in the body weight of rabbits from start of the study to the termination stage. Myung-Ja et al. (2003) reported that the body weight, liver and heart weights increased in all rabbit groups during the experimented phase, and there was no significant difference between the groups. According to him, body weight of control group increased from 1.5±0.00 to 2.45±0.28 while garlic supplemented increased 1.5±0.00 to 2.33±0.29 kg/rabbit. Yanni et al. (2003) reported that body weight of rabbits was correlated with time because in the beginning of the study the rabbit body weight was 2.88±0.06 while after the feeding of amino acids they gained body weight up to 3.52±0.05 kg/rabbit.

Hypercholesterolemic perspectives

**Total cholesterol**

From mean (Table 4), maximum cholesterol was record-
Table 5. Means for high density lipoprotein (HDL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>14 days</td>
</tr>
<tr>
<td>Control</td>
<td>17.06±0.57c</td>
<td>17.96±0.45b</td>
</tr>
<tr>
<td>1</td>
<td>17.81±0.42c</td>
<td>18.48±0.04de</td>
</tr>
<tr>
<td>2</td>
<td>17.53±0.30d</td>
<td>18.84±0.05l</td>
</tr>
<tr>
<td>3</td>
<td>16.88±0.42bc</td>
<td>18.85±0.02g</td>
</tr>
<tr>
<td>Mean</td>
<td>17.33±0.43c</td>
<td>75.64±2.61b</td>
</tr>
</tbody>
</table>

Low density lipoprotein

From mean (Figure 2) concluded that maximum LDL was recorded as 6.81±0.45 mg/dL in G₀ followed by 5.87±0.39 mg/dL in G₁ and 5.72±0.40 mg/dL in G₃ while lowest was 5.69±0.71 in G₂.

Furthermore, it also predicted that during the whole study, the LDL was reduced in all treated groups that is, 6.06±0.36 to 5.42±0.04 mg/dL in G₁, 6.31±0.28 to 4.91±0.03 mg/dL in G₂ while 5.86±0.39 to 5.26±0.02 mg/dL in G₃ from the beginning of study to the end of trial. It was noticed that low density lipoprotein was significant in the action of days, groups and their interaction at the level of significance $P<0.05$.

Fidan et al. (2007) reported that the value of LDL changed by feeding the rabbits Hypericum lysimachioides with different dose formulation. According to the studies...
LDL was reduced in all treated groups, that is, 26.6±4.7 to 20.6±62 in G1, 20.4±6.7 to 15.4±1.6 in G2, 25.6±4.7 to 14.02±4.1 and 30.2±1.32 to 48.60±16.88 in G3. Myung-Ja et al. (2003) reported that LDL level was reduced by feeding the garlic powder 666.0±151.2 in 12 weeks while control group was 1169.5±150.4. Late et al. (2003) reported that the VLDL was significantly decreased in all Moringa oleifera supplemented groups during the experimental periods as compared to the control group (P<0.05). By comparing the control group with Moringa oleifera supplemented group for 6 weeks, it showed that Moringa oleifera lowered the VLDL value from about 21.1±0.66 to 16.1±0.03 mg/dL.

These results are supporting to my project finding and also concluded that after receiving garlic extract up to 12 weeks, VLDL was reduced more as compared to the normal group.

**Very low density lipoprotein (VLDL)**

Mean values showed that maximum VLDL was recorded (6.78±0.31 mg/dL) in G0 followed by (5.69±0.43 mg/dL) G1 and G3 (5.38±0.66 mg/dL) as shown in Figure 3. It was observed from the table that maximum value for VLDL was 7.11±0.4 mg/dL in G0 while lowest value was 4.12±0.03 in G3 from the 0 days to 28th days trial. It was recorded that same trend was observed in very low density lipoprotein that was significant in the action of days, groups and their interaction at the level of significance P<0.05.

Myung-Ja et al. (2003) reported that VLDL level was reduced by feeding the garlic powder 671.3±93.5 in 12 weeks.

