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Reno-protective effect of garlic extract against immobilization stress induced changes in rats

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PEER REVIEW

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Comments

This study is novel in term of prophylactic and curative effect of crude garlic extract of *A. sativum* against immobilization stress particularly in kidney. Based on the observation of certain biomarkers and antioxidant enzymes, it is clear that crude extract of garlic has encouraging effects over deranged free radical damage by immobilization stress in kidney.
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ABSTRACT

Objective: To examine immobilization stress-induced antioxidant defense alterations in rat kidney and the antioxidant effects of aqueous garlic extract in pre and post stress extract treatments.
Methods: Albino rats were treated with aqueous extract of garlic both before and 6 h of immobilization stress. Pro-oxidant eminence of rat kidney was assessed by determining the levels of glutathione, thiobarbituric acid reactive substances, aspartate aminotransferase, alanine aminotransferase, glucose, uric acid, alkaline phosphatase and antioxidant enzymes activities.
Results: In response to 6 h of immobilization stress, a significant rise in the level of kidney enzymes was recorded. However, antioxidant enzyme activities showed a sharp decline.
Conclusions: The extract treatment before and after the stress reverted the activities of above mentioned enzymes towards their control values. Hence, garlic extract can be given as nutritional supplement for scavenging the free radicals generated in rat kidney.

KEYWORDS
Stress, Antioxidant enzymes, AST, ALT, MDA, Garlic

1. Introduction

Garlic [*Allium sativum* L. (*A. sativum*)] is used by all civilization as a source of medicines since ancient times and also used as the flavoring agent and traditional medicine for healing. Recently, there has been rising interest in abusing the biological role of different Ayurveda medicinal plants, which is due to its cost effectiveness, their natural origin and lesser side effects. Recent studies have demonstrated and validated many medicinal properties attributed to garlic. Different types of garlic supplements like garlic powder (tablets), aged garlic extracts (capsules, tablets and liquid), garlic oil (capsule) is commercially available; each being different in organosulfur compound profile. Extensive studies carried out on garlic have described the presence of two main classes of antioxidant machineries, namely flavonoids and sulfur-containing compounds (diallyl sulfide, trisulfide and allyl-cysteine)[1,2]. These are likely to play an important role in the widely demonstrated biological effects of garlic, which include antitumor, hypolipidemic, hypocholesterolemic, antiatherosclerotic, antioxidant and immunomodulatory effects[3-5]. It has been reported that this effect of garlic extract emerges as it prevents the lipid peroxidation (LPO) by ROS, the oxidative damage to DNA and protein s-allyl cysteine (SAC) is the most abundant compound in the aged garlic[6,7]. Several *in vitro* and *in vivo* studies documented the antioxidant properties of both aged crude garlic extract as well as purified SAC. Considerable number of studies reported their ability to ROS and

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reactive nitrogen species[6,8].

Life exists by sustaining a complex dynamic equilibrium, or homeostasis, that is constantly challenged by intrinsic or extrinsic forces or stressors[9]. We earlier demonstrated that the restraint or immobilization stress is an easy and simple method to induce both physical (muscle work) and psychological stress (escape reaction), which results in both, restricted mobility and subsequent aggression[10]. Several studies have reported that oxidative damage is due to enhanced free radicals in various kinds of stresses. Generation of free radicals and subsequent oxidative damage like peroxidation of lipids are the potent outcomes of the restraint stress. Moreover, stress has been suggested to decrease the level of glutathione (GSH) and vitamin C, which plays an important role in the protection of tissues from oxidative damage[11].

Biological systems have developed antioxidant defense mechanisms which disrupt the oxidation chain by providing electrons to free radicals without becoming reactive themselves[12]. Therefore, the adverse effects of free radicals depend on the balance between the speed of their generation and dynamics of their inactivation by the defense system, especially endogenous antioxidants.

