

Using The Aqueous Extract Of *Allium Sativum* In Improvement Of Some Physiological And Immunological Parameter In Albino Rats

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Abstract

The current study was designated to investigate the effect of *Allium sativum* on some physiological and immunological parameters in rats. thirty adult rats were divided into three groups (10 rat for each). G1: served as healthy control, G2 :rats were treated with 150 mg\kg of *Allium sativum*, G3: treated with 300 mg\kg of *Allium sativum*. All treated animals were given orally for 30 days. The effects of garlic on some parameters were investigated such as alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), total and differential counts of white blood cells (WBC) like Lymphocyte, Monocyte, Neutrophil, Basophil, Eosinophil, as well as the level of Malondialdehyde (MDA), C-reactive protein (CRP), catalase (CAT), glutathione (GSH) and tumor necrosis factor alpha (TNF- α), the results administrated a significant decreased in the ALT and AST level in group treated with 150 and 300 mg\kg of *Allium sativum* as compared with control also the results display no significant in LDH level in treated groups with 150 and 300 mg\kg of *Allium sativum*. The values of WBC show high significant increased in all groups treated with 150 and 300 mg\kg of *Allium sativum* compared with control while the results obtained significant decreased in neutrophil in all groups that treated with 150 and 300 compared with control, also the results investigated a significant decreased in serum level of MDA, CRP, CAT, TNF- α while the level of CAT relieved significant increased in group treated with 150 and 300 of aqueous extract of *Allium sativum*.

key words: *Allium sativum*, aqueous extract , Lymphocyte

Introduction

Medical plants have showed a wide range of valuable therapeutic properties without causing any side effects [1], plant antioxidants such as flavonoids and vitamins have been used to prevent and protect from diseases, the natural antioxidant contained in plants have active biochemical functions which prevent the oxidative damage caused by free radical species, the polyphenolic compounds have a wide spectrum of therapeutic benefits [2,3]. In view of the great therapeutic benefits, plants rich in polyphenols and antioxidants have gained wide importance as a potential alternative to synthetic pharmaceutical materials in order to increase the safety of food-derived products that have been verified by laboratory studies [4,5]. Garlic is an aromatic herbal plant that has been used for a long time in the treatment of many diseases as a traditional medicine.

[6,7], which is used against several diseases such as hypertension, cardiovascular disease, lung and stomach disorder, influenza and different infections [8]. Garlic contains antioxidants and other properties that strengthen the immune system and reduce the risk of cancer. Garlic also has the ability to fight many types of parasites and improve many symptoms and complications associated with diabetes and blood. Garlic is rich with sulfur compounds which provide anti-inflammatory, anticancer, immunomodulatory, antidiabetic and anti-atherosclerotic [9,10]. Garlic has been shown to improve the immune system and thus inhibit cancer and heart disorders. Garlic helps reduce calcification of blood vessels (atherosclerosis), which is useful for the prevention of cardiovascular disease and garlic works to reduce high levels of cholesterol and fats in the blood. Garlic contains compounds of sulfur, selenium, potassium and other substances that are known for their ability to fight many types of viruses and bacteria. [11].

The beneficial factors of garlic on health have been reported for centuries. Garlic contains compounds that have been used to improve the immune system [12,13]. Compounds of *Allium* were examined in terms of their immune functions. It is well-known that dysfunction of the immune system acts an important role in the development of

diseases and this food (garlic) may contribute to the inhibition and treatment of several disorders such as gastric ulcer, obesity, metabolic syndrome (diabetes) and heart diseases [14,15]. Garlic

might be used as an alternative drug without any side effect as compared with other chemotherapy in treating cancers caused by aflatoxin B1 [16].

The aim of current work was to estimate the effect of *Allium sativum* on ALT, AST, LDH, total and differential counts of WBC like Lymphocyte, Monocyte, Neutrophil, Basophil, Eosinophil as well as the level of MDA, CRP, CAT, GSH and TNF- α in rats.

Materials and methods

1. Preparation of plant extract

Fifty grams of garlic powder were mixed with 200 ml of the distilled water and kept on a shaker for 24 hr. at 30 °C, a filter paper was used to collect filtrate which was used for further experiments.

2. Experimental design

Thirty albino male rats weighing (160-180) grams were used in this study, divided into three groups (10 rats/group): G1: healthy control, G2: treated with 150 mg/kg of *Allium sativum*, G3: treated with 300 mg/kg of *Allium sativum*, animals were treated with extract orally as a single dose for 30 days.

3. Blood collection

Blood samples were collected under anaesthetized condition, immediately centrifuged at 3000 r.p.m for 10 min, the serum was used for further analyses. Included ALT, AST, LDH, MDA, CRP, CAT, GSH, that were estimated by using (BioSystem S, A. Costa Brava, 30.08030 Barcelona\Spain, TNF- α was done by using (TNF- α ELISA Kit, Elabscience, China, total and differential count of WBC were calculated by automated digital counter machine from Bengaluru, India).

