ORIGINAL ARTICLE

HEPATOPROTECTIVE AND ANTIOXIDATIVE EFFECTS OF *ALLIUM SATIVUM* VAR *LEHSUN GULABI* ON ACETAMINOPHEN INDUCED ACUTE HEPATITIS IN MALE ALBINO RATS

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Background: Acute hepatitis results in massive necrosis of liver cells and impairment of liver functions. Acetaminophen toxicity is the most common cause of drug-induced hepatitis. The oxidative metabolite of acetaminophen, NAPQI (*N*-acetyl-*p*-benzo-quinone imine) depletes a natural antioxidant Glutathione peroxidase (GPx). The objective of this study was to determine the hepatoprotective and antioxidative effects of ethanolic extract of *Allium sativum* var *Lehsun gulabi* on acetaminophen induced hepatotoxicity in male albino rats. **Methods:** This randomized controlled trial (RCT) was carried out in Physiology Department, SIMS, Lahore from August 2015 to February 2017 on 90 male albino rats. A single intraperitoneal dose of acetaminophen 750 mg/Kg was used to induce oxidative stress and hepatotoxicity. The rats were randomly divided into three groups of thirty each. Group A was given normal saline (negative control); group B was administered hepatotoxic dose of acetaminophen (positive control); group C (experimental) was pretreated with *Allium sativum* var *Lehsun Gulabi* extract for 7 days before receiving hepatotoxic dose of acetaminophen (Experimental). Serum ALT, AST, ALP, total proteins, albumin and glutathione peroxidase levels in each group were estimated from terminal blood sampling done 24 hours after acetaminophen administration. **Results:** *Allium sativum* var *Lehsun Gulabi* manifested hepatoprotective and antioxidative effects by producing highly significant (p=0.000) reduction in serum ALT and AST, and significant (p=0.015) reduction in serum ALP levels. This garlic extract also produced highly significant (p=0.000) increase in serum total proteins, albumin and glutathione peroxidase levels. **Conclusion:** *Allium sativum* var *Lehsun Gulabi* has potent hepatoprotective and antioxidative potential.

Keywords: *Allium sativum*, *lehsun gulabi*, Glutathione peroxidase, Antioxidative, Hepatoprotective, Acetaminophen

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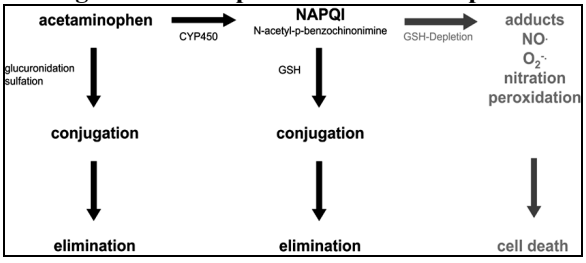
INTRODUCTION

Acute hepatitis results in massive necrosis of liver cells leading to severe impairment of liver functions.[1] An estimated 1,600 cases of acute hepatic failure occur each year in United States. Acetaminophen toxicity is the most common cause, accounting for at least 45% of the cases.[2]

Acetaminophen (AAP) is a commonly used antipyretic and analgesic. Its overdose can lead to acute liver injury and centrilobular necrosis.[3] Chronic alcohol use may greatly increase susceptibility to hepatotoxicity from acetaminophen because of depleted glutathione stores.[4]

Treatment of healthy adults with acetaminophen taken at the maximum daily recommended dose of 4 g for 4 or more days frequently cause elevations in serum aminotransferases which often persist when acetaminophen concentrations are no longer measurable in plasma.[5] Chronic ingestion of therapeutic doses may produce hepatic necrosis and hepatitis, which persist long after the drug has been discontinued.[6] There is no effective treatment other than stopping the drug, giving antidote N-acetylcystine (NAC) and providing general supportive care.[7] The oxidative metabolite of acetaminophen is more toxic than the drug. 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 contributes to tissue necrosis.[8] (Figure-1).

