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Short Communication

Comparative Effects of Aqueous and Organic Solvent Extracts of Garlic on Glucose Level and Lipid Profile of Diabetic Rats

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Authors' Contributions
MS, MS and MAM perceived, designed and supervised the study and helped in article writing. MA and AR helped in animal studies and proof reading. MAK performed the statistical analysis. FH executed the extraction and studied animals and wrote the article.

Key words
Hyperglycemic, Alloxan, Black cumin, Diabetes, Garlic, Herbal preparations.

ABSTRACT

Life time management of diabetes by synthetic drugs has been found cost effective with severe side effects as well. Herbal remedies are gaining popularity. Black cumin and garlic are found effective against hypoglycemia, hyperlipidemia, and hypercholesterolemia. Present study was designed to evaluate the relative antidiabetic potential of garlic aqueous (AGE) and petroleum ether (EGE) extracts compared to black cumin (*Nigella sativa* oil; NSO) and insulin. Alloxan induced diabetic rats were post treated with plant extracts (150 mg/kg) and serum samples were collected from overnight fasted diabetic rats on day 15 to observe thepercent changes in blood glucose levels and lipid profile. Garlic extracts have shown the significant ($p<0.001$) hypoglycemic activity with no considerable effect on mean body weight of rats (ns, $p>0.05$) as compared to diabetic rats. The AGE treatment has significantly reduced the blood glucose level (56%), however, the EGE treatment was found effective in normalizing the lipid contents of diabetic rats. The black cumin oil treatment has resulted in significant ($p<0.001$) increase (48%) in body weight of diabetic rats, but found less active in regulating other biochemical parameters. However, significant role of garlic extracts; especially the ether extract (EGE) was noted in normalizing the biochemical attributes. On the basis of findings, it is recommended that the garlic ether extract should be applied in herb-drug combinations commonly used as antidiabetic.

Insulin therapy is the usual practice in most of the diabetic patients (Inzucchi *et al*., 2012). Oral synthetic diabetic drugs such as meglitinides, sulfonylureas, and others may cause hypoglycemia. Hence, drug scheme for each diabetic complication should be devised to diminish the risk of hypoglycaemia (Inzucchi *et al*., 2012).

Pharmacological studies on herbal therapies are underway to discover more active anti-diabetic agent from natural source (Walker and Whittlesea, 2012). The antihyperglycemic, antihyperlipidemic, and antihypercholesterolemic activities of black cumin (*Nigella sativa*) are well established (Shabana et al., 2013; Ikram and Hussain, 2014; Bamosa, 2015). Garlic has also been reported as anti-inflammatory, hepatoprotective, antioxidant, antifungal and wound curative agent (Londhe., 2011). Garlic has exhibited its antihyperglycemic effects by increasing the pancreatic secretion of insulin from β-cells (Mustafa *et al*., 2007), discharging the bound insulin (Eyo *et al*., 2011), interfering with intestinal glucose absorption (Juarez-Rojop *et al*., 2012) and also by increasing the peripheral glucose utilization (Gupta *et al*., 2012). From another perspective, the garlic oil has high linolic acid contents and thus advised in condition of heart disease and high blood pressure mostly associated with diabetes (Bagudo and Acheme, 2014).

Several reviews have been published currently describing the therapeutic potential of black cumin in different diseases such as cardiovascular (Shabana *et al*., 2013), antidiabetic (Mathur *et al*., 2011), anticancer (Khan *et al*., 2011), anti-inflammatory (Woo and Kumar, 2012), as well as many other general reviews (Ahmad *et al*., 2013). It has been one of the most commonly used medicinal plants by folks for treatment to diabetes (Mathur *et al*., 2011). Therefore, black cumin seed oil is used in this

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study as herbal remedy to compare the antidiabetic effects of garlic aqueous (AGE) and petroleum ether extracts (EGE) along with the clinical therapy, insulin.

The aim of the present study was to compare the hypoglycemic and hypolipidaemic potential of garlic aqueous and ether extracts with that of black cumin seed oil (*Nigella sativa*) and insulin treatments in alloxanized rats.

