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Evaluation of ameliorative effect of allicin (diallyl thiosulfinate) on experimentally induced diabetes mellitus in albino rats

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ABSTRACT
Background: Diabetes causes increased oxidative stress, to play an important role in the pathogenesis of various diabetic complications. The present study investigated the protective potential of allicin against diabetes in rat model.

Methodology: Animals were injected a single dose of streptozotocin (55mg/kg.b.wt) to induce diabetes. Control and diabetic rats were treated with allicin (20 mg/kg.b.wt) for 30 days. Glibenclamide (10mg/kg.b.wt) was used as a positive control. After the experimental regimen, the rats were sacrificed and the blood was collected and serum was separated. Blood sugar, body weight, total protein, oxidative stress markers (TBARS, CAT, SOD and GSH) and inflammatory markers (TNF-α and IL-6) were used to bio-monitor the protective potential of allicin.

Results: The experimental animals lost weight significantly, exhibited elevated blood sugar levels, decreased protein levels (p<0.05) in comparison to control animals; these alteration were corrected after allicin therapy. The significant elevation of TBARS content, reduction in total glutathione content and declined CAT and SOD activities in experimental animals; which were normalized following allicin therapy. The altered serum inflammatory markers were significantly reduced to near normal levels. No significant alterations in allicin alone administered rats.

Conclusion: We thus conclude that allicin has potent antihyperglycemic, anti-lipidperoxidative and anti-inflammatory potential in diabetic animals.

INTRODUCTION
Diabetes is a common life-long health condition caused by an absolute or relative lack of insulin and is characterized by hyperglycemia in the postprandial and fasting state. The number of people with type I and type II diabetes are dramatically increasing on a global scale (Leila, et al. 2007). The World Health Organization (WHO) estimates that more than 180 million people in the world have diabetes and that this number will double by 2030 (WHO, 2009).
Diabetes is a chronic metabolic disorder with numerous complications such as retinopathy, neuropathy, and peripheral vascular insufficiencies (Chehade and Mooradian, 2000).

Hyperglycemia induced oxidative stress results in overproduction of reactive oxygen species (ROS) leading to an imbalance between free radical production and the ability of the antioxidant defense system to remove free radicals (Ugochukwu and Cobourne, 2003). Streptozotocin (STZ) acts as a diabetogenic agent due its capacity to destroy pancreatic β-cells, possibly through a free radical mechanism (Halliwell and Gutteridge, 1994). Various hypotheses to explain the genesis of free radicals in diabetes include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products (AGEs), and enhanced glucose flux through the polyol pathway (Tiwari and Rao, 2002).

The generated ROS produce oxidative stress and exert major effects on signaling pathways, which further influence cellular metabolism and trigger a low-grade inflammatory reaction (Dominiczak, 2003). Moreover, glucose in itself is pro-inflammatory and increases the levels of acute-phase inflammatory markers, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP). These acute-phase inflammatory markers are associated with insulin resistance and metabolic syndrome, suggesting a role for chronic low-grade inflammation in diabetes mellitus (Niehoff, et al. 2007).

Diabetes is still not completely curable by the present antidiabetic therapy. Insulin therapy is the only satisfactory approach in diabetic mellitus, even though it has several drawbacks like insulin resistance (Piédrola, et al. 2001), anorexia, brain atrophy, and fatty liver in chronic treatment (Weidmann, et al. 1993). There are several oral hypoglycemic agents used therapeutically but certain adverse effects and weak effectiveness of them have led to the search for more effective agents. WHO recommended that medicinal plant research warrant attention to search for anti-diabetic properties (WHO, 1980). Our previous studies indicated that medicinal plant modulates the effect of circulatory antioxidants against oral carcinogenesis (Sasikumar, et al. 2010). The protective effects of medicinal plants have been attributed to various phytoconstituents, such as carotenoids, vitamin C and phenolic acids (Tan, et al. 2011). In particular, several studies have also focused on the biological activities of flavonoids, a group of plant polyphenolic compounds that possess potent antioxidant effects (Xiao, et al. 2011).

Allicin, diallyl thiosulfinate (or S-(2-propenyl) 2-propene-1-sulfinothioate), is the main biologically active compound derived from garlic (Allium sativum L.). Chemical structure of allicin is show in Fig. 1.

