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Bioactive ingredients of local garlic variety from Pakistan

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Abstract

The health claims of locally grown garlic variety/line lehsan gulabi (garlic pink) for the management of serum glucose were explored. In efficacy study, garlic based diets given to the rats *i.e.* whole garlic (G_1), garlic powder (G_2) and garlic oil (G_3) resulted in reduction in glucose, serum creatinine and serum urea levels, ALT, AST and ALP and elevation in serum insulin as compared to control (G_0). The weight of rats substantially suppressed after the intake of different garlic preparations. It is deduced that garlic feeding may prove beneficial in weight management program. The whole garlic consumption proved more effective. The garlic oil caused highest glucose reduction (9.38%). Garlic oil alleviates glucose and insulin related abnormalities more efficiently. Additionally, treatments imparted significant effect on liver and kidney functioning. In the nutshell, pink garlic has potential to curtail different physiological malfunctioning. Thus diet based therapy by selecting suitable food ingredients should be encouraged as a preventive device against various metabolic syndromes among the vulnerable segments in the developing economies.

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Introduction

Phytonutrients are widespread in the human diet from the ancient times providing natural shield against several metabolic syndromes. In the contemporary dietary regime, nutritionists are striving to explore some novel approaches for improving public health and life expectancy by curtailing lifestyle related disorders. Considering various phytoremedies, garlic, onion, ginger etc. have attained forefront position to reduce the incidence of life threatening ailments (Potawale *et al.,* 2008).

Garlic (*Allium sativum* L.) is widely consumed as seasoning, flavoring and in culinary preparations. Alongside, it is utilized in folk medicines for curing various maladies (Rivlin, 2001). 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 is recommended by World Health Organization (1999).

The composition of garlic/ concentration of allicin varies with geographical location, harvesting time, agronomic practices etc. Garlic contains approximately 65% water, 30% carbohydrates and 5% other bioactive components mainly sulfur containing compounds (Milner, 2001). The most plentiful sulfur containing compound S-allyl-cysteine sulfoxide named as allicin comprises 10 mg/g fresh or 30 mg/g dry weight of garlic (Lawson *et al.,* 1995).

Diabetes mellitus is one of the leading causes of death particularly in the developing economics; Pakistan holds 6th position. The prevalence of diabetes was estimated as 2.8% in the year 2000 that will increase to 4.4% in 2030 in all age groups (Wild *et al*., 2004). The diet selection and physical exercise are the suitable strategies to manage diabetes and associated complications like immune dysfunction, degenerative and cardiovascular disorders.

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Efficacy trials have depicted garlic (Jabbari *et al.,* 2005), garlic juice (El-Demerdash *et al*., 2005) and garlic oil (Anwar and Meki, 2003) as therapeutic agent against hyperglycemia. The hypoglycemic ability of garlic is accredited to its sulfur-containing compounds that stimulate insulin secretion (Mahesar *et al*., 2010). The bioactive moieties like allyl sulfide and S-allyl-cysteine sulfoxide (allicin) have also shown hypoglycemic effects (Sukandar *et al*., 2010).

It is also reported that the diet with 2% garlic powder decreases ischemia and reperfusion-induced arrhythmias along with acute renal failure and oxidative stress induced by gentamicin (Rahman and Lowe, 2006). Keeping in view the health claims of garlic, present project was designed to characterize locally grown promising garlic variety/line lehsan gulabi (garlic pink) with special reference to its bioactive ingredients. The therapeutic role of garlic 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 obtained from Ayub Agriculture Research Institute (AARI), Faisalabad. Garlic was cleaned to remove the dirt, dust and other foreign trash. Analytical and HPLC grade reagents and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

Garlic preparations

The garlic cloves were peeled. The following garlic preparations were made. Whole Garlic: Whole garlic bulbs were crushed. Garlic powder: the bulbs were crushed, dehydrated and 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.

Bioevaluation studies

Male Sprague Dawley 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. The rats were monitored for the body weight gain throughout the trial. At the mid of study (28^{th} day) half of the overnight fasted rats in each group were scarified, while remaining decapitated at the end of trial (56^{th} day) and blood samples were collected in EDTA coated tubes. The serum was separated after centrifuging the blood for 6 min @ 4000 rpm. The whole efficacy trial was repeated for results verification.

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%). 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 ml/kg body weight) were added, respectively in the diet formulation.

