Original Article

Comparison of Hepatoprotective Effect of two Varieties of Garlic (allium Sativum Var Chinese Exotic and Allium Sativum Var Lehsun Gulabi)on Acetaminophen Induced Acute Hepatitis in Male Albino Rats

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Objective: To determine and compare the hepatoprotective effect of ethanolic extracts of two varieties of Allium sativum on acetaminophen induced hepatotoxicity in male albino rats.

Methods: This study was carried out on 120 male albino rats. A single intraperitoneal dose of acetaminophen 750mg/kg was used to induce hepatotoxicity. 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 sativumVar 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 ALT, AST and ALP levels in each group were measured from terminal blood sampling done 24 hours after acetaminophen administration.

Results: The ethanolic extract of Allium sativum Varlehsungulabi showed highly significantly (p=0.000) greater reduction in serum ALT and AST as compared to the effect of ethanolic extract of Allium sativumVar Chinese exotic, but there was non significant (p=0.526) difference in serum levels of ALP between the two varieties of garlic.

Conclusions: On comparison, Allium sativumvar Lehsun Gulabi has better hepatoprotective potential as compared to Allium sativumvar Chinese exotic.

Keywords: allium sativum, glutathione peroxidase,antioxidative, hepatoprotective, acetaminophen.

Introduction

Acute hepatitis results in massive necrosis of liver cells leading to severe impairment of liver functions.[1] An estimated 1600 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 (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.[3] Chronic alcohol use may greatly increase susceptibility to hepatotoxicity from acetaminophen because of depleted glutathione stores.[4]

It has been observed that treatment of healthy adults with acetaminophen taken at the maximum daily recommended dose of 4g for 4 or more days frequently cause elevations in serum aminotransferases which often persist when acetaminophen concentrations are no longer measurable in plasma.[5] In some patients, chronic ingestion of therapeutic doses may produce hepatic necrosis and hepatitis, which persist long after the drug has been discontinued.[6] In most cases, there is no effective treatment other than stopping the drug and providing general supportive care. N-acetylcystine (NAC) has been used as an antidote for acetaminophen toxicity.[7] 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*-benzoquinone 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, tumor necrosis factor alpha (TNF-α) that in turn contributes to tissue necrosis.[8]

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

in Punjab are Chinese (exotic), Lehson Gulabi (local).[10] Traditional uses of Allium sativum are; in intestinal disorders, diarrhea, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging[9], headache, flu, sore throat, fever and otitis media.[11]

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).[9] These sulphur compounds of garlic have proved to be promising antioxidants against drug induced hepatitis.[12 15]

Search for new drugs for limiting hepatic injury has been of interest. The present study was aimed to explore new drug for curing acute hepatitis and delaying its progression to hepatocellular carcinoma by evaluating hepatoprotective properties of Allium sativum. Garlic is a natural component of diet in Pakistan and efforts should be channelized towards bringing down incidence of acute hepatitis in our country by improving intake of this natural antioxidant. The objective of this study was to determine and compare the hepatoprotectiveeffect of ethanolic extracts of two varieties of garlic on acetaminophen induced hepatotoxicity in albino rats.

Methods

One hundred and twenty 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 0C and a light/dark cycle (lights on: 0900-2100).16 The animals were fed with commercially available standard pellet diet ad libitum and were provided with tap water in clean bottles.

Allium sativumVar Chinese exotic and Allium sativumVarLehsunGulabi were obtained from local market of Lahore. Ethanolic extract of Allium sativumVar Chinese exotic and Allium sativumVarLehsunGulabi were 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. After evaporation, a dark brown concentrate was obtained. This concentrate was kept at 4 0C prior to use. The crude extract was then dissolved in normal saline and then diluted to the desired concentration.[17,18]

A single intraperitoneal dose of acetaminophen 750 mg/kg19dissolved in normal saline was used to induce acute oxidative hepatic injury. One hundred and twenty rats were divided into 04 groups.

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/kg19dissolved in normal saline intraperitoneally. Group C (Experimental 1, n=30): was pretreated with Allium sativumVar Chinese exotic ethanolic extract in a dose of 500mg/kg body weight intra-peritoneally20 for 7 days before a single intraperitoneal dose of acetaminophen 750 mg/kg19 dissolved in normal saline. The extract was administered once as a single daily dose, while acetaminophen was administered after 12 hours overnight fast.[16] Group D (Experimental 2, n=30): was pretreated with Allium sativumVarLehsungulabi ethanolic extract in a dose of 500mg/kg body weight intraperitoneally20 for 7 days before a single intraperitoneal dose of acetaminophen 750 mg/kg19 dissolved in normal saline. The extract was administered once as a single daily dose, while acetaminophen was administered after 12 hours overnight fast.[16] After 24 hours of acetaminophen administration, each rat was anesthetized using ether. Three-milliliter blood was drawn directly from heart 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.

Serum alanine aminotransferase (ALT), (AST) aspartate aminotransferase and alkaline phosphatase (ALP) were determined.

Data was analyzed using PASW18 (formerly SPSS). The arithmetic mean and standard deviation for quantitative variables were determined. The statistical significance of difference amongst the four groups were determined by applying one way ANOVA followed by post hoc LSD (multiple comparison) 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.

