Ameolirative effects of garlic (Allium sativum L.,) in streptozotocin induced oxidative stress in diabetic brain of rat

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Abstract

The aim of the present study was to evaluate the protective effect of methanol garlic extract on the enzymes related with polyol pathway, advanced glycation end products, markers of oxidative stress and antioxidant status in brain of streptozotocin induced diabetic rats. Antioxidant capability of methanol extract of garlic was evaluated by 2,2-diphenyl-2-picrylhydrazyl hydrate radical and FOX (ferrous ion oxidation-xylenol orange) H_2O_2 scavenging test. Diabetes was induced by single i.p injection of STZ (32mg/kg per body Wt.,). Blood glucose levels and body wt, were measured on every 7th day over a period of 30 days. The diabetic rats treated with garlic extract at two doses 250mg/kg and 500mg/kg body wt., by oral administration. Diabetic rats showed significant increase in food and water intake, decrease in blood glucose levels, body weights, but could not show any recovery by garlic treatment. Garlic treatment significantly decreased aldose reductase (AR); sorbitol dehydrogenase (SD) and glutathione S-transferase (GST) enzyme activities. A decrease of malndialdehyde (MDA), Protein carbonyls (PC), Pentosidine advance oxidation protein products (AOPP), Advanced glycation end products (AGEs) was also observed. Additionally garlic administration produced a restoration of brain superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) in diabetic rats. Garlic treatment also reduced the free radical formation and progression of hyperglycemia induced diabetic complications by decreasing influx of glucose into the polyol pathway and increased activity of antioxidant enzymes. The current study reveals exerts, efficiently, an attenuating effect of methanol garlic extract exterted antihyperlgycemic, antioxidant and anti-glycating effects in a dose dependent manner in diabetic rats.

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INTRODUCTION

Diabetes is a metabolic disorder, growing at a faster pace, incidence is been increasing annually all over the world and it is predicted that this number could jump to 592 million by year 2035. The reasons for this rise is projected to be increase in urbanization, westernized diet, decreased levels of physical activities etc. Hyperglycemia and Insulin resistance is characterized feature of diabetes. Uncontrolled hyperglycemia may start degenerative processes in both Peripheral organs including peripheral nervous system and CNS (Baynes, 1991) due

to high free radical generation. Many studies documented that these free radicals result in the end organ damage induced by type-II diabetes. And many results that are conducted on animals and humans had confirmed the role of free radical mediated oxidative stress involved in the pathogenesis of damage of brain. The mechanism seems to be activation of polyo pathway by oxidative stress. Activation of polyo pathway by oxidative stress seems the main mechanism involved in diabetes, along with nonenzymatic glycation of proteins yielding advanced glycation end products (AGEs), glucose autooxidation (Hunt et al., 1990), disturbance in oxidative stress markers and diminishes in the antioxidant enzymes.

Brain is extremely susceptible to chronic hyperglycemia in diabetic condition there are many evidence indicating that ROS and other free radicals has crucial role in neurodegeneration especially in cortex region, in which high content of neurons are much more sensitive to ROS damage and associated cognitive decline in aging (Ranjbar et al., 2010). Superoxide radicals or nonradical H_2O_2 are generated as part of metabolism. Lipid peroxidation, inactivation of many antioxidant enzymes and DNA rupture are the results of imbalance between antioxidant defense mechanism and ROS, which is caused by oxidative stress.

Brain synaptosomes of diabetic rats are fictile to oxidative stress (Devi et al., 2007). Morphological, Neuropsychological alterations and Neurophysiological changes are involved in complications of diabetes. Free radical scavengers protect neurons against neurodegenerative conditions. There is strong evidence that biological antioxidants could prevent diabetes induced complications (Koçak et al., 2000). Hence currently researchers are involved in developing potent pharmacological agents with an ability to protect the tissues from hyperglycemia and its secondary complications without any side effects.

In accordance of severity of fundamental progression of hyperglycemia and its complications, there is active search for novel therapies through out the world. There are various investigations on traditional treatment methods for hyperglycemia. To confirm the grandness of garlic (*Allium sativum Linn*) as a folk remedy for hyperglycemia the present work was planned. It belongs to Liliaceae family, which is used as spice in Indian food, with many medicinal uses for health issues in man.