Hypoglycemic perspectives

At intervals (4^{th} and 8^{th} week) glucose concentration was estimated by GOD-PAP method (Katz *et al*., 2000). Insulin level was estimated by following the instructions of Ahn *et al*. (2011).

Liver functioning tests

Alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP) were estimated (Basuny *et al.*, 2009). ALT and AST levels were measured by dinitrophenylhydrazene (DNPH) through Sigma Kits 58-50 and 59-50, respectively. Alkaline Phosphatas-DGKC was used for ALP assessment.

Kidney functioning tests

The serum samples were analyzed for urea by GLDH-method and creatinine by Jaffe-procedure via commercial kits (Thomas, 1998; Jacobs *et al*., 1996).

Statistical Analysis

The data were subjected to statistical analysis (Costat-2003, Co-Hort, v 6.1). Level of significance was determined (ANOVA) using 2-factor factorial CRD (Steel *et al.* 1997).

Results and discussion

Bioevaluation studies

Biological evaluation was carried out through rodent modeling to explore the functional/ nutraceutical worth of garlic preparations with special reference to glycemic response, liver and kidney functioning perspective.

Body weight

Statistical analysis explicated that body weight affected substantially with treatments and study intervals in both trials. Means in Fig. 1. (trail I and II) illustrated that body weight in different rat groups for treatments G_0, G_1, G_2 and G_3 significantly increased with the passage of time.

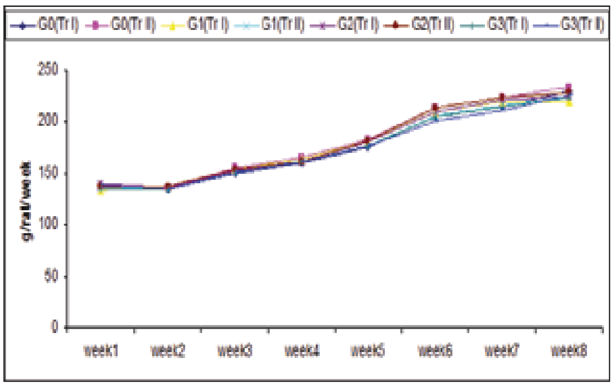

Fig. 1. Body weight of rats (g/rat/week)

The findings pertaining to body weight gain are in agreement with Elkayam *et al.* (2003), who reported obesity, hypercholesterolemia and hyperglycemia as leading detrimental outcomes of lack of physical activity and high caloric diet in daily routine. They illuminated a significant reduction in body weight of rats fed on garlic based diet. Kang *et al.* (2008) explicated substantial decline in the rats weight fed on garlic powder. Earlier, Wu *et al.* (2001) analyzed the role of organosulfur compounds from garlic on the body weight using rodents modeling. The findings indicated an inverse association of garlic active moieties with net body weight gain.

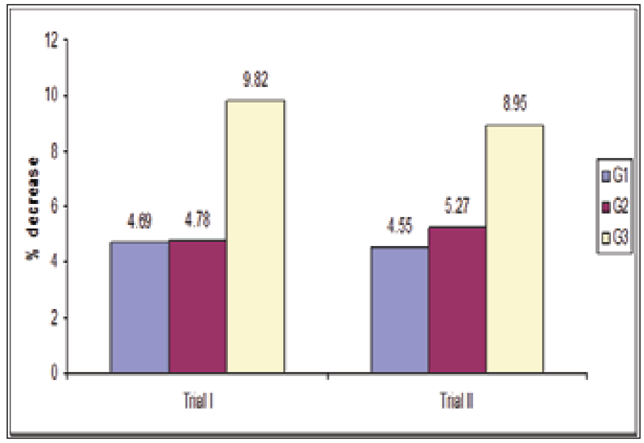

Fig. 2. Percent decrease in glucose level of rats

Glucose

Serum glucose showed significant variations due to treatments and time intervals, however their interaction imparted non-significant differences (Table I and II). Means regarding glucose (Table I) showed maximum value 85.40±5.12 mg/dL in G_0 that substantially reduced to 81.12±5.23, 84.25±5.68 and 84.34±4.98 mg/dL in G_3, G_2 and G_1, respectively. From Fig. 2 it is concluded that treatment G_3

Table I. Effect of treatments on glucose, insulin and liver and kidney functions of rats