![Chemical structure of allicin](image)

Fig. 1: Chemical structure of allicin [S-(2-propenyl) 2-propene-1-sulfinothioate or diallyl thiosulfinate].

However, no evidence is available on the ability of allicin on ameliorative effects associated with free radical scavenging and inflammatory response on diabetes. Hence, the present study was designed to evaluate the therapeutic efficacy of allicin on diabetic animals.

MATERIALS AND METHODS

Animals
Male albino rats (Wistar Stain), weighing 250–290 g were used for the study. The animals were obtained from the Central Animal House, King Saud University, Riyadh, KSA. The rats were housed in polypropylene cages and were maintained in controlled atmosphere (temperature of 22±2°C and 55±5 % humidity) with a 12 hr light:dark (LD) cycles in an experimental room. The normal laboratory diet (Manufactured by Grain Silos and Flour Mills Organization, Riyadh, KSA) and water given ad libitum.

Chemicals
Streptozotocin was obtained from Sigma Aldrich Chemical Pvt Limited, Bangalore, India. Allicin was purchased from Shanghai Harvest Pharmaceutical Co., Ltd. (Shanghai, China). All other chemicals used were of analytical grade.

Induction of diabetes
The STZ was dissolved in citrate buffer (pH 4.5), and rats were made diabetic by injection of a single dose of STZ (55 mg/kg, i.p). They were given 5% of glucose in drinking water for the first 24 h to encounter any initial hypoglycemia. On the 3rd day animals were checked for serum blood glucose levels and those with more than 300 mg/dl were used in the study (Rathod, et al. 2008).

Experimental design
The rats were divided into 5 groups of 6 rats each as follows. Group I – Normal animals injected with vehicle (citrate buffer), Group II, III and IV rats were induced diabetes. After diabetic induction group II received vehicle only. Group III diabetic rats were treatment with allicin (20 mg/kg body weight, orally) for 30 days by gastric intubation using force-feed needle. Group IV diabetic received glibenclamide (10 mg/kg, p.o.). Group V rats received oral administration of allicin alone throughout the experimental period. After the experimental regimen, the rats were fasted overnight and were sacrificed by cervical dislocation under light ether anesthesia, and the blood was collected on decapitation and serum was separated by centrifugation (20 min at 2000 rpm) and stored at –20°C for biochemical assays.

Determination of oxidative stress markers
Thiobarbituric acid reactive substances (TBARS) were assayed in plasma according to the methods of Yagi, (1987). Plasma was deproteinised with 10% phosphotungstic acid and the precipitate was treated with thiobarbituric acid at 90°C for 1 hour. After cooling, 5.0ml of n-butanol was added and the mixture was shaken vigorously and centrifuged at 1000g for 15 minutes. The pink color formed gives a measure of TBARS. Reduced glutathione (GSH) was determined by the method of Beutler and Kelly, (1963). The technique involved in protein precipitation by meta phoshphoric acid and spectrophotometric assay at 412 nm of the yellow derivative obtained by the reaction of supernatant with 5,5'...
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dithio-bis-2-nitrobenzoic acid (DTNB). Superoxide dismutase (SOD) activity was assayed by the method of Kakkar, et al. (1984). The assay of superoxide dismutase was based on the inhibition of formation of NADH-phenazine methosulphate nitroblue tetrazolium formazan. The colour formed at the end of the reaction was extracted into butanol layer and measured at 520nm. Catalase (CAT) activity was assayed using the method of Sinha, (1972). The method is based on the utilization of H$_2$O$_2$ by the enzyme. The color developed was read at 620 nm.

**Estimation of sugar and protein**

Blood glucose was determined by the method of Sasaki and Matsy, (1972) using O-toluidine reagent. The protein content was estimated using a previously reported method (Lowry, 1951).

**Determination of inflammatory markers**

Serum TNF-α concentration was estimated by using the CYTELISA rat TNF-α obtained from CYTIMMUNE Sciences Inc., Maryland, USA. The intra assay variation was 6.7%. To avoid inter assay variation all samples were run at one time. Optical density of each well was determined by using a microplate reader (Thermo Labsystems, Finland). Serum IL-6 was estimated by using the ELISA Quantikine rat IL-6 immunoassay kit obtained from R and D Systems Inc., Minneapolis, MN, USA. The intra assay variation was 5.5%. To avoid inter assay variation all samples were run at one time. Optical density of each well was determined by using a microplate reader (Thermo Labsystems, Finland).

