BIOCHEMICAL STUDIES ON THE EFFECT OF AGED GARLIC EXTRACT ON ACETAMINOPHEN INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Aged garlic extract has been shown to protect rats against acetaminophen induced liver injury through its antioxidant property. Aged garlic extract was given orally to rats for a period of 28 days. Liver damage was induced by inoculating acetaminophen at 500 mg/kg on 12th day of the experimental study. In acetaminophen treated rats there was significant increase in serum amino transferases (ALT and AST), but these enzymes were in the normal range in aged garlic extract (AGE) treated group indicating the protective effect of aged garlic extract against acetaminophen induced injury.

KEY WORDS : Aged garlic extract, Acetaminophen, Amino transferases, Rats

INTRODUCTION

Aged Garlic Extract (AGE) is an alternative source to fresh garlic which is richer in anti-oxidants and is produced by aqueous extraction of fresh organic garlic stored at room temperature (37°C) for 20 months. Paracetamol is an extensively used analgesic and antipyretic drug. Although safe, when used at therapeutic doses is associated with significant hepatotoxicity when taken in overdose (Ramsay *et al.,* 1989). It is believed that aged garlic extract exerts hepatoprotective effect through its antioxidant property.

MATERIALS AND METHODS

The study was conducted at the Department of Veterinary Pathology, Veterinary College, Bangalore. Forty Albino rats of Wistar strain weighing between 150 to 300 g were used for the present study. They were maintained under standard laboratory conditions and were fed with standard commercial rat feed obtained from M/S Amruth feeds, Bangalore. The animals were kept for acclimatization in Laboratory Animal House, for 12 days, before commencing the experiment. The experiment was carried out for a period of 28 days. The rats were divided into four groups having ten rats in each group . No treatment was given to rats of group I and treated as normal control . To induce hepatotoxicity in rats of group II, commercially available paracetamol preparation (Febrinil® 150mg/ml) was administered by intraperitoneal injection @ 500 mg/kg body weight. Aged garlic extract was obtained from M/S Vet Care Pvt. Ltd, Yelahanka, Bangalore. The rats belonging to group III were administered aged garlic extract (AGE) @ 200mg /kg body weight, orally for 28 days and group IV animals were administered paracetamol intraperitoneally after fasting overnight at the dose rate of 500 mg/kg body weight and 200 mg /kg AGE on 12th day of the experiment and the control group (I) received same volume of normal saline intraperitoneally. Blood samples of about one ml were collected from all the rats in each group through retro orbital plexus on days 0, 14, 21 and 28 of the experiment. Samples of blood collected were allowed to clot and centrifuged at 1500 rpm for 5 min. The sera, thus obtained were stored at 4^{0}C for further use. The Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and creatinine levels were estimated by the method of International Federation of Clinical Chemistry (IFCC) using semi-automatic analyser and further, the data subjected to two way analysis of variance (ANOVA) test using Graph Pad Prism version 5 for windows.

RESULTS AND DISCUSSION

The results on the "Biochemical studies on the effect of aged garlic extract in acetaminophen induced liver damage in rats" are presented in table 1-3 .The results indicate that there were significant ($P \leq 0.05$) elevation in the level of ALT and AST at 14 and 21 days in group II rats whereas non significant elevation in enzyme activity was observed at 28 day as compared to 0 days activities of the enzyme in group II animals . The level of enzyme activity in group I remains more or less similar upto 28 days . In group III animals the level of ALT and AST was significantly ($P \leq 0.05$) lower as compared to group II and were more or less similar to group I with a slight marginal variation. The enzyme activity in group IV rats were slightly higher although non significant at day 14 and was similar at day 21 and 28 . No significant difference was observed in the creatinine, between treated and control group during the entire period of study and was depicted in table 3.

Table 1: Mean values of ALT (U/L) of control, paracetamol and aged garlic extract treated groups at different intervals of time.

Groups	Days of experimental study			
	Day 0	Day 14	Day 21	Day 28
I	37.70±2.81 (n=10)	35.60±1.78 (n=10)	43.13±5.65 (n=8)	39.62±3.78 (n=6)
II	43.20±3.26 (n=10)	81.50±3.66 [a] (n=10)	76.75±3.24 [a] (n=8)	53.67±2.75 (n=6)
III	37.20±2.29 (n=10)	41.40±2.98 [b] (n=10)	36.12±3.77 [b] (n=8)	38.66±4.72 (n=6)
IV	41.40±4.08 (n=10)	49.10±4.31 [a b] (n=10)	43.25±3.99 [b] (n=8)	40.22±3.89 (n=6)

Table 2: Mean values of AST (U/L) of control, paracetamol and aged garlic extract treated groups at different intervals of time.

Groups	Days of experimental study			
	Day 0	Day 14	Day 21	Day 28
I	68.10±4.40 (n=10)	69.50±4.75 (n=10)	72.00±4.23 (n=8)	66.67±7.57 (n=6)
II	69.20±4.27 (n=10)	240.70±13.94 [a] (n=10)	190.63±4.09 [a] (n=8)	85.50±3.94 (n=6)
III	67.90±4.25 (n=10)	69.20±3.80 [b] (n=10)	71.13±3.95 [b] (n=8)	62.50±7.16 (n=6)
IV	69.20±3.80 (n=10)	69.80±3.68 [b] (n=10)	73.38±4.14 [b] (n=8)	63.50±8.14 (n=6)

From the above results it is revealed that AGE administration revoke the toxic effects due to paracetamol ,further if AGE administered along with paracetamol there is no symptoms of toxicity

as revealed by normal level of ALT and AST, the two enzymes are indicator of hepatic and cardiac damage.

The paracetamol has been reported to cause hepatotoxicity with disruption of calcium homeostasis, mitochondrial dysfunction and oxidative stress (Donnelly *et al.,* 1994,). Hence elevation in the ALT and AST values in present study could be directly attributed to the induction of hepatotoxicity by the acetaminophen. ALT and AST levels remained within the normal range in aged garlic extract treated rats (Group IV). It could be due to protective action of aged garlic extract on the hepatocytes through its antioxidant property. These finding are in accordance with the findings of Sumioka *et al.* (2001) and Ezeala *et al.,* (2009). No significant difference was observed in the levels of creatinine between treated groups and control group.

Table 3: Mean values of creatinine (mg/dl) of control, paracetamol and aged garlic extract treated groups at different intervals of time.

Groups	Days of experimental study			
	Day 0	Day 14	Day 21	Day 28
I	0.57±0.04 (n=10)	0.56±0.05 (n=10)	0.74±0.10 (n=8)	0.73±0.07 (n=6)
II	0.59±0.07 (n=10)	0.55±0.06 (n=10)	0.58±0.06 (n=8)	0.63±0.11 (n=6)
III	0.52±0.06 (n=10)	0.61±0.05 (n=10)	0.49±0.04 (n=8)	0.52±0.05 (n=6)
IV	0.48±0.05 (n=10)	0.65±0.09 (n=10)	0.61±0.09 (n=8)	0.72±0.15 (n=6)

The hepatoprotective effect could be attributed to antioxidant and anti-lipid peroxidative effects of garlic. Oxidative stress and lipid peroxidation are key processes in the toxicity of acetaminophen. Garlic contains several organosulfur compounds such as allicin, diallyl sulfide, and diallyl disulfide which are valuable precursors for glutathione biosynthesis and garlic prevents glutathione depletion induced by acetaminophen overdose by up regulating its biosynthesis in the liver (Ezeala *et al,* 2009)

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