Original Article

COMPARISON OF EFFECTS OF TWO VARIETIES OF ALLIUM SATIVUM (ALLIUM SATIVUM VAR CHINESE EXOTIC AND ALLIUM SATIVUM VAR LEHSUN GULABI) ON SERUM GLUTATHIONE PEROXIDASE IN ALBINO RATS

Sauda Usmani[1], Hamid Javaid Qureshi[2]

ABSTRACT:

Objective: Glutathione peroxidase (GPx) is a family of multiple isozymes that catalyze the reduction of H_2O_2 or organic hydro peroxides to water or corresponding alcohols using reduced glutathione (GSH) as an electron donor. GPx competes with catalases for H_2O_2 as a substrate to protect against mild oxidative stress. Different medicinal plants and their active ingredients possess the ability to prevent decrease in GPx in oxidative stress. The objective of this study was to compare the effects of two varieties of Allium sativum on an antioxidative biomarker, serum glutathione peroxidase in albino rats.

Subjects and methods: It was a randomized controlled trial (RCT)

This study was conducted at Physiology Department, Services Institute of Medical Sciences (SIMS), Lahore from August 2012 to February 2014.The study was carried out on 120 male albino rats. The rats were randomly divided into four groups of thirty each. Group A was given normal saline (control); group B was administered hepatotoxic dose of acetaminophen (negative control); group C was pretreated with Allium sativum Var Chinese exotic extract for 7 days before receiving hepatotoxic dose of acetaminophen (Experimental 1); and group D was pretreated with Allium sativum Var Lehsun Gulabi extract for 7 days before receiving hepatoprotective dose of acetaminophen (Experimental 2). Serum glutathione peroxidase levels in each group were measured from terminal blood sampling done 24 hours after acetaminophen administration after ether anesthesia.

Results: Highly significant (p=0.000) less reduction in serum glutathione peroxidase were manifested in experimental group D pretreated with ethanolic extract of Allium sativum Var Lehsun Gulabi as compared to reduction in this parameter in experimental group C pretreated with ethanolic extract of Allium sativum Var Chinese exotic.

Conclusion: Allium sativum Var Lehsun Gulabi has better antioxidative potential as compared to Allium Sativum Var Chinese exotic.

Key Words: Garlic, Glutathione peroxidase, Catalase

INTRODUCTION

Glutathione peroxidase (glutathione: H_20_2 oxido-reductase E.C. 1.11.1.9) was discovered by Mills in 1957 in his search for the factors that function in the protection of erythrocytes against oxidative hemolysis.[1]

Glutathione peroxidase is the general name for a family of multiple isozymes that catalyze the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols using reduced glutathione (GSH) as an electron donor. Glutathione peroxidase is involved in protection against oxidative stress, and thus uses glutathione as a substrate. It participates in amino acid transport through the plasma membrane, scavenges hydroxyl radical and singlet

[1]Assistant Professor Physiology, Pak Red Crescent Medical, College, Lahore.
[2]Professor Physiology, AMDC, Lahore.

oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of GPX. Glutathione is able to regenerate the most important antioxidants; vitamins C and E back to their active forms. The intracellular content of glutathione depends on environmental factors and functions as a balance between its utilization and synthesis. Exposure to ROS (involving H_2O_2)/RNS, or to compounds which can generate ROS, can increase the content of GSH by increasing the rate of GSH synthesis. Significantly, GPx competes with catalase for H_2O_2 as a substrate. Glutathione redox cycle is a major source of protection against mild oxidative stress, whereas catalase becomes increasingly important in protection against severe oxidative stress.[2]

Allium sativum, or "garlic" is widely used in culinary preparations.[3] Two varieties of Allium sativum grown in Punjab are Chinese (exotic), Lehson Gulabi (local).[4]Traditional uses of Allium sativum include; use in intestinal disorders, diarrhea, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging[3], headache, flu, sore throat, fever and otitis media.[5]