Apart from having antidiabetic properties garlic has many protective medical properties such as antihypertensive Potential; Wound Healing Potential, Anti-cancer Potential, Hepatoprotective Potential and antioxidant potential etc. There are not many studies on animals that prove, the role of garlic extract on polyol pathway enzymes, inhibiting AGEs, oxidative stress markers and antioxidant enzymes. This current study reports the protective effect of garlic on neuronal tissue of brain in diabetic rat.

MATERIALS AND METHODS

Chemicals and Reagents

Streptozotocin (STZ), TBA (2-thiobarbituric acid, TEP (1,1,3,3-tetra ethoxy propane), OPT (O-Pthaladehyde), NEM (N-ethyl malemide), DNPH (2,4-dinotrophenylhydrazine), Glutathione (GSH) were bought from Sigma Chemical (USA). All other chemicals and reagents used were of AR grade unless otherwise specified.

Animals

Albino Wistar rats (male) of 200-225g each of aged about 2 to 3 months were brought from NCLAS (NIN), Hyderabad. They were housed in cleaned individual plastic cages with (22-24 °C) controlled temperature, at 12h light/12h dark cycle and standard pellet diet (NIN) was provided as food, water ad libitum. Monitoring of Food and water intake (daily) was done. One week of acclimatization to laboratory conditions was done. Every effort was made to minimize the suffering of the animals and the number of animals used. At most care was taken in the care and treatment of animals' throughsout the experiment. (CPCSEA No: 383/01/a/CPCSE).

Preparation of extract

Fresh garlic (bulbs) was bought from the market (Hyderabad, Telangana) and sliced into pieces and to take out moisture they were air-dried. The garlic-methanol extract was prepared in the ratio of 3:1 (300 ml of methanol-100g of garlic) by subjecting for 72 hours in soxhlet apparatus. After filteration of this extraction, it was lyophilized and was kept in -80C until used. Sterile water was to used dissolve the residual extract of garlic for further investigation.

Induction of diabetes

Single dose of (freshly) prepared streptozotocin (32 mg/ kg body wt.,) in 0.1 M citrate buffer of pH 4.5 was used to induce diabetes by intraperitoneal injection. Confirmation tests of fasting blood glucose were conducted after 72 hrs. Only animals above 180 mg/dL of blood glucose levels were used for further experimentation. Rats were kept in separate cages (2-3 per cage) due to excess urination in STZ induced rats. The bedding of the cage was changed

every day. After initiation of the experiment, body weights and blood glucose levels were measured at certain intervals.

Experimental design

In each group randomly selected animals which were divided into four experimental groups for 30 days (n=8)

Groups-I: Control, received citrate buffer as a vehicle.

Group-II: Diabetic rats, intraperitoneal injection of STZ.

Group-III: Animals received methanol garlic extract (250 milligram/kg bodyweight in sterile water).

Group-IV: received methanol garlic extract (500 milligram/kg body weight in sterile water).

Experimental protocol

Antioxidative property of extract

DPPH radical scavenging assay:- Scavenging activity of Garlic was measured by the method of Brand-Williams et al. (1995), (modified) with ascorbic acid as standard. FOX (ferrous ion oxidation-xylenol orange) H_2O_2 scavenging capacity assay: Garlic extracts efficacy as scavenging H_2O_2 was done by the FOX assay of Long et al. (1999) with pyruvate as standard. All the IC_{50} values were obtained from a dose-response curve.

Tissue extraction and processing

GOD-POD method was used for estimation of blood glucose on weekly basis. Animals were sacrificed by CO_2 asphyxiation after 4 weeks of experimentation; brains were extracted and kept in -80°C. A 10% homogenate of brain was done and used for MDA and total proteins were estimated. All the biochemical studies were done with 750Xg and 25,000Xg supernant at 4°C and necessary centrifugation depending on the assay. The tissue proteins estimated by Lowry et al. (1951).

Biochemical estimations

Polyol pathway Enzymes

The AR activity by Hayman and Kinoshita (1965). SDH activity was done by Gerlach and Hiby (1974).

Non-enzymatic glycation

The accelerated glycooxidation was measured by quantifying the Maillard reaction products i.e AGEs by Sell and Monnier (1989).

Oxidative stress markers

LPO was estimated by indirect method of measuring malondialdehyde using HPLC by Templar (1999) method. Protein carbonyls were estimated

spectrophotometrically by Uchida et al. (1998). AOPP determination was done by spectrophotometer by Kalousová et al. (2002) method.

Antioxidant enzymes

Catalase was estimated by Aebi (1984) method. Superoxide dimutase in the tissue was done by Marklund and Marklund (1974) Glutathione peroxidase activity was measured by Martinez et al. (1979) method.