Parameters	Trials	G_0	G_1	G_2	G_3
Glucose (mg/dL)	T_I	$85.40±5.12^a$	$84.34±4.98^a$	$84.25±5.68^a$	$81.12±5.23^b$
	T_{II}	$88.68±5.20^a$	$85.54±5.21^b$	$85.03±4.95^b$	$82.98±5.14^b$
Insulin (IU/L)	T_I	$9.09±0.81^b$	$9.22±0.76^b$	$9.41±0.88^a$	$9.52±0.92^a$
	T_{II}	$9.08±0.51^c$	$9.39±0.49^b$	$9.48±0.52^b$	$9.63±0.63^a$
Alanine	T_I	$40.89±2.45^a$	$38.58±2.81^b$	$38.91±3.19^{ab}$	$39.45±2.34^a$
Transferases (ALT)	T_{II}	$42.60±2.98^a$	$39.21±2.47^a$	$40.03±2.64^a$	$40.21±2.53^b$
Aspartate	T_I	$139.78±8.34^a$	$124.09±9.92^c$	$133.93±8.04^b$	$134.25±9.45^b$
Transferases (AST)	T_{II}	$138.22±8.06^a$	$123.45±7.64^c$	$132.73±7.90^b$	$132.74±7.85^b$
Alkaline	T_I	$167.83±11.83^a$	$158.43±9.84^b$	$160.24±11.83^a$	$162.04±10.20^a$
Phosphatase (ALP)	T_{II}	$165.73±11.83^a$	$160.13±9.84^b$	$160.84±11.83^a$	$161.61±10.20^a$
Serum creatinine	T_I	$0.71±0.03^a$	$0.63±0.05^a$	$0.64±0.03^b$	$0.65±0.02^c$
(mg/dL)	T_{II}	$0.69±0.03^a$	$0.64±0.05^b$	$0.66±0.03^b$	$0.67±0.02^a$
Serum Urea	T_I	$37.85±2.66^a$	$34.32±2.96^b$	$36.32±2.87^a$	$37.50±3.09^a$
(mg/dL)	T_{II}	$38.92±2.66^a$	$35.23±2.96^b$	$35.35±2.87^b$	$37.31±3.09^a$

resulted highest glucose reduction by 9.82 and 8.95% (mean 9.38%) followed by G_2 4.78 and 5.27% and G_1 4.69 and 4.55% in trial I and II, respectively. The findings of Cheng *et al.* (2006) are in harmony with the current study, they reported substantial decrease (11.02%) in glucose of experimental rats after consuming garlic oil. They deduced that the presence of S-allyl-cysteine sulfoxide in garlic oil alleviates abnormalities in glucose and insulin secretion thus acts as a hypoglycemic agent.

The outcomes of different scientific explorations are advocating the hypoglycemic ability of garlic and its different forms. This effect is endorsed due to the presence of organosulphur compounds capable to perform anti-inflammatory and anti-hyperglycemic activities (Eyo *et al.,* 2011). They administrated different doses of garlic *i.e.* 100, 125, 150 200 250 and 300mg/kg body weight of rats for 6 weeks, which indicated 79.7% reduction in glucose level.

The enhancement in insulin secretion from pancreas, interfering with glucose absorption and saving the insulin utilization are the three possible routes by which garlic and its active constituents perform anti-diabetic activity. A significant effect of garlic supplementation for reducing glucose related variables was observed (Asaduzzaman *et al.* 2010; Balasubramaniam *et al.* 2010). Ugwuja *et al.* (2010) observed 16.25% decrease in glucose level of rats after consuming garlic enriched diet for a period of 6 weeks. Later, Mirunalini and Krishnaveni (2011) expounded significant effect of garlic on plasma glucose concentration in rats after the consumption of garlic (1.2g) for 30 days.

A substantial decline 18.06% in the glucose level of garlic fed rats was noticed (Djankpa *et al.,* 2012). They ascribed that garlic may exert beneficial effect on insulin by increasing its secretion from β-cells thus caused a decrease in blood glucose. Similarly, Patil *et al.* (2012) determined the anti-diabetic effect of garlic in combination with other herbal formulations. The garlic exhibited strong α-amylase and α-glucosidase inhibiting activity. It is inferred from the discussion that garlic supplementation is suitable to cope with glucose related abnormalities. No doubt, all garlic forms were beneficial nevertheless garlic oil is more effective to address glucose related disorders.