The present study was carried out to investigate the preventive effect of a single dose of aqueous garlic extract on the immobilization stress induced oxidative damages. We investigated and compared activities of superoxide dismutase (SOD), catalase (CAT) and GSH-S-transferase (GST) as endogenous antioxidant enzymes, and the level of thiobarbituric acid reactive substances (TBARS), GSH, alkaline phosphatase (ALP), glucose, uric acid, aspartate aminotransferase (AST) [serum glutamic oxaloacetic transaminase (SGOT)] and alanine aminotransferase (ALT) [serum glutamic pyruvic transaminase (SGPT)]. The results of the study are likely to contribute to understanding the potential of garlic extract in preventing/ alleviating stress induced diseases involving oxidative damage to cellular constituents.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin, 1-chloro-2, 4-dinitrobenzene, and thiobarbituric acid were purchased from Sigma (St Louis, MO, USA); 5-5'-dithiobis-2-nitrobenzoic acid, hydrogen peroxide and pyrogallol were procured from E-Merk (Darmsradt, Germany). All other chemicals used were of analytical grade and obtained from commercial sources.

2.2. Preparation of garlic extract

One kilogram of garlic cloves (*A. sativum* L.) was purchased from the local market, peeled and grounded with an electric mincer until an aqueous suspension was obtained. It was diluted in double distilled water at 4 g/mL on the basis of the weight of the starting material and centrifuged (Beckman J20) for 15 min at 6500 r/min at 4 ℃. The supernatant was aliquot and stored at -80 ℃ until use. Garlic mainly contains organosulfur compounds such as allicin, ajoene, diallyl disulphide, diallyltrisulfide, SAC, SAC sulfoxide and flavonoids, phenolics and anthocyanins. It also contains carbohydrates, proteins, fatty acids, glycolipids, phospholipids, fiber, saponins, glycosides, lectins, and vitamin B1, B2, B6, C and E[13-15].

2.3. Animal stress procedure and treatments

Adult male Albino Wistar rats weighing in the range of 180-200 g were housed in group cages, Purina diets were supplied to them and tap water ad libitum. Experimental protocols adhered to the guidelines of the animal welfare committee of the University. Prior to commencement and throughout the experiment the rats were housed at (24 ± 3) ℃ room temperature and 12 h light/ dark cycles.

A preliminary dose dependent study (n=3) was performed to find out the best therapeutic dose of garlic, which can modulate deranged free radical metabolism (results not shown). It was observed that the extract at 100 mg/kg body weight dose for 2 h had the most preventive effect on oxidative stress changes in the kidney. To elucidate the effect of immobilization stress induced pro-oxidant changes and its attenuation by garlic extract, 40 rats were selected and divided into 5 groups (n=8 per group). The groups were: group with no stress and no treatment (Control group); a group with immobilization stress without any treatments (Stressed group); group without stress and pre-treated with single dose garlic extract (Garlic extract group); a group pre-treated with single dose garlic extract followed by stress (Pre-stress Garlic); and lastly, a group which was treated with garlic extract after the stress (Post-stress Garlic). The dose of the garlic extract was given with the help of catheter for the duration of 2 h. Rats were subjected to immobilization stress between 9 a.m to 3 p.m for 6 h by placing them in the individual wire mesh cages of appropriate size attached to a wooden board, as reported by us[11]. Rats were deprived of food and water during the stress procedure. Animals were sacrificed using pentobarbital (*i.p.* 50 mg/kg body weight) 30 min after the completion of the stress procedure. Non-stressed control animals (with or without garlic extract) were handled at the same time similar to stressed groups but were not immobilized.

2.4. Preparation of kidney samples

All the rats were carefully dissected and the kidneys excised, cleared of adhering connective tissues, weighed and homogenized in ice cold 1.15% KCl (1 g tissue/3 mL) using a Potter-Elvenhjem type homogenizer. The homogenates were then centrifuged at 5000 rpm for 15 min at 4 ℃ and aliquots of the supernatant were used for biochemical assays.