**Plasma triglycerides**

From mean (Table 6) maximum plasma triglycerides were recorded (79.11±1.51 mg/dL) in G0 followed by (75.46±2.66 mg/dL) G1 and G3 (74.78±3.68 mg/dL). Furthermore, it was also predicted that during the whole study plasma triglycerides was reduced in all treated groups from 77.93±1.74 to 72.63±0.02 in G1, 75.86±0.36 to 69.36±0.03 in G2 while 78.33±0.46 to 70.97±0.04 in G3. It was observed that plasma triglycerides were significant in the action of days, groups and their interaction at the level of significance P<0.05.

Myung-Ja et al. (2003) reported that triglyceride was significantly decreased in all garlic supplemented groups during the experimental period. Aqueous garlic extract significantly lowered the triglyceride as compared to the control group (P<0.05). By comparing the control group

### Table 6. Means for plasma triglycerides.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>14 days</th>
<th>28 days</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.65±0.86c</td>
<td>79.01±0.90d</td>
<td>80.66±0.76a</td>
<td>79.11±1.51a</td>
</tr>
<tr>
<td>1</td>
<td>77.93±1.74c</td>
<td>75.81±0.47de</td>
<td>72.63±0.02j</td>
<td>75.46±2.66b</td>
</tr>
<tr>
<td>2</td>
<td>75.86±0.36g</td>
<td>72.68±0.03j</td>
<td>69.36±0.03h</td>
<td>72.63±3.25c</td>
</tr>
<tr>
<td>3</td>
<td>78.33±0.46bc</td>
<td>75.04±0.02e</td>
<td>70.97±0.04g</td>
<td>74.78±3.68d</td>
</tr>
</tbody>
</table>
| Mean   | 77.44±1.08a | 75.64±2.61b | 73.41±5.01c | }
with garlic supplemented group for 6 weeks, it showed that garlic lowered the triglyceride level to about 23% and total cholesterol 14%. These results are supporting to my project findings and also concluded that after receiving garlic extract up to 12 weeks, triglyceride and total cholesterol values lowered by 34 and 24% as compared to the normal group.

Hyperglycemic perspectives

Serum glucose

From mean (Table 7) investigated serum glucose was recorded as (7.35±0.1 mg/dL) in G₀ followed by (6.43±0.2 mg/dL) G₃ and G₁ (6.30±0.6 mg/dL). Furthermore, it also predicted that during the whole study the serum glucose was reduced in all treated groups from 6.50±0.2 to 6.10±0.4 in G₁, 6.60±0.2 to 5.80±0.3 in G₂ while 6.70±0.3 to 6.20±0.3 in G₃ in whole study. It was recorded that serum glucose was significant in the action of days, groups and their interaction at the level of significance p < 0.05.

Mikail (2009) reported that serum glucose level decreased by providing garlic extract compared to non treated ones. It was observed that serum glucose was reduced by the supplementation of garlic intake dose from 7.6±0.5 to 5.0±0.3. Metwally (2009) also reported that feeding of garlic to the fish *Tilapia nilotica* significant reduced glucose level. It was further investigated that different groups like control group, fed natural garlic, garlic oil and garlic powder reduced blood glucose as 115.5.1±4.18, 96.49±1.58, 85.75±1.58 and 82.56±1.49, respectively. Moreover, glucose concentration decreased in blood serum by consuming different preparations of garlic.

### CONCLUSION

Garlic (*Allium sativum* L., *Liliaceae*) is an essential vegetable that has been widely utilized as seasoning, flavoring, culinary and in herbal remedies. The consumption of traditional plants especially garlic has progressively increased worldwide because of their great effectiveness, fewer side effects and relatively low cost. Garlic is well known to acquire an array of phytochemicals. These bioactive molecules are playing pivotal role in maintaining human health and having potential to reduce various ailments. The present project was an attempt to explore the functional/nutraceutical role of garlic against various threats including hypercholesterolemic and hyperglycemia perspectives using rabbit's experimental modeling. It was concluded that using 3 ml garlic extract per day can attenuate these physiological threats because it reduced the total cholesterol, triglycerides and serum glucose level.

### REFERENCES