**Statistical Analysis**

The analysis was done on SPSS 20.0 statistical package. The data are expressed as mean±SD. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). The results were considered statistically significant at p-values less than 0.05.

**RESULTS**

Fig. 2 shows the effects of allicin on body weights of normal and experiment animals. Body weights were similar in all the groups at baseline. The body weights of 30 days diabetic rats were significantly lower than non-diabetic controls. The rats started regaining weight after instituting allicin therapy and reached their baseline levels within 1 month. Administration of allicin alone did not show any effect on body weights.

![Fig. 2: Effects of allicin on body weight in diabetes induced albino rats.](image-url)
The blood sugar and total protein levels of control and experimental animals in each group are depicted in Table 1. Blood glucose levels were elevated in the diabetic animals compared to control animals. However, this increase in blood sugar levels in allicin and reference drug (Glibenclamide) treated rats were significantly (p<0.05) decreased. The levels of serum protein were significantly decreased (p<0.05) in diabetic rats compared to control rats, whereas in allicin treated rats, these levels were significantly increased (p<0.05) compared to group II rats. The altered levels of blood sugar and serum protein were normalized by treated with glibenclamide. No significant alterations in these parameters were noted in allicin alone administered group of rats.

Table 1: Effects of allicin on blood sugar and serum protein in diabetic induced albino rats (Mean±SD; n = 6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Normal)</th>
<th>Group II (Diabetic)</th>
<th>Group III (Diabetic + allicin)</th>
<th>Group IV (Diabetic + Glibenclamide)</th>
<th>Group V (allicin alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood sugar (mg/dl)</td>
<td>99.33±7.56a</td>
<td>335.89±22.92b</td>
<td>126.65±11.78c</td>
<td>116.91±11.21d</td>
<td>109.23±8.62ad</td>
</tr>
<tr>
<td>Serum protein (g/dl)</td>
<td>7.09±0.44a</td>
<td>4.03±0.37b</td>
<td>6.83±0.59a</td>
<td>6.98±0.56a</td>
<td>6.45±0.55a</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly at p<0.05.

Table 2 represents the activity of oxidative stress markers in the different experimental groups. There was significant elevation in the TBARS content, with a concomitant reduction in the total glutathione content in the diabetic rats. Treatment with allicin and glibenclamide reverses the TBARS content back to normal level and increases the GSH content. The activities of CAT and SOD enzymes were also found to be declined in the plasma of STZ-induced rats which was restored to normal levels on allicin and glibenclamide treatment. Allicin only administered rats registered no significant changes (group V).

Table 2: Status of TBARS and antioxidants in plasma of control and experimental animals (Mean±SD; n=6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Normal)</th>
<th>Group II (Diabetic)</th>
<th>Group III (Diabetic + allicin)</th>
<th>Group IV (Diabetic + Glibenclamide)</th>
<th>Group V (allicin alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmoles/mL)</td>
<td>3.22±0.20a</td>
<td>4.93±0.38b</td>
<td>3.57 ± 0.27c</td>
<td>3.32 ± 0.25ac</td>
<td>3.21 ± 0.24a</td>
</tr>
<tr>
<td>CAT (nmol/min/ml)</td>
<td>84.39±6.43a</td>
<td>53.61±3.47b</td>
<td>80.83±5.38a</td>
<td>83.48±4.88a</td>
<td>83.01±6.32a</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>21.90±1.67c</td>
<td>13.83±1.28b</td>
<td>19.73±1.3c</td>
<td>20.71±1.41ac</td>
<td>21.78±1.66c</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>104.48±6.52a</td>
<td>70.00±5.33b</td>
<td>98.57±6.32a</td>
<td>102.02±7.77a</td>
<td>103.87±6.33a</td>
</tr>
</tbody>
</table>

*The amount of enzyme required to inhibit 50% nitroblue tetrazolium (NBT) reduction. Values not sharing a common superscript letter differ significantly at p<0.05.

Table 3 shows the effects of allicin compared with normal group I animals showed a significant rise in serum acute-phase inflammatory markers (TNF-α and IL-6). Treatment with allicin on inflammatory markers. STZ-treated rats and Glibenclamide significantly reduced the serum TNF-α and IL-6 to near normal levels compared with group II diabetic animals. Administration of allicin alone
did not show any effect on inflammatory markers.