2.5. SOD assay

SOD activity was measured according to the method described by Marklund and Marklund[16]. This procedure depends upon the auto-oxidation of pyrogallol (8 nmol/L) in the presence of 0.05 mol/L tris succinate buffer pH 8.2. The inhibition of pyrogallol auto-oxidation by SOD was monitored at 412 nm. One unit of the enzyme was defined as the amount of enzyme required to inhibit the rate of pyrogallol oxidation by 50%.

2.6. CAT assay

Catalase activity was assayed according to the method of Beers and Seizer[17] with hydrogen peroxide (30 mmol/L) as substrate. One unit

of CAT activity was defined as the micromoles of hydrogen peroxide consumed/min/mg of protein sample.

2.7. GST assay

GST was assayed according to the method of Habig *et al*[18]. Using 1-chloro-2, 4-dinitrobenzene (CDNB) [1.0 mmol/L] as a substrate. The GST activity assay utilizes CDNB, upon conjugation with the thiol group of GSH to CDNB substrate, there is an increase in the absorbance at 340 nm.

2.8. LPO assay

Lipid peroxidation of kidney tissues was measured according to the method of Halliwell and Chirico[19]. One molecule of malondialdehyde (MDA) reacted stoichiometrically with two molecules of 0.69% 2-thiobarbituric acid at pH 3.5. The pink chromogen was detected spectrophotometrically with an extinction coefficient of 156 mmol/L/cm at 532nm.

2.9. Total GSH assay

The method of Sedlak and Lindsay[20] was used to measure the GSH level in the kidney homogenate. The assay is based on the reduction of 0.01 mol/L 5-5'-dithiobis-2-nitrobenzoic acid by sulfhydryl groups of GSH to form 2-nitro-5-mercaptobenzoic acid per moles of GSH.

2.10. ALT, AST, glucose and uric acid assays

The AST and ALT activities were measured using a kit from Reckon Diagnostics Pvt. Ltd (Delhi, India).

2.11. Protein estimation

Protein in the kidney tissues was estimated according to method of Lowry *et al.*[21] using bovine serum albumin as standard.

2.12. Statistical analysis

All the data are expressed as mean ± SD. Statistical analyses were performed using Graph Pad Prism version 4.0 software (Graph Pad Software, La Jolla, CA). Since post-treatment of garlic extract is more logical as the dietary supplement, analysis of variance with interaction was used. One-way ANOVA was also used to compare control group versus the stressed group and Stress+pre and post-extract-group, using Dunnett's test. Statistical significance was determined at $P < 0.05$. Means with different symbols are significantly different.

3. Results

The present study revealed that 6 h of immobilization stress caused a significant decrease in the activities of SOD, CAT, GST and the levels of GSH and glucose (Figures 1 and 2) with increase in the levels of LPO,