Garlic contains sulfur-containing constituents like γ-glutamyl-S-alkyl-l-cysteine and S-alkyl-l-cysteine, sulfoxides, allicin, steroidal glycosides, lectins, prostaglandins, fructan, pectin, essential oil, adenosine, vitamins B_1, B_2, B_6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, anthocyanins, flavonoids, phenolics and essential amino acids.Allicin and other thiosulfinates instantly decompose to other compounds, such as diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyltrisulfide (DAT), dithiins and ajoene. At the same time, γ-glutamylcysteines are converted to S-allylcysteine (SAC).[17]These sulphur compounds of garlic have proved to be promising antioxidants against drug induced hepatitis.[6-9]

The United States National Cancer Institute tested the toxicity of SAC vs. other typical garlic compounds and found that it has 30-fold less toxicity than allicin and DADS.[10] A study carried out to determine LD_{50} of ethanolic extract of garlic in lab mice showed that the LD_{50} in mice after oral ingestion was 8000 mg/kg.[11]

Acetaminophen (APAP), which is also named paracetamol, is a commonly used antipyretic and analgesic. Overdose of acetaminophen can lead to acute liver injury and histopathological changes characterized by centrilobular necrosis.[12] Chronic alcohol use may greatly increase susceptibility to hepatotoxicity from acetaminophen because of depleted glutathione stores.[13] The oxidative metabolite of acetaminophen is more toxic than the drug. Hepatotoxic doses of paracetamol deplete the normal levels of hepatic glutathione. The hepatic cytochrome P450 enzyme system metabolizes paracetamol, forming NAPQI (N-acetyl-p-benzo-quinone imine). NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione. Conjugation depletes glutathione, a natural antioxidant. The highly reactive active metabolite NAPQI appears to mediate much of the acetaminophen-related damage to liver tissue by forming covalent bonds with cellular proteins and subsequent activation of inflammatory mediator TNF-α that in turn contribute to tissue necrosis.[14] (Figure 1)

The liver is a vital organ and a number of chemical agents and drugs that are used on a routine basis produce cellular as well as metabolic liver damage. Paracetamol is a well-known hepatotoxic drug. It damages liver cells mainly by inducing lipid peroxidation and oxidative stress. The scenario becomes complex while prescribing it to a patient on anti-tuberculous or anti-convulsive drug therapy or in case of patients with renal failure, diabetes mellitus or chronic hepatitis.

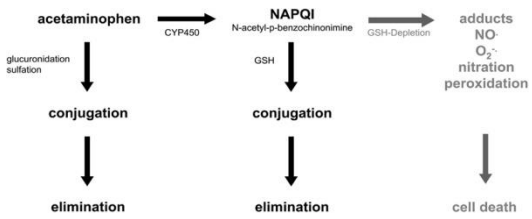

Figure 1: Decomposition of acetaminophen

There is a need to develop new, safer drugs for hepatitis patients suffering from multi-organ failure. Search for new drugs for limiting hepatic injury has been of interest recently. Garlic is a natural component of diet in Pakistan and efforts should be channeled towards bringing down the incidence of acute hepatitis in our country by improving intake of this natural antioxidant. We, in our study used ethanolic extracts of two varieties of garlic to determine and compare their effects on an antioxidative biomarker, serum glutathione peroxidase in albino rats.

METHODS

One hundred twenty male albino rats weighing 200-250 grams were obtained from were obtained from National Institute of Health (NIH), Islamabad. Animals were housed in groups of 30 per cage for at least one week before the start of the experiments. Housing conditions were thermostatically maintained at 26±2 ^{0}C and a light/dark cycle (lights on: 0900-2100).[15] The animals were fed with commercially available standard pellet diet ad libitum and were provided with tap water in clean bottles.