Glutathione-S-Transferase was done by method assayed spectrophotometrically using 1-Chloro-2, 4-dinitrobenzene.

Glutathione activity in the tissue was done by Hissin and Hilf (1976) method.

Statistical analysis of data

For statistical analysis was done by one way Anova, followed by Post Hoc test (multiple comparisons). The differences were considered significant if p was <0.05.

Table 1: Effect of garlic extract on total protein levels in different groups

Groups	mg/g tissue
G-I	304.80±2.30
G-II	151.27±4.68*
G-III	172.64±1.95*
G-IV	269.73±2.4377*

The data are the mean ± SD (n=8). * denotes that datais significantly different from group-I at P<0.05

Table 2: Effect of garlic extract on food and water intake in different groups

Groups	Food intake g/24h	Water in take ml/24h
G-I	24.62±0.31	34±1.140
G-II	33.46±0.230*	106±3.049*
G-III	33.96±0.260*	118±2.121*
G-IV	33.52±0.277*	128±1.483

The data are the mean ± SD (n=8). * denotes that datais significantly different from group-I at P<0.05

RESULTS AND DISCUSSION

Free-radical quenching capacity of methanol-garlic extract

As Shown in Figure 1 and Figure 2 the IC_{50} (inhibitory concentration 50%) value of the $\alpha_1\alpha$-diphenyl-β-picryl-hydrazyl and H_2O_2 radical scavenging activity of methanol garlic extract were 424.02±4.375 μg/ml and 768.86±1 μg/ml.

Table 3: Effect of garlic extract administration on polyol pathway enzyme in streptozotocin induced diabetic rat brain tissue.

Groups	AR μmolesNADPHoxidized/h/100/mg protein	SDH μmolesNADPHoxidized/h/100/mg protein
G-I	15.12±0.59	2.06±0.08
G-II	48.80±1.14*	8.33±0.02*
G-III	38.12±1.11*	5.16±0.15*
G-IV	27.58±1.06*	3.75±0.12*

The data are the mean $\pm$ SD (n-8). * denotes that datais significantly different from group-I at P<0

Table 4: Effect of garlic extract administration on oxidative stress markers in streptozoticin induced diabetic rat brain tissue.

Groups	LPO nmole/gm tissue	Protein carbonyls nmole/gm tissue	AOPP nmole/gm tissue	Pentosidine Fluorescence units (au)
G-I	3.75±0.064	2.11±0.008	2.85±0.019	33.73±0.80
G-II	8.83±0.063*	6.63±0.024*	4.97±0.021*	61.19±1.03*
G-III	5.54±0.091*	5.18±0.010*	4.02±0.021*	51.43±1.21*
G-IV	4.59±0.480*	4.65±0.010*	3.30±0.021	41.02±0.72*

The data are the mean $\pm$ SD (n-8). * denotes that datais significantly different from group-I at P<0.05.

Table 5: Effect of garlic extract administration on Antioxidant enzymes in streptozoticin induced diabetic ratbrain tissue.

Groups	Catalase μmole H$_2$O$_2$ decomposed min^{-1} mg^{-1} protein	Superoxide dismutase (SOD) Unit/min/100 mg tissue	Glutathione peroxidase (GPx) μmole GSSG formed min-1 mg-1 protein	Glutathione S-Transferase (GST) μmole CDNB conjugated min-1 mg-1 protein	Glutthione reduced (GSH) Nmol g-1 wet weight tissue
G-I	3.45±0.008	7.62±0.0015	3.42±0.011	1..272±0.011	375.19±6.35
G-II	1.21±0.0008*	4.83±0.020*	2.05±0.019*	1.822±0.013*	171.88±5.32
G-III	2.98±0.008*	6.92±0.011*	2.61±0.015*	1.582±0.090*	304.95±3.54
G-IV	3.07±0.008*	7.35±0.032*	2.96±0.015*	1.494±0.033*	330.78±10.83

The data are the mean $\pm$ SD (n=5). * denotes that datais significantly different from group-I at P<0.01.

Body weight, Blood Glucose levels, Food and water intake

STZ induced diabetes lead to distinctive decrease in body weight (Figure 3), rise in blood glucose level (Figure 4), total proteins (Table 1), food (Polyphagic) and water (polydipsic) intake (Table 2). Fasting glucose levels of diabetic rats was significantly decreased by treated with garlic of 500 mg/kg body wt., rather than 250 mg/kg body wt. There was no affect of garlic treatment on body weights.