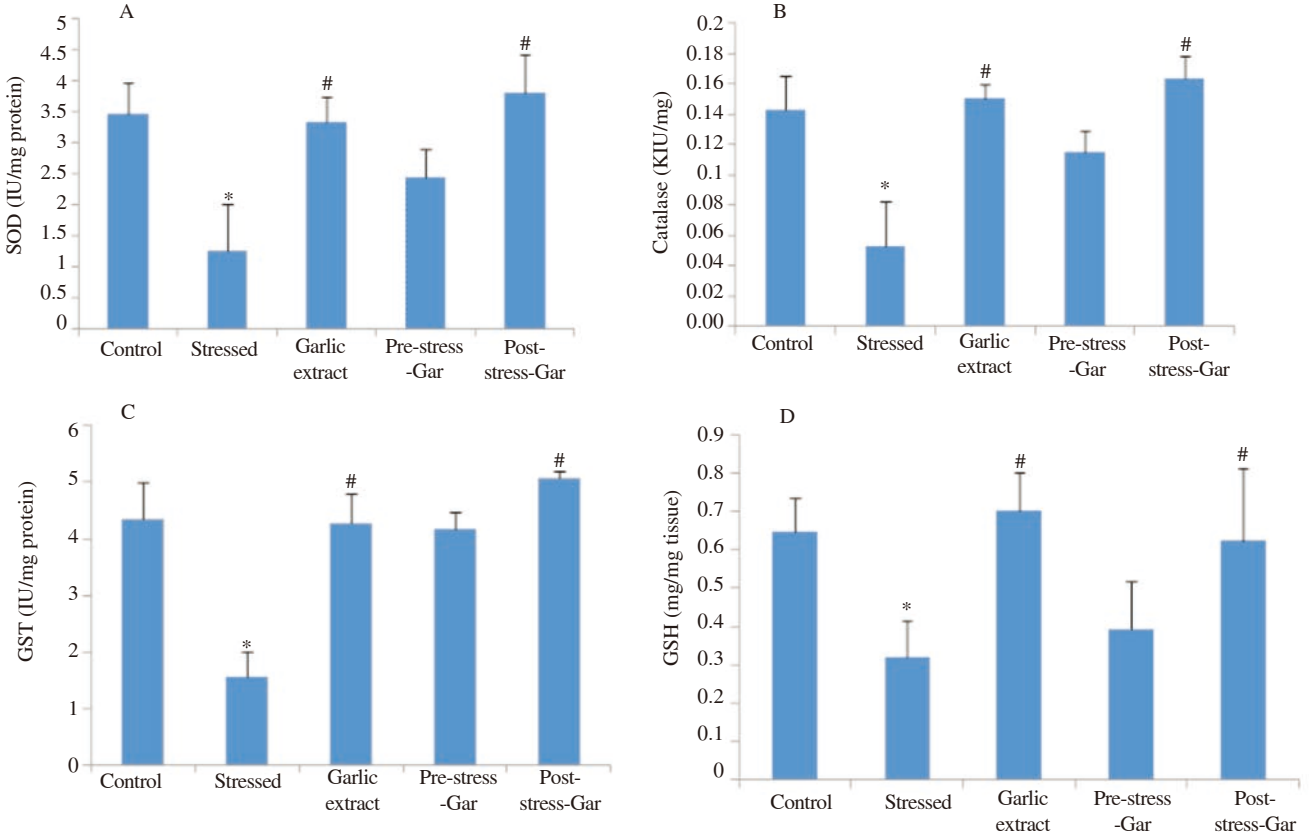

Figure 1. Effect of treatment with garlic on immobilization stress induced changes in kidney tissue levels. A: SOD; B: Catalase; C: GST; D: GSH. Significant decreased in antioxidant enzyme activities and GSH level after the immobilization stress. The pre and post stress treatment of garlic extract revert the deranged free radical system to their normal values with a relative dominance by later; *: $P < 0.05$ compared with controls; #: $P < 0.05$ compared with stressed rats.