Figure-1: Decomposition of acetaminophen[8]



Allium sativum, or 'garlic' is widely used in culinary preparations.[9] Two varieties of *Allium sativum*

grown in Punjab are Chinese (exotic), *Lehsun Gulabi* (لہسن گلابی) (local).[10] Traditional uses of *Allium sativum* include use in intestinal disorders, diarrhoea, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging, headache, flu, sore throat, fever and otitis media.[9]

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.[9] Allicin and other thiosulfinates instantly decompose to other compounds, such as diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DAT), dithiins and ajoene. At the same time, γ-glutamylcysteines are converted to *S*-allylcysteine (SAC).[9] These sulphur compounds of garlic have proved to be promising antioxidants against drug induced hepatitis.[11–13]

The liver is a vital organ, so searching new drugs for limiting hepatic injury has been of interest. A case study reported for the first time that liver injury, marked by elevated liver function tests, occurred secondarily to garlic supplementation.[14] The present study was aimed to explore hepatoprotective and antioxidant properties of *Allium sativum*. Garlic is a natural component of diet in Pakistan and the *Lehsun gulabi* variety is commonly available in market. The objective of this study was to determine the hepatoprotective and antioxidative effects of ethanolic extract of *Allium sativum* var *Lehsun gulabi* on acetaminophen induced hepatotoxicity in albino rats.

METHODOLOGY

Ninety male albino rats weighing 200–250 grams 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 experiments. Housing conditions were thermostatically maintained at 26±2 °C and a light/dark cycle (lights on: 0900–2100 Hours).[15] The animals were fed with commercially available standard pellet diet ad libitum and were provided with tap water in clean bottles.

Allium sativum var *Lehsun gulabi* was obtained from local market of Lahore and its ethanolic extract was made and standardized using facilities available at Applied Chemistry Research Centre, PCSIR labs, Lahore. The extract obtained, was filtered and the solvent (ethanol) evaporated in vacuum with a rotary evaporator and a dark brown concentrate was obtained. This concentrate was kept at 4 °C prior to use. The crude extract was then dissolved in normal saline and then diluted to the desired concentration.[16]

A single intraperitoneal dose of acetaminophen 750 mg/Kg[17] dissolved in normal saline was used to induce acute oxidative hepatic injury. Ninety male albino rats were divided into:

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

Group B (Positive Control, n=30): was given a single dose of acetaminophen 750 mg/Kg[17] dissolved in normal saline intraperitoneally.

Group C (Experimental, n=30): was pretreated with *Allium sativum* var *Lehsun gulabi* ethanolic extract in a dose of 500 mg/Kg body weight intraperitoneally[18] daily for 7 days before a single intraperitoneal dose of acetaminophen 750 mg/Kg[17] dissolved in normal saline.

After 24 hours of acetaminophen administration, each rat was anesthetized using ether. Three mL blood was drawn by cardiac puncture and was kept in the test tube for about 15–20 minutes, and allowed to clot. After 15–20 minutes, samples were centrifuged at 5,000 rpm for 15 minutes. The serum, thus obtained, was preserved in labelled polypropylene storage tubes.

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein and albumin were determined on the same day of blood sampling. About 0.5 mL of each of these samples were stored at -20 °C for determination of serum glutathione peroxidase at later stage.

Data was analyzed using PASW-18. The arithmetic mean and standard deviation for quantitative variables were calculated. The statistical significance of difference amongst the three groups were determined by applying one way ANOVA followed by post hoc LSD (multiple comparison) test. The values were considered significant if the p was <0.05; and, highly significant if the p was <0.001.

RESULTS

After pretreatment with ethanolic extract of *Allium sativum* var *Lehsun gulabi* followed by acetaminophen hepatotoxicity, there was highly significant ($p<0.000$) decrease in liver enzymes including serum ALT, AST and ALP, and highly significantly ($p<0.000$) less decrease in serum total protein, albumin and glutathione peroxidase in experimental group as compared to both negative and positive control groups (Table-1).

The positive control group (group B) having acetaminophen toxicity showed highly significantly ($p=0.000$) raised values of serum ALT, AST and ALP, and highly significant ($p=0.000$) decrease in serum total protein, albumin and glutathione peroxidase as compared to the negative control group (group A) as depicted in Table-2.