Enzymes of Polyol pathway

Aldose Reductase and Sorbitol dehydrogenase activity was significantly higher in Group -II (diabetic) rats in comparison of control (G-I) rats and treatment of garlic to diabetic rats decreased the activity of enzymes, but not up to extent of control rats (Table 3). And dosage dependent manner the 500 mg/kg body weight garlic treated rats were found to be more effective in reducing the AR and SDH activities when compared to 250 mg/kg body weight garlic treated group.

Non-enzymatic glycation

Significant increase in AGEs and pentosidine in diabetic rats (G-II) was seen by maximum fluorescence emission (Figure 5). Interestingly, methanol garlic extract inhibited AGEs causing a reduction in the intensity of fluorescence. Maximum inhibition was observed in 500 mg/kg body weight garlic treated rats were when compared to 250 mg/kg body weight garlic treated group.
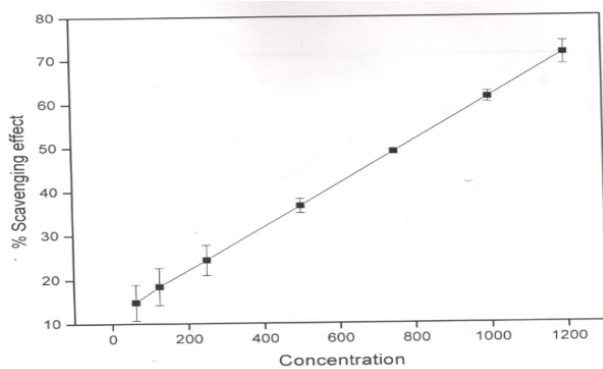
Methanol extracts of garlic scavenged H_2O_2 radical with an IC_{50} (inhibitory concentration) value of $768.86 \pm 1.76\mu g/ml$.

**Figure 1: The IC50 (inhibitory concentration 50%) value of the DPPH ($\alpha_1\alpha$-diphenyl-β-picryl-hydrazyl)**
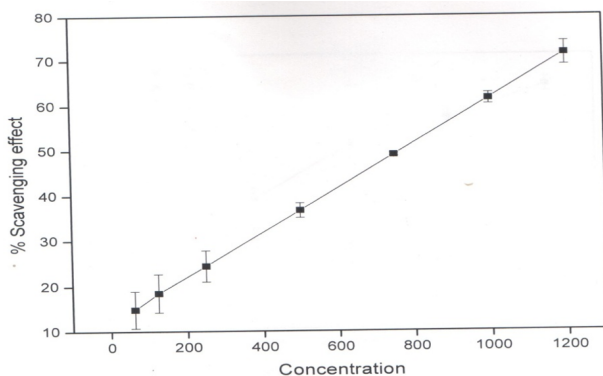
Methanol extracts of garlic scavenged H_2O_2 radical with an IC_{50} (inhibitory concentration) value of $768.86 \pm 1.76\mu g/ml$.

Figure 2: H_2O_2 scavenging capacity by ferrous ion oxidation-xylenol organe (FOX) assay
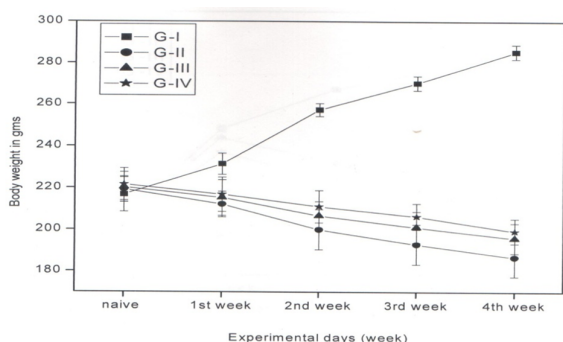
The data are the mean ± SD (n=8) that is significantly different from group-I at P<0.05

Figure 3: The effect of Garlic extract on body weights of different experimental groups
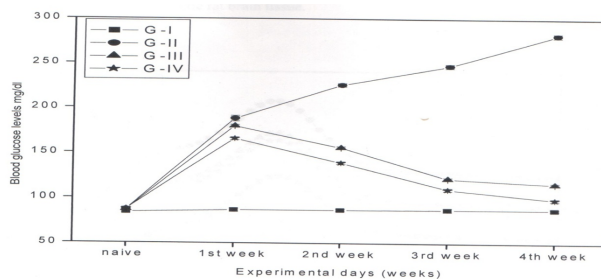
The data are the mean ± SD (n=8) that is significantly different from group-I at P<0.05