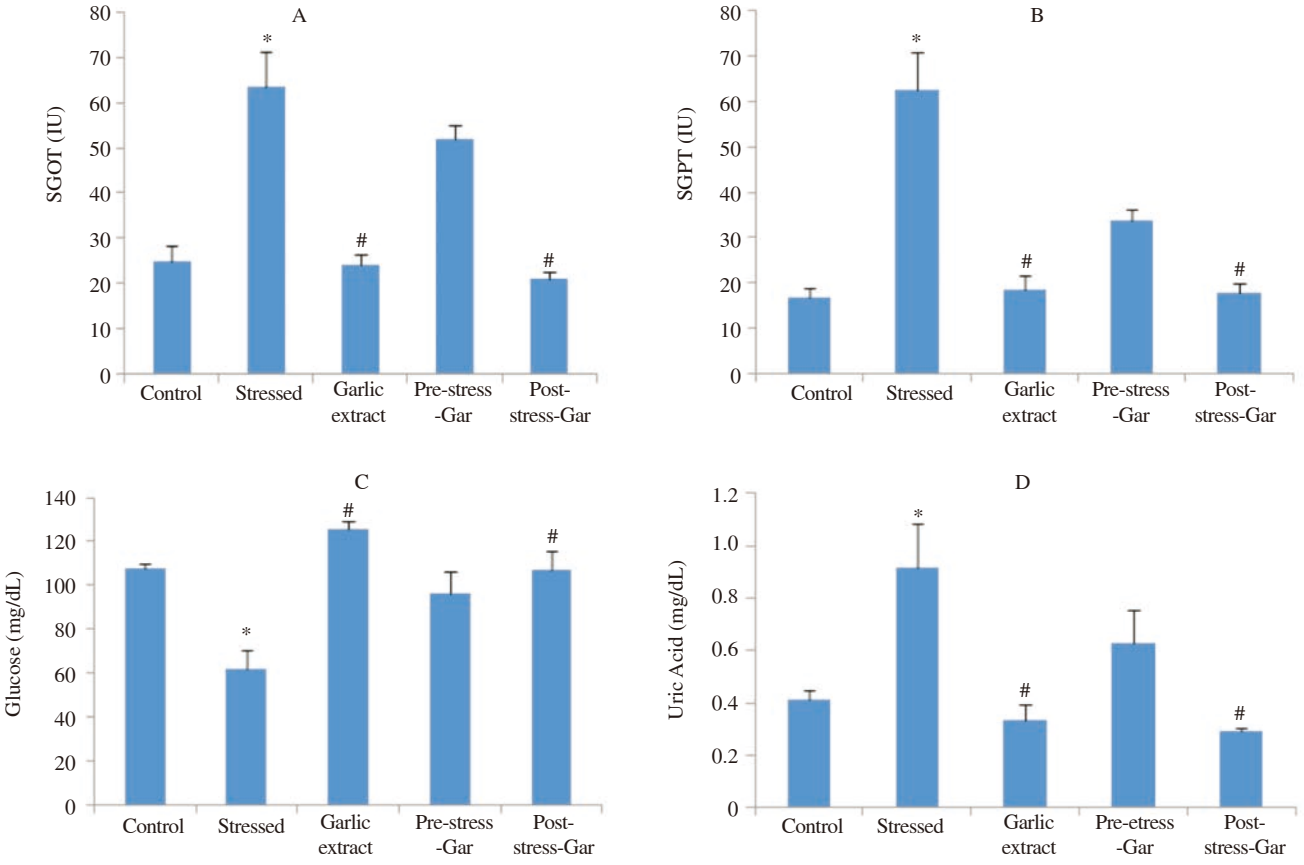

Figure 2. Effect of treatment with garlic on immobilization stress induced changes in kidney tissue levels. A: SGOT; B: SGPT; C: Glucose; D: Uric acid. The decreased in glucose content was observed with a significant increase in SGOT, SGPT and uric acid after the immobilization stress. The pre and post stress treatment of garlic extract resulted in a significant increase in glucose content with a decrease in SGOT, SGPT and uric acid, The post stress treatment of extract was found more effective than pre stress treatment in combatting the oxidative stress induced changes; *: $P < 0.05$ compared with controls; #: $P < 0.05$ compared with stressed rats.