After pretreatment with ethanolic extract of *Allium sativum* var *Lehsun gulabi* followed by acetaminophen toxicity, the experimental group C showed highly significant ($p=0.000$) decrease in serum

levels of ALT and AST, significant (p=0.015) decrease in serum level of ALP and highly significant (p=0.000) increase in serum levels of albumin, total proteins and glutathione peroxidase as compared to the positive control group (group B) (Table-3).

Table-1: Serum ALT, AST, ALP, total protein, albumin and glutathione peroxidase in groups (One way ANOVA) (Mean±SD)

Parameters	Group A (n=30)	Group B (n=30)	Group C (n=30)	*p*
Serum ALT (U/l)	53.53±4.46	177.50±6.53	58.73±3.68	0.000*
Serum AST (U/l)	65.80±3.46	102.43±7.19	61.70±4.46	0.000*
Serum ALP (U/l)	124.30±5.81	575.90±4.69	572.47±3.54	0.000*
Serum total proteins (g/dl)	6.78±0.21	4.20±0.12	4.44±0.14	0.000*
Serum albumin (g/dl)	3.45±0.13	1.40±0.16	3.59±0.12	0.000*
Glutathione peroxidase (ηg/ml)	21.49±0.79	4.62±0.60	15.03±1.25	0.000*

*highly significant

Table-2: Serum ALT, AST, ALP, total protein, albumin and glutathione peroxidase in groups A and B (Post hoc LSD) (Mean±SD)

Parameters	Group A (n=30)	Group B (n=30)	*p*
Serum ALT (U/l)	53.53±4.46	177.50±6.53	0.000*
Serum AST (U/l)	65.80±3.46	102.43±7.19	0.000*
Serum ALP (U/l)	124.30±5.81	575.90±4.69	0.000*
Serum total proteins (g/dl)	6.78±0.21	4.20±0.12	0.000*
Serum albumin (g/dl)	3.45±0.13	1.40±0.16	0.000*
Serum glutathione peroxidase (ηg/ml)	21.49±0.79	4.62±0.60	0.000*

*highly significant

Table-3: Serum ALT, AST, ALP, total protein, albumin and glutathione peroxidase in groups B and C (Post hoc LSD) (Mean±SD)

Parameters	Group B (n=30)	Group C (n=30)	*p*
Serum ALT (U/l)	177.50±6.53	58.73±3.68	0.000**
Serum AST (U/l)	102.43±7.19	61.70±4.46	0.000*&
Serum ALP (U/l)	575.90±4.69	572.47±3.54	0.015*
Serum total proteins (g/dl)	4.20±0.12	4.44±0.14	0.000**
Serum albumin (g/dl)	1.40±0.16	3.59±0.12	0.000**
Serum glutathione peroxidase (ng/ml)	4.62±0.60	15.03±1.25	0.000**

*Significant, **highly significant,

DISCUSSION

Our study evaluated the effects of ethanolic extract of *Allium sativum* var *Lehsun gulabi* on experimentally induced acetaminophen hepatotoxicity and noted effects on liver enzymes (ALT, AST and ALP), serum albumin, total proteins and serum glutathione peroxidase in male albino rats. The study showed that pretreatment of rats with ethanolic extract of this variety of garlic grown in Pakistan prevented the increase in liver enzymes and decrease in serum albumin, total proteins and glutathione peroxidase, due to acetaminophen toxicity. This adds to several reports on the pharmacological usefulness of garlic extracts as liver protective agents.