Figure 4: The effect of Garlic extract on blood glucose levels in STZ induced diabetic rats and other experimental groups.
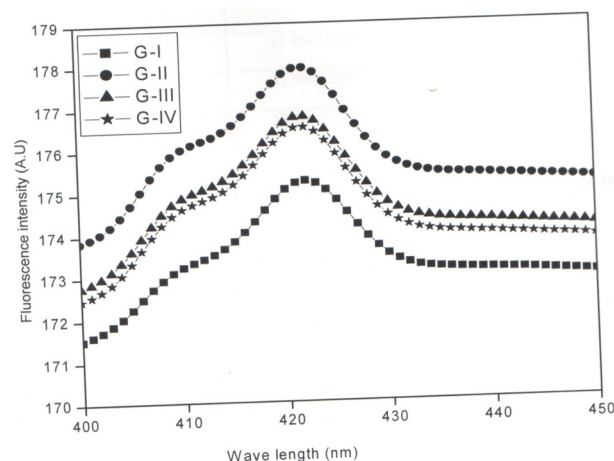

Figure 5: The effect of Garlic extract on AGE's in brain tissue of STZ induced diabetic rats and other experimental groups.

Oxidative stress markers

LPO, AOPP and protein carbonyls all the oxidative stress markers were significantly elevated in diabetic group indicating some percentage of release of oxy-radicals (Table 4). However, above oxidative markers were significantly restored in the rats that were administrated with 250 mg/kg body weight and 500 mg/kg body weight garlic though not to control levels.

Antioxidant enzymes

In diabetic rats significant decrease in GSH, GPx, CAT and SOD and GST activity was increased and levels of these enzymes were restored to some extent but not controls levels by administration of garlic with most potency in 500mg/kg body wt., garlic treated groups when compared to 250mg/kg body weight garlic treated groups rats (Table 5).

DISCUSSION

Neuropathy is a serious result of chronic hyperglycemia. According to Maiese *et al.* (2007) hyper-

glycemia is the main culprit for increasing number of ROS and plays vital role in ontogeny of diabetes and its complications. Hyperglycemia do affect Brains metabolism (glucose). In the present investigation we have reported methanolic extract of garlic 250 and 500 mg/kg body wt., doses, which were effectual in reducing blood glucose and this effect was even more pronounced in dose-dependent way higher the dose the more better the results. These results of plalsma glucose are consistent with the findings of Khayatnouri *et al.* (2011), and recent study shown the aged garlic is more potent in reducing blood glucose content (Jang *et al.*, 2018).

The diabetic group showed enhance in food and water intake confirms the polyphagic and polydipsic nature of the animal and decrease in body weight attribute to the rapid catabolism of lipids and proteins in the tissues with muscle wasting. The decline of total protein content in diabetic rats shows the insolubilization and aggregation of the proteins. Garlic treated diabetic rats showed the recovery of proteins content, which may be due its enhancing insulin release effect. This in turn increases the protein content due to its anabolic nature. These results are similar to the results of Mahdi *et al.* (2003), which also reported in increase of protein content in diabetic animal treated with crude extract of garlic bulbs.

Most of the studies reported that of all the organic extract preparations, methanol-garlic extract has been proved to be more potent as antioxidant. In the present study methanol garlic extract reveals dose dependent manner in DPPH radical scavenging activity, which is because of hydrogen-donating abilities (Chen and Ho, 1995). Heating of methanol garlic extract may decrease H_2O_2 scavenging capacity of the extract when compared to pyruvate but does not cut the radical scavenging activity suggesting that the compounds are thermostable. Other studies where garlic is used as one of the ingredients in herbal medicine also showed similar scavenging activity, but the potency of aged garlic was proved by study conducted by Jang *et al.* (2018).

Reactive Oxygen Species have vital role in conversion of amino acids of proteins carbonyl groups. Protein carbonyls and AOPP levels are markers of protein oxidation in diabetic patients (Çakatay, 2005). Diabetic rats showed increased in PCO and AOPP which indicates depletion of antioxidant enzymes. Garlic treatment of diabetic rats attenuated the PCO and AOPP showing the protective effect in antioxidant property.