PREPERATION OF EXTRACT

Allium sativum Var Chinese exotic and Allium sativum Var Lehsun Gulabi were obtained from the local market of Lahore and were identified by a qualified taxonomist. Ethanolic extract of Allium sativum Var Chinese exotic and Allium sativum Var Lehsun gulabi were made and standardized using Facilities available at the Applied Chemistry Research Centre, PCSIR labs, Lahore. Their bulbs were first dried in the shade and then crushed into a coarse powder using an electric grinder. This powder was then extracted in a Soxhlet extractor with 99.9% ethanol. The extract thus obtained, was filtered and the solvent (ethanol) evaporated in vacuum with a rotary evaporator. After evaporation a dark brown concentrate was obtained. This concentrate was kept at 4 ^{0}C prior to use. The crude extract was then dissolved in normal saline and then diluted to the desired concentration.[16, 17]

Induction of acetaminophen toxicity: A single intraperitoneal dose of acetaminophen 750 mg/kg[18]dissolved in normal saline was used to induce acute oxidative hepatic injury.

Group A (Negative Control, n=30): was given normal saline 10ml/kg body weight intraperitoneally for 7 days.

Group B (Positive Control, n=30): was given a single dose of acetaminophen 750 mg/kg[18]dissolved in normal saline intraperitoneally.

Group C (Experimental 1, n=30): was pretreated with Allium sativum Var Chinese exotic ethanolic extract in a daily dose of 500mg/kg body weight intra-peritoneally[19](Figure 2) for 7 days before a single intraperitoneal dose of acetaminophen 750 mg/kg[18] dissolved in normal saline.[15]

Group D (Experimental 2, n=30): was pretreated with Allium sativum Var Lehsun Gulabi ethanolic extract in a dose of 500mg/kg body weight intraperitoneally[19]for 7 days before a single intraperitoneal dose of acetaminophen 750 mg/kg[18] dissolved in normal saline.

After 24 hours of acetaminophen administration, each rat was anesthetized using ether. The needle of 5 ml disposable syringe was inserted directly into heart taking care that it may not pierce its posterior wall. Three-milliliter blood was drawn and was kept in the test tube for about 15-20 minutes, and allowed to clot. After 15-20 minutes, samples were centrifuged at 5000 RPM for 15 minutes. The serum, thus obtained, was preserved in labeled polypropylene storage tubes and stored at -

20 ^{0}C for determination of serum glutathione peroxidase.

STATISTICAL ANALYSIS

Data was analyzed using PASW18. The arithmetic mean and standard deviation for quantitative variable, Serum glutathione were calculated. The statistical significance of difference amongst the four groups was determined by applying one way ANOVA followed by post hoc LSD (multiple comparisons) test. The values were considered significant if the p value was less than 0.05; and, highly significant if the p value was less than 0.001.

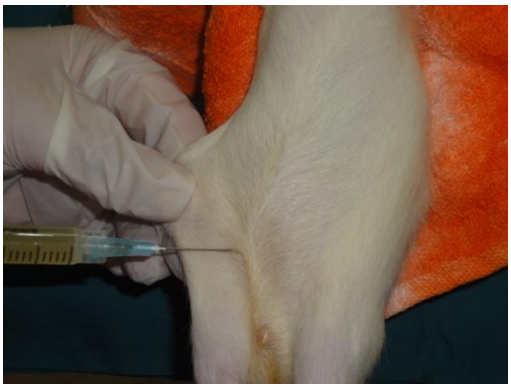

Figure 2: Administration of intraperitoneal dose of extract.

RESULTS

After pretreatment with ethanolic extract of Allium sativum followed by acetaminophen hepatotoxicity, there was highly significant (*p<.000) less decrease in glutathione peroxidase in both experimental groups as compared to both negative and positive control groups. (Table 1)

The positive control group (group B) having acetaminophen toxicity showed highly significant (p=0.000) decrease in serum glutathione peroxidase as compared to the value in the negative control group (group A) as depicted in (Table 2).