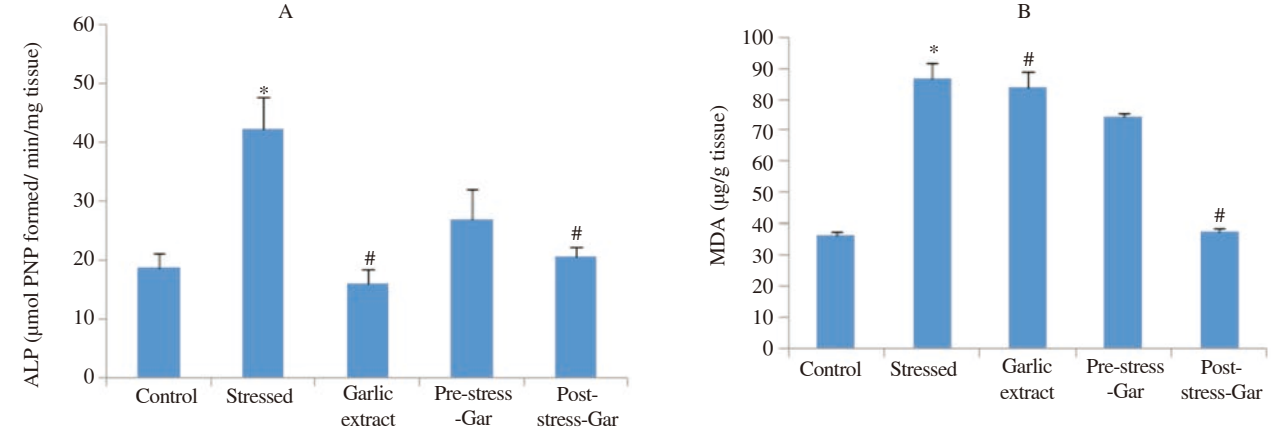

Figure 3. Effect of treatment with garlic on immobilization stress induced changes in kidney tissue levels. A: ALP; B: MDA.
The increase in ALP and MDA content was observed after the immobilization stress. The pre and post stress treatment of garlic extract resulted in a significant decrease in ALP and MDA, The post stress treatment of extract was found more effective than pre stress treatment in combatting the oxidative stress induced changes; *: $P < 0.05$ compared with controls; #: $P < 0.05$ compared with stressed rats.

AST, ALT, ALP and uric acid compared with non-stressed control rats (Figures 2 and 3).

A single dose of *A. sativum* extract alone (100 mg/kg body weight) did not cause significant change in these biochemical parameters (results not shown) in unstressed normal control rats. Oral administration of garlic extract both before (pre-stress treatment) and after (post-stress treatment) immobilization stress treatment resulted in a significant alteration of these parameters as compared to stress treated rats and reverted these parameters towards their control values. However, the post-stress oral treatment of extract (100 mg/kg body weight) was found-more effective in restricting stress induced decrease of SOD, GST, CAT, GSH and glucose (Figures 1 and 2) and increase in the level of TBARS, AST, ALT, ALP and uric acid as compared to stress alone or pre-stress extract treatments (Figures 2 and 3).

4. Discussion

In biological systems, oxidative stress refers to an imbalance between pro-oxidants and antioxidants in favor of the former, which is very harmful to cells[12,22]. Defense against oxidative stress is primarily dependent upon an orchestrated synergism between several endogenous (free radical scavenging enzymes, SOD, GST, CAT and total GSH) and exogenous (vitamins such as A, E and C) antioxidants[10]. Lipid peroxidation of membrane polyunsaturated fatty acid, leading to the formation of MDA, is implicated in the pathogenesis of liver injury. The decrease in the fluidity of the biomembrane due to LPO may impair major metabolic functions and depends upon membrane structure and integrity[23,24]. Immobilization stress is a convenient method of inducing both psychological (escape reaction) and physical (muscle work) stress as a result of restricted mobility and aggression. Immobilization stress has been also reported to bring out the antioxidant defense changes in rat kidney. SOD, GST and CAT play an important role in scavenging oxy-radicals and their products. In order to maintain the stability in living organism, it is necessary to maintain balance between the oxidative and antioxidant defense[25].

In the present study, 6 hours of stress resulted in generation of free radicals in rat's kidney, which resulted decrease in GSH, glucose level and decline in the antioxidant enzymes activities. Furthermore, ROS enhances the level of pro-oxidants such as uric acid and TBARS, an indicator of peroxidation of lipids which additionally aggravates the ROS generation. The depletion in the GSH content observed in the present study might be the result of decreased activities of the free radical scavenging enzymes SOD, CAT and GST, further causing upsurge LPO as shown by the elevated TBARS level. In the last one decade, a number of natural products and neutraceuticals have been investigated and reported for their beneficial effects in humans. However, the anti-stress profile of garlic has not been clearly outlined. For the first time, the present study reports modulatory affects and subsequent biochemical adoptive role of garlic extract against stress induced ROS.