6. Statistical Analysis

The data of the experiment were calculated by using one-way analysis of difference and the group differences were investigated using Duncan multiple range test, data are presented as mean \pm SM, the different small letters investigate a significant difference ($P < 0.05$).

Results

As shown in table 1, the results of ALT were significant ($P \leq 0.05$) decreased in group treated with 150 and 300 mg/kg of *Allium sativum* extract (26.33 \pm 1.20, 20.67 \pm 1.85) U/L as compared with control group (29.33 \pm 1.45 U/L). On the other hand, the results were relieved significant ($P \leq 0.05$) decreased in AST activity in groups treated with 150 and 300 mg/kg of *Allium sativum* extract (26.67 \pm 1.20, 19.33 \pm 1.85) U/L as compared with control group (30.00 \pm 1.52 U/L), also

the results show no significant in LDH in treated groups with 150 and 300 mg/kg of Allium sativum extract ($133.67 \pm 4.48, 109.00 \pm 21.22$ U/L) respectively

Table1: effect of Garlic on ALT, AST and LDH in rats

Group	Mean \pm SE		
	ALT (U/L)	AST (U/L)	LDH (U/L)
Control	29.33 ± 1.45 a	30.00 ± 1.52 a	155.33 ± 7.42 a
Allium 150	26.33 ± 1.20 b	26.67 ± 1.20 b	133.67 ± 4.48 b
Allium 300	20.67 ± 1.85 c	19.33 ± 1.85 c	109.00 ± 21.22 c
Differences small letters are significant ($P < 0.05$) as comparison between columns			

The values of WBC which illustrated in table 2 show high significant increased in all groups treated with 150 and 300 mg/kg b.wt 6.06 ± 0.42 and 7.33 ± 0.93 cells $\times 10^3$ compared with control group 4.90 ± 0.73 cells $\times 10^3$, in addition, the results obtained significant decreased in neutrophil in all groups that treated with 150 and 300 mg/kg b.wt (37.26 ± 0.17 and 33.86 ± 7.74)% compared with control group 64.60 ± 4.60 %. The percentage of Lymphocyte obtained significant increased in groups treated groups with 150 and 300 mg/kg b.wt. 53.06 ± 5.15 and 56.56 ± 6.31 % Compared with control group cells 26.26 ± 3.31 %

Table2: effect of Garlic on total and differential counts of WBC in rats

Groups	Mean \pm SE					
	WBC	Neutrophil	Lymphocyte	Monocyte	Basophil	Eosinophil
Control	4.90 ± 0.73 b	64.60 ± 4.60 a	26.26 ± 3.31 b	2.67 ± 0.36 b	1.933 ± 0.23 a	4.50 ± 3.10 a
Allium 150mg/kg	6.06 ± 0.42 a	37.26 ± 0.17 b	53.06 ± 5.15 a	2.96 ± 0.98 b	2.63 ± 1.24 a	3.70 ± 3.70 a
Allium 300mg/kg	7.33 ± 0.93 a	33.86 ± 7.74 b	56.56 ± 6.31 a	3.80 ± 1.05 a	1.81 ± 3.26 a	2.40 ± 2.37 b
Differences small letters are significant ($P < 0.05$) as comparison between columns						

The statistical analysis of the results in table 3 show that, there was a significant decreased in serum MDA in group treated with 150 and 300mg\kg of aqueous extract of Allium sativum(1.383 ±0.48,0.56 ±0.03) nmol\ mL respectively compared with control group 2.96 ±0.11. The mean value of CRP significantly decreased in groups treated with 150 and 300 mg\kg of aqueous extract of Allium sativum(3.33 ±0.88 , 2.67 ±0.20) as compared with control group 5.00 ±0.57 , the results of GSH was significant decreased in group treated with 150 and 300 mg\kg of extract (respectively 122.67 ±5.04, 105.00 ±3.21 μmol/L as compared with control group 164.00 ±5.03 μmol/L, on the other hand the results were relieved significant increased in CAT in group treated with Allium extract 1.0 96 ±0.14, 0.973 ±0.14 as compared with control group 0.973 ±0.14. Also the value of TNF-α revealed significant decreased in groups treated with aqueous extract of Allium sativum(278.67 ±8.77, 242.64 ±10.83) pg/ml as compared with control group 323.01 ±12.22 pg/ml .