Insulin

Treatments and study intervals affected insulin level significantly (Table I and II), nonetheless influence of interaction remained non-momentous. Means concerning insulin (Table 1) showed that the G_0 exhibited lowest insulin level 9.09±0.81 and 9.08±0.51 IU/L (in trial I and II respectively). It is evident from Fig. 3 that the provision of

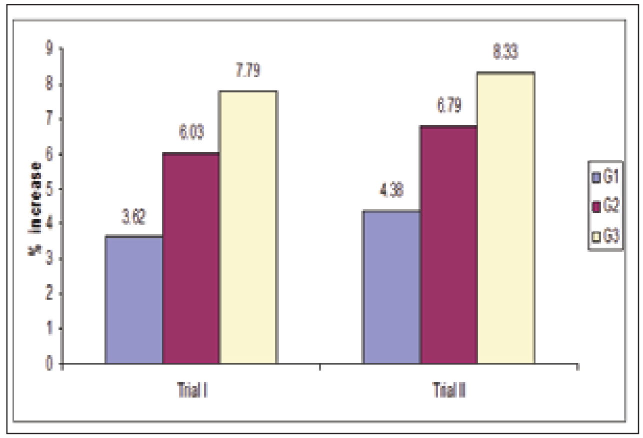

Fig. 3. Percent increase in insulin level of rats

garlic supplemented diets caused elevation in serum insulin as compared to the control. In this context, the consumption of garlic oil (G_3) exhibited maximum increase as 7.79 and 8.33% followed by garlic powder (G_2) 6.03 and 6.79% and whole garlic (G_1) 3.62 and 4.38% in both trials, respectively.

Enhancement in the insulin level with garlic preparations especially with garlic oil in current investigation are in accordance with the findings of Mustafa *et al.* (2007). They observed 30.12% insulin increase in male albino rats after administrating 10% V/V garlic for the period of 30 days. Khayatnouri *et al.* (2011) observed that garlic oil supplementation caused insulin elevation in rats plasma due to its organosulphur compounds. The bioactive moieties in garlic like allicin and S-allyl cysteine sulphoxide (SACS) caused deactivation of inflammatory mediators after producing nitric oxide (NO) thus facilitate in reversing insulin sensitivity (Khayatnouri *et al.,* 2011; Djankpa *et al.,* 2012). Earlier, Mariee *et al.* (2009) carried out a rodent trial and noticed that garlic enhances insulin secretion alongside reducing blood glucose, triglycerides, cholesterol and LDL. Islam and Choi (2008) reported that ginger and garlic administration proved beneficial for insulin release in diabetic patients.

The role of allicin as an insulin enhancing compound is obvious from many studies conducted both on humans and animals models. It increases insulin secretion from the β-cells. Moreover, its consumption reduces the triglyceride and glucose concentration and protects LDL from oxidation. All these properties resulted a balance in fat, protein and carbohydrate metabolism, facilitate toxin removals, manage the key enzymes in insulin secretion thereby enhance insulin secretion (Birdee and Yeh, 2010). From the above discussion it is deduced that different garlic preparations especially garlic oil is effectual to alleviate insulin related malfunctioning thus can be utilized to improve the glycemic response in the vulnerable segment.

Liver functioning

ALT, AST and ALP concentrations showed substantial differences owing to treatments and study intervals (Table I and II).

Alanine transferase (ALT)

A significant decrease in ALT level with the use of garlic preparations was observed. The present outcomes are consistent with the previous findings of Eidi *et al.* (2006). They investigated the impact of garlic against diabetes in rats. The ALT level in diabetic rats was significantly reduced by the use of garlic extract over two weeks. Later, D'Argenio (2010) assessed liver functioning in rats with CCl_4-induced fibrosis. Garlic treatment showed momentous decrease in ALT level among rats with liver fibrosis. The study affirmed the ability of garlic extract to mitigate liver damage and strengthen the curative potential of the diallyldisulphide compound. Likewise, the study of Dkhil *et al.* (2011) highlighted the beneficial impact of garlic against liver dysfunction as it lowers the elevated ALT towards normal level.