Materials and methods

Fresh bulbs of garlic (*Allium sativum*) were blended and dried in hot air oven at a temperature 60-80°C for 24 h. Dried garlic paste was reduced to fine powder using pestle and mortar and the powder was extracted successively with petroleum ether using soxhlet apparatus. The extracted liquid was evaporated to obtain dry crude petroleum ether exract of garlic (Suleiman and Abdallah, 2014). Aqueous extract of garlic was prepared by homogenizing it in a mixer. Immersed in 1 L distilled water, and allowed to stand for 24 h with shaking. After filtration, filtrate was dried and then responded in standard saline (0.85% NaCl) at concentration of 1 g/mL (Mahesar *et al*., 2010).

Albino rats of either sex weighing 150-250 g obtained from the local market were fed with standard pellet diet and water; the rats were maintained on standard laboratory conditions of room temperature, humidity and under 12 h dark-light cycle. The animals were acclimatized to the conditions for two weeks before experimentation.

To determine the metabolic changes related to blood glucose and lipid profile in diabetic rats, diabetes was induced in animals by an intraperitoneal injection of alloxan (150 mg/kg of a 10% alloxan solution in isotonic NaCl) (Sigma Chemical Co., St Louis, MO, USA). The rats were then kept on 5% oral glucose for next 24 h. After 72 h, the animals with glucose levels higher than 200 mg/dl were considered as diabetic (Pund *et al*., 2012; Qazi *et al*., 2014).

Thirty rats (25 surviving diabetic rats and 5 normal rats) were randomized into six groups (n=5) and administered daily doses of following: Group I served as control and received physiological saline (0.9%); Group II served as diabetic control; Group III received orally the garlic aqueous extract (150 mg/kg); Group IV ingested with garlic ether extract (150 mg/kg); Group V injected subcutaneously with NPH insulin (Eli Lilly & Co.) (6 to 8 IU) at 12 h interval (Geetha *et al*., 2011). Group VI were fed with black cumin oil (NSO) (150 mg/kg). After two weeks, the blood withdrawn via cardiac puncture using sterile syringes and needles was collected in kept in plain bottles. Recovered blood was then centrifuged at 3000 rpm for 30 min for subsequent analysis.

Data are presented as mean ± standard error of means. For establishing significant differences between groups, data were analyzed by one-way ANOVA. The statistical significance was determined using SPSS (Statistical Package for Social Sciences) software, version 20.0.

Results and discussion

Table I shows the effect of different plants extracts treatments (150 mg/kg/day, single dose) for two weeks on body weight, blood glucose and lipid profile in control and alloxan-induced diabetic rats. The diabetic rats (GII) were compared to control (GI) animals, while the rest of the

Table I.- Effect of different test treatments on alloxan induced diabetic rats after two weeks.

Parameters	GI (Control)	GII (Alloxan)	GIII (AGE)	GIV (EGE)	GV (Insulin)	GVI (NSO)
Body weight (g)	250±0.90 (100%)	200± 3.90 (-22%)***	196 ±3.75 (-1%)^{ns}	202 ±2.82 (-3%)^{ns}	246 ±1.27 (23%)$^{###}$	296±5.46 (48%)$^{###}$
Blood sugar level (mg/dl)	86.375±0.47 (100%)	367.3±75.6 (324%)***	161.6±122.5 (-56%)$^{###}$	196.43±76.98 (-46%)$^{###}$	78±0.67 (-27%)$^{###}$	267±0.67 (-78%)$^{###}$
Cholesterol (mg/dl)	105.5±1.59 (100%)	116.1±1.61 (10%)***	74.26±1.42 (-36%)$^{###}$	100.46±1.51 (-13 %)$^{###}$	87±1.58 (-25%)$^{###}$	78±1.58 (-33%)$^{###}$
Triglycerides (mg/dl)	96.25±1.47 (100%)	98±1.58 (-2%)^{ns}	55.5±1.54 (-43%)$^{###}$	93.73±1.49 (-4%)^{ns}	39± 1.58 (-60%)$^{###}$	38± 1.58 (-61 %)$^{###}$
HDL-C (mg/dl)	40.0±1.62 (100%)	29.9±1.37 (-26%)***	30.2±1.03 (-2 %)^{ns}	39.4±1.7 (-32%)$^{###}$	15 ± 1.58 (-50%)$^{###}$	11± 2.14 (-63%)$^{###}$
LDL-C (mg/dl)	46±1.58 (100%)	50±1.58 (9%)^{ns}	39.9±1.48 (-39%)$^{###}$	43.5±1.64 (-13%)^{ns}	69.4± 1.80 (38%)$^{###}$	61± 1.58 (22%)$^{###}$
VLDL-C (mg/dl)	19.4± 1.5 (100%)	26.3± 1.52 (36 %)*	11.13±1.8 (-58%)$^{###}$	18.5± 1.58 (-30%)$^{##}$	11± 1.58 (-58%)$^{###}$	7± 1.58 (73%)$^{###}$