Table3: effect of Garlic on TNF-α and some antioxidant enzymes in rats

Group	Mean ± SE				
	MDA (nmol\ mL)	CRP	CAT IU\ml	Glutathion: GSH (μmol/L)	TNF-α (pg/ml)
Control	2.96 ±0.11	5.00 ±0.57	2.280 ±0.15	164.00 ±5.03	323.01 ±12.22
Allium 150	1.383 ±0.48 b	3.33 ±0.88	1.0 96 ±0.14	122.67 ±5.04	278.67 ±8.77
Allium 300	0.56 ±0.03	2.67 ±0.20	0.973 ±0.14	105.00 ±3.21	242.64 ±10.83
Differences small letters are significant (P<0.05) as compression between columns					

Discussion

The current research indicated that, the administration of garlic extract at 150 and 300 mg/day for 15 days improved hepatic enzymes (table 1), Garlic contains different types of sulfur compounds, minerals and enzymes such as allinase. allinase enzyme converts alliin to allicin following damage to the garlic bulb, allicin has anti-inflammatory and antioxidant activities through inhibiting production of nitric oxide and superoxide anion. Elevated levels of liver enzymes are considered to be important indicator of liver damage. However, results reported that garlic has a hepatoprotective activity by decreasing the level of ALT, AST enzyme [17,18]. Oxidative stress is generated due to over production of cellular oxidants due to a poor antioxidant defense system. Oxidative stress and oxidative damages have

been linked with hepatic injury. Furthermore, antioxidant treatment has become a therapeutic strategy for decreasing the risk of liver disease caused by free radicals [19,20]. Moreover, garlic extract treatment facilitated a significant increase in all tested antioxidant enzymes levels. This supports the notion that garlic extract possesses hepatoprotective potential which may be due to its potential to increase the level of antioxidant enzymes [21].

The results presented in table 2 investigate that, there was a significant increase in total and differential count of WBC. Garlic has the ability to stimulate immune system by stimulating the function of organs related to blood cell formation such as bone marrow, spleen, and thymus. Garlic promotes immune system functions by mechanisms including macrophage activation, phagocytosis, immunoglobulin synthesis and modulation of cytokine production which leads to stimulate immune cells like eosinophils, natural killer cell, dendritic cell and lymphocyte. It has been reported that garlic extract has an effective role in variety of leukocyte cytokine production, in some inflammatory condition such as inflammatory bowel disease (IBD) garlic and its derivatives reduce the T-helper1 cells inflammatory cytokine production, on the other hand garlic extract possesses both stimulatory and inhibitory action on lymphocyte proliferation and Lipopolysaccharide induced TNF- α generation by inducing the production of IL10- which controls inflammatory condition [22]. The active compounds (allicin) present in garlic enhance the immune system [23]. Allicin has antibacterial activity which can affect a different type of virulent bacteria [24]. According to another study, red and white blood cells, packed cell volume, phagocytic activity, Hb%, respiratory burst, lysozyme, and were enhanced in garlic-fed groups compared to the control [25].

Antioxidant enzymes represent the first line of defense against free radicals to maintain the function of immune system [26]. According to the results presented in table 3, there was a significant decrease in the value of MDA, CRP, GSH and TNF- α also the results display a significant increase in the value of CAT as comparison with control, garlic possess antioxidant property (SOD, CAT, and GPX) by reducing free radicals and enhancing the antioxidant enzymes, the administration of garlic promotes antioxidant activities either by increasing the endogenous antioxidant synthesis or reducing the production of free radical, it is justified to assume that the antioxidant effect of garlic might be through modulation of ROS, increasing glutathione and cellular antioxidant enzymes. The use of garlic increases the effectiveness of antioxidants and reduces the harmful effects of oxidation by reducing the production of free radicals or increasing the production of enzymatic antioxidants, thus

protecting tissues from free radical damage. Studies have also shown that garlic increases the activity of some antioxidant enzymes in the liver tissues of rats [27,28]. The present result investigated the beneficial and important effects of garlic extract in the health sciences as it opens new fields in drug synthesis and treatment of different diseases.

Upon bacterial infection, host cells will generate reactive oxygen species (ROS) as signaling molecules for homeostasis and the uncontrolled production of ROS lead to oxidative stress which damages DNA, lipids, proteins, loss of cell function and programmed cell death [29,30]. To prevent cell damage from excess ROS, the antioxidant defense system (SOD) can catalyze the dismutation of superoxide into oxygen and hydrogen peroxide (H_2O_2) and then there may be further reduction by CAT to H_2O and oxygen [31]. Garlic extracts stimulated the synthesis of IL-10, an anti-inflammatory cytokine in LPS-treated human whole-blood cultures, and suppressed the monocyte production of pro-inflammatory cytokines, including TNF- α , IL-6 and IL-8 [32], another study showed that garlic extracts stimulated IL-10 while they reduced TNF- α production in LPS-stimulated human placental cells [33]. Actually, we speculate that the anti-inflammatory effects exerted by the garlic hydroalcoholic extract could also be related, at least in part, to the phenol and flavonoid content. In particular, the presence of garlic acid and protocatechuic acid is consistent with the reduction in the tested pro-inflammatory biomarkers [34,35,36].

Conclusion

From the results obtained in this present study, it could be safely concluded that garlic extract at a dose of 150 and 300 mg/kg b.wt be useful in the treatment of liver damage, stimulate immune system and antioxidant enzyme.

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