**Table 3:** Effects of allicin on inflammatory markers in diabetic induced albino rats (Mean±SD; n=6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Normal)</th>
<th>Group II (Diabetic)</th>
<th>Group III (Diabetic + allicin)</th>
<th>Group IV (Diabetic + Glibenclamide)</th>
<th>Group V (allicin alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>100.01±7.62a</td>
<td>199.90±15.22b</td>
<td>115.99±8.83c</td>
<td>103.92±6.93ac</td>
<td>111.89±8.52ac</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>381.04±29.01a</td>
<td>596.46±36.54b</td>
<td>402.05±30.61a</td>
<td>399.61±30.43a</td>
<td>401.07±30.54a</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly at p<0.05.

**DISCUSSION**

Rats treated with STZ display many features seen in human beings with uncontrolled diabetes, including hyperglycemia, hypoinsulinemia, increased urinary glucose levels, and consequently polyurea as well as weight loss (De Angelis, *et al.* 2002). The destruction of β-cells during diabetes ultimately causes physic-metabolic abnormalities such as decrease in body weight gain and increase in food and water intake (Rodriguez, *et al.* 1997). In addition, diabetic rats showed a clear muscle atrophy involving a decrease in both skeletal muscle mass and protein content. These changes were related to important alterations in protein turnover in skeletal muscle (Pepato, *et al.* 1996). Hence, a notable decrease in the body weight change observed in the diabetic group of rats (group II) might be the result of protein wasting due to the unavailability of carbohydrates for energy metabolism (Brodsky, 1998). The improvement in body weight gain in diabetic rats supplemented with allicin highlight the body glucose homeostasis which in turn promotes the body weight gain.

Hyperglycemia causes oxidative stress by inducing the overproduction of ROS, which results in an imbalance between free radical production and the ability of the antioxidant defense system to remove free radicals (Ugochukwu and Cobourne, 2003). Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications (Ceriello, 2000). Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses (Saxena, *et al.* 1993). ROS results in lipid peroxidation and subsequently increased in TBARS levels leading to degradation of cellular macromolecules. A marked increase in the concentration of TBARS in STZ-induced diabetic rats indicates enhanced lipid peroxidation (LPO) leading to tissue injury and failure of the antioxidant defense mechanisms to prevent overproduction of ROS. It has been proposed that streptozotocin (STZ) acts as a diabetogenic agent owing to its ability to destroy pancreatic β-cells, possibly by a free radical mechanism (Halliwell and Gutteridge, 1994). The level of LPO in cell is controlled by various cellular defense mechanisms consisting of enzymatic and non-enzymatic scavenging systems which are altered in diabetes. Moreover, disturbances of antioxidant defense systems in diabetes showed alteration in antioxidant enzyme levels, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) along with impaired glutathione (GSH) metabolism (McLennan, Heffermen and Wright, 1991).

SOD and CAT are the two major scavenging enzymes that remove free
Evaluation of ameliorative effect of allicin (diallyl thiosulfinate) on experimentally diabetic animals. A decreased activity of these antioxidants of group II animals can lead to an excess availability of superoxide anion (O2-) and hydrogen peroxide (H2O2), which in turn generate hydroxyl radicals (·OH), resulting in initiation and propagation of LPO (Jin, et al. 2008). The SOD and CAT play a prominent role in scavenging free radical and restoring antioxidant activities in the plasma and it protects cell protein and cell membranes against oxidative stress (Rahimi, et al. 2005) of diabetic animals. These observations may clearly indicate that the increased levels of SOD and CAT in allicin treated animals has free radical scavenging activity, which may exert a beneficial effect against pathological alterations caused by reactive oxygen species.

Glutathione plays an important role in the endogenous non-enzymatic antioxidant system. Primarily, it acts as reducing agent and detoxifies hydrogen peroxide in presence of an enzyme, glutathione peroxidase (Ketterer, et al. 1976). The depleted GSH may be due to reduction in GSH synthesis or degradation of GSH by oxidative stress (Loven, et al. 1986) in diabetic induced animals. Treatment with allicin increased the activity of antioxidants, which scavenge the free radicals generated during diabetes.