Values are expressed as mean ± SE (n = 5); P values: $^{###,***}$<0.001;$^{##,**}$<0.01;$^{#,*}$<0.05; *GI vs GII, $^{#}$ GII vs GIII,GIV,GV,GVI, ^{ns}Non-significant comparison. (GI, Control; GII, Diabetic control; GIII, Alloxan+AGE; GIV, Alloxan+EGE; GV, Alloxan+insulin; GVI, Alloxan+NSO). The data were analyzed using Oneway-ANOVA analysis and Tukey's post hoc test.

treatment groups were compared to GII. The rats have shown the highly significant reduction (~22%, $p<0.001$) in mean body weight of animals compared to control values. Both garlic aqueous (AGE) and petroleum ether extracts (EGE) have revealed the non-significant reduction ($p>0.05$, ~1% and ~3% respectively) in mean body weight of animals (GIII and GIV, respectively) when compared to the diabetic GII, but exhibited the marked reduction in body weight in comparison to the GI. The highly significant ($p<0.001$) increase in mean body weight of animals was also observed in insulin treated group (GV) as compared to the diabetics (-20 to +23%). However, the NSO treatment showed the highly significant effect (up to 48%, $p<0.001$) in increasing the mean body weight of GVI animals.

However, Tripathi *et al*. (2013) have described that the administration of aqueous extract of *Allium sativum* (ASE) has increased the body weight of diabetic rats (28 days treatment) when administered alone or supplied in combinations with metformin. Our findings were in agreement with Alimohammadi *et al*. (2013), who also reported a significant weight loss in the diabetic rats which have gained the weight after the NSO treatment.

The diabetic animals have shown the highly significant ($p<0.001$) and abrupt increase in blood glucose concentrations (up to 324%) compared to the control rats. However, the plant extract treatments to diabetic rats (GIII, GIV, and GVI) have highly significantly ($p<0.001$) reduced the blood glucose levels (-56%, -46% and -27%, respectively) as compared to GII. While comparing the plant extracts, the AGE was found to be more effective. However, the insulin treatment has showed the most significant ($p<0.001$) and regulatory effect in reducing the blood glucose contents (-78%), near to normal values, in group V animals.

Ikram and Hussain (2014) have reported that alloxan monohydrate has raised significantly the blood-glucose levels of diabetic rabbits (265.6 ± 30.26 mg/dl; $p<0.05$) as compared to the non-diabetic animals (99.5 ± 13.12 mg/dl). Our results have shown the significant blood glucose lowering effects of garlic extract treatment. To compare the anti-diabetic effects of daily administration of garlic and onion extracts, Tripathi *et al*. (2013) have also reported hypoglycaemic effect of the antidiabetic drugs.

Majority of the studies have shown hypoglycaemic effect of black cumin and thymoquinone (Bamosa, 2015), however, according to our findings, the NSO treatment has not proved effective in normalizing the mean blood glucose levels of diabetic rats. Thus, the herbal preparations should be tested further to get more safe medical formulations.