Results

After pretreatment with ethanolic extract of Allium sativum followed by acetaminophen hepatotoxicity, there was reduction in liver enzymes including serum ALT, serum AST and ALP 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 significantly (p=0.000) raised values of serum ALT, AST and ALP as compared to these values in negative control group (group A). **(Table 2)** After pretreatment with ethanolic extract of Allium sativumVarChinese exotic followed by acetaminophen toxicity, the group C showed highly significant (p=0.000) decrease in serum levels of ALT and AST but non significant (p=0.335) change in serum ALP. **(Table 3)** After pretreatment with ethanolic extract of Allium sativum VarLehsun Gulabi followed by acetaminophen toxicity, the experimental group D showed highly significant (p=0.000) decrease in serum levels of ALT and AST and non significant (p=0.015)decrease in serum level of ALP, **(Table 4).** The experimental group D pretreated with ethanolic extract of Allium sativumVarlehsungulabi showed highly significant (p=0.000) greater reduction in

Table-1: Comparison of serum ALT, AST, ALP and glutathione peroxidase in groups A, B, C and D. (one way ANOVA).

Parameters	Group A (n=30)	Group b (n=30)	Group C (n=30)	Group D (n=30)	P-value
Serum ALT (U/l)	53.53±4.46	177.50±6.53	82.83±6.36	58.73±3.68	0.000*
Serum AST (U/l)	65.80±3.46	102.43±7.19	83.03±5.87	61.70±4.46	0.000*
Serum ALP (U/l)	124.30±5.81	575.90±4.69	574.00±2.80	572.47±3.54	0.000*

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

Table-2: Comparison of serum ALT, AST, ALP and glutathione peroxidase in groups A and B. (Post hoc LSD.

Parameters	Group A (n=30)	Group B (n=30)	P-value
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*

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

Table-3: Comparison of serum ALT, AST, ALP and glutathione peroxidase in groups B and C. (Post hoc LSD).

Parameters	Group B (n=30)	Group C (n=30)	P-value
Serum ALT (U/l)	177.50±6.53	82.83±6.36	0.000*
Serum AST (U/l)	102.43±7.19	83.03±5.87	0.000**
Serum ALP (U/l)	575.90±4.69	574.00±2.80	0.335***

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

Table-4: Comparison of serum ALT, AST, ALP and glutathione peroxidase in groups B and D. (Post hoc LSD)

Parameters	Group B (n=30)	Group D (n=30)	P-value
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**

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

Table-5: Comparison of serum ALT, AST, ALP and glutathione peroxidase in groups A, B, C and D. (one way ANOVA).

Parameters	Group C (n=30)	Group D (n=30)	P-value
Serum ALT (U/l)	82.83±6.36	58.73±3.68	0.000*
Serum AST (U/l)	83.03±5.87	61.70±4.46	0.000*
Serum ALP (U/l)	574.00±2.80	572.47±3.54	0.526***

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

serum ALT and AST as compared to reduction in these parameters in experimental group C pretreated with ethanolic extract of Allium sativumVar Chinese exotic but there was no significant (p=0.526) difference in serum levels of ALP among the two experimental groups, (Table 5).

Discussion

Our study compared the effects of ethanolic extracts of two varieties of garlic (Allium sativumVar Chinese exotic and Allium sativumVarLehsunGulabi) on experimentally induced acetaminophen hepatotoxicitythat manifested as increased liver enzymes.This study showed that pretreatment of rats with ethanolic extract of two varieties of garlic grown in Pakistan prevented the increase in liver enzymesdue to acetaminophen toxicity. This effect was exhibited more strongly by LehsunGulabi extract when compared to that of Chinese exotic. 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 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 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.[21] Allyl methyl disulfide (AMDS) has been identified as one of the bioactive components in fresh garlic pastealleviates APAP-induced elevation of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels, significantly ($p < 0.05$) reduced the maleic dialdehyde (MDA) level in liver tissues and restored the activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase and glutathione towards normal levels. [22]

Allium sativumreduced ALT and total serum bilirubin in a dose dependent fashion whereas it reduced AST, ALP and LDH level in a dose independent manner. The reduction in ALP and LDH levels by extracts may suggest repairing of rat liver by Allium sativumextracts. Thus 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 sativumextracts may be through their antioxidative effects that are capable of free radical scavenging in living system.[23] Rashedet 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 reducing serum elevations of ALT, AST, ALP, LDH, MDH, hepatic CYP2E1 activity and preventing the decreased hepatic glutathione content as a result of APAP toxicity. This ability of garlic to lower the raised levels of ALT, AST and ALP after APAP toxicity was in accordance with results of the present study. 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.[24] The hepatoprotectiveeffects of aged black garlic (ABG) in rodent modules of liver injury was investigated. 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.[25] This finding was in accordance with our study regarding inability of the ethanolic extract of Allium sativumvar Chinese exotic to reduce significantly this elevated serum ALP. The ethanolic extract of Allium SativumVarLehsunGulabi produced significant reductions in elevated ALP. Possible explanation for these varied results of two varieties of garlic grown in Pakistan could be the difference in their chemical composition.

Conclusion

On comparison, Allium SativumVarLehsunGulabi has better hepatoprotectivepotential as compared to Allium sativumvar Chinese exotic. Thus garlic may be considered as a useful dietry supplement topatients treated with regular high doses of paracetamol such as of tuberculosis, cancer, dengue fever and arthritis.

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