Table 1- Comparison of serum glutathione peroxidase in groups A, B, C and D. (One way ANOVA)

Parameter	Group A (n=30)	Group B (n=30)	Group C (n=30)	Group D (n=30)	p-value
Glutathione peroxidase (ng/ml)	21.49±0.79	4.62± 0.60	5.27± 0.71	15.03±1.25	0.000*

Values are presented as mean± SD
*p<.000 highly significant

Table 2- Comparison of serum glutathione peroxidase in groups A and B. (Post hoc LSD)

Parameter	Group A (n=30)	Group B (n=30)	p-value
Serum glutathione peroxidase (ng/ml)	21.49±0.79	4.62±0.60	0.000*

Values are presented as mean± SD
*p<.000-highly significant

After pretreatment with ethanolic extract of Allium sativum Var Chinese exotic followed by acetaminophen toxicity, the experimental group C had significant (p=0.025 significant) increase in serum glutathione peroxidase as compared to the value in negative control group (group B), (Table 3).

Table 3- Comparison of serum glutathione peroxidase in groups B and C. (Post hoc LSD)

Parameter	Group B (n=30)	Group C (n=30)	p-value
Serum glutathione peroxidase (ng/ml)	4.62±0.60	5.27±0.71	0.025**

Values are presented as mean± SD
**p<.05-significant

After pretreatment with ethanolic extract of Allium sativum Var Lehsun Gulabi followed by acetaminophen toxicity, the experimental group D showed highly significant (p=0.000) increase in serum levels of glutathione peroxidase as compared to those in the positive control group (group B), (Table 4).

Table 4- Comparison of serum glutathione peroxidase in groups B and D. (Post hoc LSD)

Parameter	Group B (n=30)	Group D (n=30)	p-value
Serum glutathione peroxidase (ng/ml)	4.62±0.60	15.03±1.25	0.000*

Values are presented as mean± SD
*p<.000-highly significant

Highly significantly (p=0.000) less reduction in serum glutathione peroxidase was manifested in experimental group D pretreated with ethanolic extract of Allium sativum Var Lehsun Gulabi as compared to reduction in these parameters in experimental group C pretreated with ethanolic extract of Allium sativum Var Chinese exotic (Table 5).

Table 5- Comparison of serum glutathione peroxidase in groups C and D.
(Post hoc LSD)

Parameter	Group C (n=30)	Group D (n=30)	p-value
Serum glutathione peroxidase (ng/ml)	5.27±0.71	15.03±1.25	0.000*

Values are presented as mean± SD
*p<.000-highly significant
**p<.05-significant

DISCUSSION

Our study compared the effects of ethanolic extracts of two varieties of garlic (Allium sativum Var Chinese exotic and Allium sativum Var Lehsun Gulabi) on experimentally induced hepatotoxicity and compared their effects on an antioxidative biomarker, serum glutathione peroxidase in albino rats. Acetaminophen was used to produce hepatotoxicity and oxidative stress, which manifested as reduced serum glutathione peroxidase.

This study showed that pretreatment of rats with ethanolic extract of two varieties of garlic grown in Pakistan prevented the decrease in serum glutathione peroxidase, due to acetaminophen toxicity. This effect was exhibited more strongly by Lehsun Gulabi extract when compared to that of Chinese exotic. This adds to several reports on the pharmacological usefulness of garlic extracts as liver protective agents.