EGE was found very effective in normalizing the lipid profile of animals, comparable to that of the normal values. The diabetic rats have shown the significantly high levels ($p<0.001$) of total cholesterol contents compared to the normal values (10%). The test plant extracts including the insulin treatment showed the marked decrease in total cholesterol levels compared to diabetic rats. A highly significant (36%, $p<0.001$) reduction in total cholesterol levels was noted in diabetic rats treated with AGE. However, comparatively less decrease (-13%, $p<0.001$), but near to normal values, was observed in diabetic rats treated with EGE.

The diabetic rats have shown the non-significant (2%, $p>0.05$) increase in serum total TG-C contents compared to the control values. Administration of AGE and NSO treatments have revealed the highly significant (-43% and -61% respectively, $p<0.001$) effects in lowering the total TG-C contents of animals as compared to diabetic rats. However, non-significant (4%, $p>0.05$) decrease in serum TG-C contents was observed in rats treated with EGE. Similar to NSO, the insulin treatment has shown the most significant (60%, $p<0.001$) reduction in serum TG-C contents. Again, the EGE treatment has enabled the diabetic rats to effectively maintain their serum triglycerides levels comparable to that of normal rats.

The diabetic rats have shown the highly significant (-26%, $p<0.001$) decrease in levels of serum high density lipoproteins (HDL-C) when compared to the normal rats. Non-significant differences in HDL values were observed in rats treated with AGE, while all other treatments have shown the highly significant (EGE; 32%, Insulin; 50%, and NSO; 63%, $p<0.001$) decrease in HDL-C contents when compared with diabetic rats. The hypolipidaemic effect of insulin and NSO was observed for HDL-C contents in present findings. However, again the EGE treatment was found effective in maintaining the normal levels of good fats (HDL) in diabetic rats.

The non-significant (9%, $p>0.05$) increase in low density lipoproteins (LDL-C) and slightly significant increase (36%, $p<0.05$) in very low density lipoproteins (VLDL-C) contents was noted in diabetic rats compared to the normal rats. Treatments of diabetic rats with AGE and EGE has reduced most significantly ($p<0.001$) the serum LDL-C (39% and 12%, respectively) and serum VLDL-C contents (57% and 29%, respectively) of treated rats when the values were compared to the diabetic rats. However, the insulin and NSO treatments to diabetic rats have resulted in highly significant (39% and 22%, respectively, $p<0.001$) elevation in LDL-C levels while the most significant (-58% and -73%, respectively, $p<0.001$) reduction in VLDL-C levels was observed in comparison to the diabetic rats.

Interestingly, while observing the effect of test treatments to lipid profile, the EGE treatment was found the most effective in maintaining the normal values for all the lipid attributes.

Earlier, the garlic powder treatment has shown the significant reduction in the LDL and cholesterol levels, while the garlic oil has shown significant elevation in the HDL cholesterol (Ried *et al*., 2013). In the present study, the lipid profile of diabetic rats was severely altered. Marked signs of abnormalities in lipid metabolism were exhibited by the diabetic animals as indicated by the elevated levels of TC, TG-C, LDL-C and VLDL-C, and more importantly the low levels of good fat, HDL-C. While describing the various properties of black cumin seeds such as its therapeutic potential, clinical aspect, and toxicity, Tembhurne *et al*. (2014) have reported the lipid lowering activity of black cumin seeds. Contrary to these findings, we have noted that the treatments of diabetic rats with insulin and NSO have shown the significant reduction in serum HDL contents, and elevation of LDL contents, not comparable to both the diabetic control and the normal values. However, both the garlic extracts have shown the better results, but the animals treated with EGE have shown the significant improvement in normalizing all the lipid contents analyzed.

Conclusions

In this study, we found the more rapid hypoglycemic and hypolipidemic effects of treatment of AGE, while the EGE has normalized the lipid profile comparable to that of the control values. The lipid profile has shown that the NSO treatments were equivalent in potential, but the effect was slow. Thus, we conclude that the garlic extracts substantiate the cell's glycemic and lipidemic status in diabetes and may be proposed to potentiate the antihyperglycemic effect of plant preparations and/or synthetic drugs.

Statement of conflict of interest

The authors have no conflict of interest.

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