The decrease in AR and SDH enzymes was observed in the garlic treated diabetic group. This indicates

that garlic inhibited high glucose influx through the polyol pathways, which is seen in diabetic rats. Hyperglycemia activates polyol pathway which consumes NADPH that is necessary for the reduction of GSH, which further results in diminished levels of the biologically active reduced form of antioxidant metabolite which further exacerbate oxidative stress. Ii *et al.* (2004), had shown that over expression of AR in lens of transgenic mice do deplete reduced GSH. Depletion of reduced GSH does effect in GSH peroxidase catalyzed removal of peroxide which is involved in scavenging of SOD on oxygen free radicals. The probable mechanism of garlic extract in decreased polyol enzymes is through the binding of bioactive organosulfur substances to the enzyme's active site. Garlic treatment showed decreasing effect of on tissue fructose content in eye (Sood *et al.*, 2003).

Apart from the role of polyol pathway, even AGE's also induce oxidative stress in diabetes. Nishikawa *et al.* (2000), have shown that hyperglycemia can disrupt Electron Transport Chain in the mitochondria, give rise to overproduction of superoxide anions. Hyperglycemia induce non-enzymatic glycation which also stimulate oxidative stress. According to Yim *et al.* (2001), the process of formation of AGE's also initiate ROS generation, their interaction with receptors further increase ROS production. Hence, inhibiting the formation of AGE's in diabetes activates antioxidant enzymes and sensitize antioxidant defense. Hence, in our study diabetic rats treated with garlic have shown in decreased level of AGE's formation and pentosidine. The probable mechanism of ginger may be due to chelate ion of transition metals. The Brain is highly sensitive to oxidative stress induced by oxygen free radicals because the Brain has highest demand for oxygen and also the brain is not enriched with antioxidant defense in comparison of other organs. An increase in LPO levels will be high in the brain because CNS is a reservoir of lipids and may be the primary target of free radical-mediated lipid peroxidation. Increased LPO in brain may be due to free radical generation (Pari and Latha, 2004). The changes in LPO in the brain of diabetic rats show decreased grade of antioxidant enzymes, catalase, glutathione peroxidase, SOD, reduced glutathione, and glutathione-S-transferase. The role of these enzymes is vital in cleaning the metabolites of incomplete oxidation.

The dismutation of $O_2{}^-$ and decomposition of H_2O_2 are due to a rise in antioxidants (GPx; CAT), that further progrates LPO by generating hydroxyl radicals. Decreased LPO in diabetic rats with garlic treatment confirmed the antioxidant potential of garlic against oxidative injury which is similar to

the result shown by (Durak *et al.*, 2002). Decrease of dismutase O_2^- and decomposition of H_2O_2 is vital feature affiliated with garlic treatment and this result is in accordance with Wei and Lau (1998); Geng and Lau (1997). The reduced activities of CAT in diabetic rats resulted in accumulation of O_2^- and H_2O_2 by autooxidation of glucose and non-enzymatic glycation which has deleterious effects (Mazunder *et al.*, 2005). Diabetic rats showed decreased level of SOD, but these diabetic animals when treated with garlic extract SOD was recovered which suggest the free radical scavenging activity (Pedraza-Chaverrí *et al.*, 2007) which could wield good activity against pathological alteration caused by the presence of O_2^-, H_2O_2 and OH. The high GST level in these rats is possibly having an exact defense mechanism by detoxifying the toxic metabolites (ketone bodies) by scraping radicals. However, the increased enzyme activity might not be in the same proportion as that of free radicals and this could be a source for an imbalance in the internal milieu and, which is in accordance to the study conducted by El-Demerdash *et al.* (2005) in diabetic condition. Reduced GSH was seen decreased in diabetic rats which suggests an increased level of impairment caused by oxidative stress. But after garlic extract treatment there was significant recovery of GSH which suggests its protection from reactive oxygen species (Rastogi *et al.*, 2008).

CONCLUSION

All studies of garlic in humans and animals have revealed the antioxidant property. But current study not only confirms the antioxidant property of garlic by studying oxidative stress markers and increased antioxidant defense mechanism but also the other pathway which triggers the oxidative stress in the form of the polyol pathway. If natural product has the potency of antioxidant, antidiabetic property, which can also inhibit the polyol pathway and AGE's can be concluded as potent drug for treating diabetic and its complications, and the present study show that garlic has all the above said properties.

ACKNOWLEDGEMENT

Conflict of interest

The authors declare that they have no conflict of interest for this study.

Funding support

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