Aspartate transferase (AST)

A decrease in AST values was noted in different garlic diet groups (G_1), (G_2) and (G_3) while an increase was noted in control group. El Shenawy *et al.* (2008) observed the potential of garlic against liver and kidney damage induced by mercury chloride in rats and recorded significant reduction in AST concentrations after treating diseased rats with garlic (63 mg/kg) during one month. Current data is also comparable to the earlier investigations of D'Argenio *et al.* (2010), which observed a reduction in serum AST of rats treated with garlic extract. It is inferred from the study of Madkor *et al.* (2011) that garlic powder resulted significant reduction in serum AST of diabetic rats.

Alkaline phosphatase (ALP)

A significant decrease in ALP levels with the use of garlic preparations was observed. Current findings are comparable with the earlier research investigations. Sharma *et al.* (2010) illustrated the effect of garlic extract against lead nitrate toxicity in male mice. Lead nitrate caused elevated ALP levels however, a considerable decline in ALP levels was observed in mice treated with ethanolic garlic extract (250 and 500 mg/kg body weight). Likewise, D'Argenio *et al.* (2010) evaluated the effect of garlic against liver fibrosis. It was revealed that administration of garlic extract normilized the value of ALP in CCl_4-induced rats. Later, Dkhil *et al.* (2011) explicated that garlic treatment momentously reduced the elevated serum ALP. Recently, D'Argenio *et al.* (2012) studied the consequences of rat liver fibrosis in response to garlic preparations and observed beneficial effects. However, they found non-significant effect of garlic on ALP value.

Kidney functioning

Serum creatinine and urea showed momentous differences due to treatments and time intervals during bioevaluation trial.

Serum creatinine

Considerable reduction in Serum creatinine was recorded in groups given diet supplemented with garlic preparations (Table I, II). G_0 exhibited higher creatinine level that substantially reduces in groups fed on G_1, G_2 and G_3 diets. The G_1 showed maximum reduction as compared to control (Table I). Serum creatinine is measured to evaluate renal functioning; higher level is an indicator of damaged nephrons while normal range indicates proper metabolism. The present data was comparable with the previous findings showing a decline in serum creatinine level after garlic administration. Jabbari *et al.* (2005) found that serum creatinine was notably reduced with garlic intake thus protecting nephrotoxicity. According to El-Shenawy and Hassan (2008), garlic treatment prevented increase in serum creatinine level of rats and caused considerable decline (17%) in creatinine level of rats with liver and kidney damage, induced by mercury chloride ($HgCl_2$). El Shenawy *et al.* (2008) illustrated the effect of garlic extract on urea nitrogen and creatinine concentrations to evaluate the kidney functioning. Significant decrease was observed in garlic treated groups with and without *Nigella sativa*. However, certain studies showed contradictions and revealed non-momentous effect of garlic intake on serum creatinine level (Durak *et al.,* 2004; Higashikawa *et al.,* 2012).

Serum urea

Rats fed whole garlic (G_1) showed maximum decrease in urea level (Table I and II). El-Shenawy and Hassan (2008) stated that garlic administration prevents elevation in serum urea in normal rats while caused notable reduction for this trait in rats with induced liver and kidney damage. Blood urea nitrogen concentration was increased in rats with damaged liver and kidney and use of garlic (63 mg/kg) showed momentous decline in blood urea level. Similarly, El Shenawy *et al.* (2008) observed positive effect of garlic supplementation on blood urea reduction in a rodent trial. In contrary, some of the researchers did not observe any considerable changes in the serum urea level by the use of garlic preparations (Jabbari *et al.,* 2005; Higashikawa *et al.,* 2012).

Conclusion

The efficacy study concluded that garlic based diets given to the rats *i.e.* whole garlic (G_1), garlic powder (G_2) and garlic oil (G_3) resulted in reduction in glucose, serum creatinine and serum urea levels, ALT, AST and ALP. It is deduced that garlic feeding may prove beneficial in weight management program. The whole garlic consumption proved more effective. The provision of garlic oil caused highest glucose reduction (9.38%). Garlic oil alleviates glucose and insulin related abnormalities more efficiently. Garlic also imparted significant effect on liver and kidney functioning. Thus locally grown garlic variety/line lehsan gulabi (garlic pink) has potential to curtail different physiological malfunctioning. The diet based therapy by selecting suitable food ingredients should be encouraged as a preventive device against various metabolic syndromes among the vulnerable segments in the developing economies.

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