Increased oxidative stress is supported by raised cytokine levels (Esposito, et al. 2002). High amounts of circulating inflammatory cytokines, such as TNF-α and IL-6, contribute significantly to insulin resistance in muscle and adipose tissue (Hotamisligil, et al. 1995) of diabetic animals. Tumor necrosis factor-alpha (TNF-α) plays a key role in the pathogenesis diabetes (Argile’s, et al. 1994). TNF-α inhibits insulin action in animal and in vitro models (Bruun, et al. 2003). Human cross-sectional studies have shown that elevated circulating TNF-α level are associated with insulin resistance and diabetes mellitus (Mishima, et al. 2001; Fernandez-Real, et al. 2002).

Many mechanisms may account for the metabolic effect of TNF-α, including the down regulation of genes related to normal insulin action and direct effects on glucose transport, and pancreatic β-cells (Grimble, 2002). IL-6 was among the first cytokines implicated as a predictor or pathogenetic marker of insulin resistance and cardiovascular disease (Fernández-Real and Ricart, 2003). Therefore, these mediators have detrimental effects on glucose metabolism. In the present study, TNF-α and IL-6 were observed to be significantly increased in diabetic rats and reverted back to normal after allicin and glibenclamide therapy. Allicin treatment reduced the serum TNF-α and IL-6 levels of diabetic rats, suggesting anti-inflammatory activity of the bark extract. Furthermore, the allicin was found to be as ameliorative potential of the reference drug glibenclamide.

The results obtained in this study clearly indicate that oral administered allicin to diabetic rats showed significant antihyperglycemic, anti-lipidperoxidative effects and this effect may be attributed at least in part to its anti-inflammatory properties as evident by attenuating TNF-α and IL-6 levels in diabetic rats. We thus conclude that allicin have potent ameliorative potential in STZ-induced diabetic rats.

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Evaluation of ameliorative effect of allicin (diallyl thiosulfinate) on experimentally


**ARABIC SUMMARY**

تقييم الخصائص الإصلاحية لمادة الأليسين (ثاني الأليل ثيو سلفونيت) ضد داء السكري المستحث تجريبياً في الفئران

ساسي مومار دونا راوس

قسم الكيمياء الحيوية، كلية الطب، جامعة حائل، المملكة العربية السعودية.

خلفية: من المعروف أن داء السكري يزيد من الجهد التأكسدي مما يحتم عليه الكثير من مضاعفات المرضية. وبحث هذه الدراسة الحالية في الإمكانيات الوقائية لمادة الأليسين ضد داء السكري في نموذج الفئران المحميات: سحقن الفئران بالمادة "المستبرتوتوسين" (5 ملجم/كمجم) لإحداث داء السكري. ثم تعال جمجمة الفئران المحمية والفاشياء بمادة الأليسين لمدة 30 يوماً. وقد تم استخدام مادة غليبي ككلاميد (1 أجم/كمجم) كمجمة مجمعة تحكم موجبة بعد النظام التجربي ثم جمعت عينات الدم من الفئران بعد ذبحها لفصل المصل عن الدم. واستخدمت كل من قياسات سكر الدم وزرن الجسم والبروتينات الكلية والكاملة والكبدية ودلال الإجهاد التأكسدي والالتهابات لرصد الحيوي لتعريف السكر الوقائي للعينات.

النتائج: توجد في حيوانات التجربة قدر معيون لألسبيس نوارا وارتفاع في سكر الدم مع انخفاض في البروتينات الكلية (وقال عند درجة ممونية 100%) مقارنة بمجمعة التحكم. بل إن هذه المثمانية تصبح جائحة بعد العلاج SOD وCAT يتم تعديل محتوي TBARS المحمية، في مجموعة الفئران و CAT في تقليل تأكسدي الكبدية ونشاط الأنزيمي TBARS و SOD في الفئران المحمية مادة الأليسين عن الفئران المحمية. تغيرا في الفئران المحمية مادة الأليسين بعده.

الخاتمة: إذا استنتج أن الأليسين كمصمم مضاد للالتهابات في سكر الدم ومضاد لأكسدة الدهون ومضاد للالتهابات في المحميات الحيوية. كلمات مفتاحية: أليسين، الجهد التأكسدي، داء السكري، انخفاض تأكسدي الدهون، بروكسيسين الدوب.