Lee et al (2016) investigated the protective effect of fermented garlic extract by lactic acid bacteria (LAFGE) against acetaminophen induced acute liver injury in rats. Their findings revealed that LAFGE modulates the signaling pathways involved in hepatic apoptosis through cellular redox control, as indicated by the inhibition of lipid peroxidation, glutathione and ATP depletion, and the elevation of antioxidant enzyme activities. These findings indicate that LAFGE ameliorates AAP-induced liver injury by preventing oxidative stress-mediated apoptosis, thereby establishing LAFGE as a potential supplement in the treatment of AAP-induced liver injury.[20]

Allyl methyl disulfide (AMDS) is as one of the bioactive components in fresh garlic paste and was investigated for its Hepatoprotective effect against acetaminophen (APAP) -induced acute liver damage in mice. Results reveal that AMDS significantly ($p < 0.05$) reduced the Maleicdialdehyde (MDA) level in liver tissues and restored the activities of antioxidant enzymes SOD, GSH-PX and GSH towards normal levels.[21]

Hepatoprotective effects of Allium sativum methanolic extracts on paracetamol induced hepatotoxic rats were investigated and it was suggested that the possible mechanism of action may be by the active ingredients in Allium sativum (allyl propyl disulfide) that could have increased the levels of glutathione to bind with the toxic metabolites of paracetamol such as N-acetyl- p- benzoquinone imine (NAPQI) and increased its rate of excretion from the body. It might also have inhibited the levels of the cytochrome P- 450 enzyme system that decreased the formation of NAPQI from ingested paracetamol. These possible mechanisms of action of Allium sativum extracts may be through their antioxidative effects that are capable of free radical

scavenging in living system.[22]

Sumioka et al studied the mechanism of protection by S-Allylmercaptocysteine (SAMC) against acetaminophen induced liver injury in mice. SAMC, one of the water-soluble organosulfur compounds in ethanol extracts of garlic, suppressed the plasma ALT activity and prevented reductions in hepatic glutathione levels.[15]

Rashed et al (2014) investigated the effect of garlic oil (GO) alone or in combination with low dose total body gamma ((γ) -irradiation (LDR) against paracetamol (APAP) - induced hepatotoxicity in rats. Findings showed that the combination of GO and LDR produced considerable comparable effects to either treatment alone in preventing the decreased hepatic glutathione content as a result of APAP toxicity. This remarkable synergistic protection against APAP-induced hepatotoxicity might be attributed partly to the suppressive effect of both GO constituents and LDR on lipid peroxidation by free radical scavenging properties or by restoration of glutathione content and cytochrome P4502E1 enzyme in the liver.[23]

CONCLUSION

Allium sativum Var Lehsun Gulabi has better antioxidative potential as compared to Allium sativum Var Chinese exotic.

RECOMMENDATION

Thus garlic may be considered as a useful dietry supplementary compound to patients treated with regular high doses of paracetamol such as of tuberculosis, cancer, dengue fever and arthritis. The antioxidative potential of Allium sativum should be further investigated in human studies. The medical implication of this finding could be that consumption of this variety of garlic might be a useful prophylactic and therapeutic strategy against oxidative stress of toxic hepatitis in Pakistan.

REFERENCES

1. Mills GC. Hemoglobin catabolism I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. Journal of Biological Chemistry. 1957 Nov 1;229(1):189-97.
2. Amagase H, Petesch BL, Matsuura H, Kasuga S,Itakura Y. Intake of garlic and its bioactive components. J Nutr 2001; 131: 955-62.
3. Mahmood T, Hussain SI, Khokhar KM, Bhatti MH, Laghari H. Comparative performance of garlic cultivars. Asian J. Plant Sci. 2002;1(2):160-1.
4. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes and infection. 1999 Feb 1;1(2):125-9.
5. Shin JH, Lee CW, Oh SJ, Yun J, Kang MR, Han SB, Park H, Jung JC, Chung YH, Kang JS. Hepatoprotective effect of aged black garlic extract in rodents. Toxicological research. 2014 Mar;30(1):49
6. .Hosono-Fukao T, Hosono T, Seki T, Ariga T. Diallyl trisulfide protects rats from carbon tetrachloride-induced liver injury. The Journal of nutrition. 2009 Oct 7;139(12):2252-6..
7. Shaarawy SM, Tohamy AA, Elgendy SM, Elmageed ZY, Bahnasy A, Mohamed MS, Kandil E, Matrougui K. Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. International journal of biological sciences. 2009;5(6):549.
8. Pal R, Vaiphei K, Sikander A, Singh K, Rana SV. Effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats. World Journal of Gastroenterology: WJG. 2006 Jan 28;12(4):636.
9. Colín-González AL, Santana RA, Silva-Islas CA, Chánez-Cárdenas ME, Santamaría A, Maldonado PD. The antioxidant mechanisms underlying the aged garlic extract-and S-allylcysteine-induced protection. Oxidative medicine and cellular longevity. 2012 May 17;2012.
10. Rashid EM. The activity of alcoholic extract of Garlic on the growth of Staphylococcus aureus with estimation of median lethal dose in lab. Mice. AL-Qadisiyah Journal of Veterinary Medicine Sciences. 2010;9(2):53-9.