The intra-gastric administration of crude extract of garlic significantly increased the circulating activities of SOD, CAT and GST, and the levels of glucose and GSH while the circulating levels of TBARS (LPO), AST, ALT, ALP and uric acid was found to be decreased. Garlic extract was found to prevent and normalize oxidative stress generated by immobilization stress, which was evident by the reversal of deranged antioxidant enzymatic activities and kidney functions including glucose and uric acid towards their normal values. This is possibly due to the organosulfur contents in the garlic like allicin, alliin, and two major organosulfur compounds SAC and S-allylmercaptocysteine which are potent free radical scavengers[13]. These antioxidant compounds present in the garlic might act as double-edged swords. They upregulate the antioxidant enzymatic activities during stress as well as GSH to scavenge the free radicals, and down regulate LPO too. Indeed, similar benefits of garlic extract after ingestion has been reported in cardiac muscles due to increased GSH content, SOD, CAT and GST activities[14].

The activity of AST and ALT are sensitive indicators of acute hepatic functional impairment and the ALP level is known as an indicator of hepatobiliary disease[26]. The decrease in these surrogate biomarkers in the plasma indicates degenerative changes, metabolic alterations and hypo function of heart and liver, which are adversely effected by immobilization stress[27]. Our findings further support the notion that the intra-gastric garlic extract treatment (both pre- and post-stress) reverses the reduced function of kidney, heart and liver.

Kidney level of glucose is found significantly decreased in response to immobilization stress. This could be due to enhanced catecholamine levels due to immobilization stress. The catecholamines secretion evokes an initial repression of insulin secretion, followed by rebound hyper secretion of pancreatic hormone, subsequently leading to hypoglycemia, which can decrease kidney glucose level during immobilization stress, either by enhancing peripheral glucose uptake or by interacting directly with β-cells of the pancreas[28]. Six hours of stress resulted into decreased glucose concentration in kidney which was reverted to their control values by the both pre- and post-stress garlic extract treatment.

Uric acid is considered as non-enzymatic antioxidant, but increased production in response to immobilization stress can cause increase in free radical generation due to activation of xanthine oxidase enzymes system[29]. This increase could be detrimental under depleted GSH level, which often happens due to immobilization stress. The treatment with garlic extract resulted in a significant decrease in the uric acid level in both pre- and post-extract treatments with a relative dominance by later. The increase in uric acid concentration in immobilization stress could be due to body's natural response to combat enhanced free radicals produced due to decreased activities of scavenging enzymes, increased xanthine oxidase activity[25] and/ or also due to high levels of catecholamines during oxidative stress, as some studies shown that catecholamines increase purine catebolites[29].

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Stress causes most of the dysfunction in the system and kidney is an important organ suffers during stress. Preventive and prophylactic measures of certain antioxidant plant result the recovery from the stress. Hence, the study carried out is potential to restore the distorted kidney function under stressed condition.

Research frontiers

The present study narrates that the preventive and curative effects of crude extract of *A. sativum* against the renal immobilization stress in term of antioxidant enzymes and related markers enzymes.

Related reports

Immobilization stress caused free damage to the system has been

published online but on the kidney's effect and the effect of particular plants extract has not been published.

Innovations and breakthroughs

Hepato-protective activity of *A. sativum* has been done and published. In the present study the authors have demonstrated the prophylactic and curative effect of *A. sativum* against immobilization stress in the renal system.

Applications

Based on the published studies, it is clear that the usage of *A. sativum* in certain amount is beneficial to humans. Thus, the present study supported and suggests the use of this plant as reno-protective agent against immobilization stress.

Peer review

This study is novel in term of prophylactic and curative effect of crude garlic extract of *A. sativum* against immobilization stress particularly in kidney. Based on the observation of certain biomarkers and antioxidant enzymes, it is clear that crude extract of garlic has encouraging effects over deranged free radical damage by immobilization stress in kidney.

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