Lee *et al*[19] investigated the protective effect of fermented garlic extract by lactic acid bacteria (LAFGE) against acetaminophen induced acute liver injury in rats. Their findings indicated lowered plasma ALT levels, inhibition of lipid peroxidation, glutathione and ATP depletion, and the elevation of antioxidant enzyme activities. These findings indicate that LAFGE ameliorated 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.[19] Allyl methyl disulfide (AMDS) has been identified as one of the bioactive components in fresh garlic paste and alleviates AAP-induced elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels, significantly reduces the maleic dialdehyde (MDA) level in liver tissues and restores the activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase and glutathione towards normal levels.[20]

Ozougwu *et al*[21] investigated hepatoprotective effects of *Allium sativum* methanolic extracts on paracetamol induced hepatotoxic rats. *Allium sativum* reduced ALT and total serum bilirubin in a dose dependent fashion whereas it reduced AST, ALP and LDH level in a dose independent manner. Acetaminophen hepatotoxicity leads to leakage of cellular enzymes into the plasma such as ALT, AST, ALP and LDH showing increased permeability and necrosis of hepatocytes. These significantly increased levels of serum AST and ALT are due to hepatocellular damage because these enzymes are normally located in the cytoplasm and released into the circulation after cellular damage. The mechanism of action of garlic could be through preventing the intracellular enzyme release and by membrane stabilizing effects. This is because garlic is rich in antioxidants. The reduction in ALP and LDH levels by extracts may suggest repairing of rat liver by *Allium sativum* extracts. Thus it was suggested that the active ingredients in *Allium sativum* (allyl propyl disulfide) 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.[21]

Another study[22] determined the antioxidative effects of *Allium sativum* methanolic extract against paracetamol induced liver toxicity. It was evident that garlic extract was able to significantly raise the intracellular contents of glutathione peroxidase.

Rashed *et al*[23] investigated the effect of garlic oil (GO) alone or in combination with low dose total body gamma (γ)-irradiation (LDR) against paracetamol (AAP) induced hepatotoxicity in rats. Findings showed that the combination of GO and LDR produced considerable comparable effects to either treatment alone in reducing serum elevations of ALT, AST, ALP, LDH, MDH, hepatic CYP2E1 activity and preventing the decreased hepatic glutathione content as a result of AAP toxicity. This ability of garlic to lower the raised levels of ALT, AST and ALP and to prevent decrease in levels of glutathione after AAP toxicity was in accordance with results of the present study. This remarkable synergistic protection against AAP-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]

Shin[24] investigated the hepatoprotective effects of aged black garlic (ABG) in rodent modules of liver injury. ABG inhibited carbon tetrachloride induced elevations of ALT and AST. D-galactosamine induced hepatocellular damage was also suppressed by ABG treatment. However, ABG did not effect the elevations of ALP in this study.[24] The ethanolic extract of *Allium sativum* var *Lehsun gulabi* produced significant reductions in elevated ALP. Possible explanation for these varied results could be the difference in the chemical composition of garlic grown in different geographical locations and this could be confirmed by quantitative analysis of allium compounds.

Sharma *et al*[25] studied amelioration of lead-induced hepatotoxicity by *Allium sativum* extracts in Swiss albino mice. Oral treatment with lead nitrate induced a significant increase in the levels of hepatic AST, ALT, and ALP. Hepatic protein levels in lead-exposed mice were significantly depleted. Aqueous garlic extract and ethanolic garlic extract restored the deranged parameters significantly.[25] This result was in accordance with our study regarding serum parameters of ALT, AST, ALP and total proteins.

Modulatory effects of dietary inclusion of garlic (*Allium sativum*) on gentamycin-induced hepatotoxicity and oxidative stress in rats were studied by Ademiluyi *et al*[26]. Hepatic damage, as revealed by significant elevation of liver damage marker enzymes (AST and ALT) and reduction in plasma albumin level, were restored following consumption of diet containing garlic. Elevations of serum levels of ALT indicated necrotic lesions in the liver cells while decreased serum albumin indicated that there was an impairment in both synthetic and excretory activities of liver.[26] These results were in accordance with our results as ethanolic extract of garlic *Lehsun gulabi* variety prevented the decrease in plasma albumin while restoring liver damage marker enzymes to nearly normal values.

CONCLUSION

Garlic may be considered as a useful dietary supplementary compound to patients treated with regular high doses of paracetamol such as of tuberculosis, cancer, dengue fever and arthritis. Consumption of this variety of garlic might be a useful prophylactic and therapeutic strategy against oxidative stress of toxic hepatitis in Pakistan. The antioxidative and hepatoprotective potential of *Allium sativum* should be further investigated in human studies.

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