11. Rappaport AM. Physioanatomic considerations. In: Schiff L, Schiff ER, editors. Disease of the liver. 5th ed. Philadelphia: JB Lippincott 1982:1-57.
12. Lauterburg BH, Velez ME. Glutathione deficiency in alcoholics: risk factor for paracetamol hepatotoxicity. Gut. 1988 Sep 1;29(9):1153-7..
13. Mayuren C, Reddy VV, Priya SV, Devi VA. Protective effect of Livactine against CCl4 and paracetamol induced hepatotoxicity in adult Wistar rats. North American journal of medical sciences. 2010 Oct;2(10):491.
14. Sumioka I, Matsura T, Kasuga S, Itakura Y, Yamada K. Mechanisms of protection by S-allylmercaptocysteine against acetaminophen-induced liver injury in mice. The Japanese Journal of Pharmacology. 1998;78(2):199-207.
15. Johnson MG, Vaughn RH. Death of Salmonella typhimurium and Escherichia coli in the presence of freshly reconstituted dehydrated garlic and onion. Appl. Environ. Microbiol.. 1969 Jun 1;17(6):903-5.
16. Gupta AD, Dhara PC, Dhundasi SI, Das KK. Effect of garlic (Allium sativum) on nickel II or chromium VI induced alterations of glucose homeostasis and hepatic antioxidant status under sub-chronic exposure conditions. Journal of basic and clinical physiology and pharmacology. 2009;20(1):1-4.
17. Katyare SS, Satav JG. Impaired mitochondrial oxidative energy metabolism following paracetamol-induced hepatotoxicity in the rat. British journal of pharmacology. 1989 Jan 1;96(1):51-8.
18. Drobiova H, Thomson M, Al-Qattan K, Peltonen-Shalaby R, Al-Amin Z, Ali M. Garlic increases antioxidant levels in diabetic and hypertensive rats determined by a modified peroxidase method. Evidence-Based Complementary and Alternative Medicine. 2011;2011.
19. Lee HS, Lim WC, Lee SJ, Lee SH, Yu HJ, Lee JH, Cho HY. Hepatoprotective effects of lactic acid-fermented garlic extract against acetaminophen-induced acute liver injury in rats. Food science and biotechnology. 2016 Jun 1;25(3):867-73.
20. Zhang Y, Zhang F, Wang K, Liu G, Yang M, Luan Y, Zhao Z. Protective effect of allyl methyl disulfide on acetaminophen-induced hepatotoxicity in mice. Chemico-biological interactions. 2016 Apr 5;249:71-7.
21. Ozougwu JC, Eyo JE, Clarence OK, Soniran O, Kelechukwu DM. Investigationof the Antihepatotoxic Effects of Allium sativum Extracts Against Acetaminophen Intoxicated Rattus novergicus. World Journal of Medical Sciences. 2014;11(3):397-404.
22. Rashed RR, El-Ghazaly MA, Kenawy SA. Protective effect of Garlic oil alone or combined with low-dose gamma irradiation on paracetamol-induced hepatotoxicity in rats. EJBMSR. 2014 Sep;2(3):1-27.