HEPATO-PROTECTIVE EFFECT OF ASCORBIC ACID ON OXIDATIVE STRESS IN MICE EXPOSED TO CYPERMETHRIN

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ABSTRACT

Objectives: To evaluate protective effect of ascorbic acid on liver parameters in mice exposed to Cypermethrin.

Study Design: Laboratory based randomized control trial.

Place and Duration of Study: Research was conducted in Army Medical College's biochemistry and molecular biology department in association with department of pathology, Army Medical College, Rawalpindi and National Institute of Health, Islamabad, Pakistan from 19 May 2013 to 17 June 2013.

Material and Methods: Thirty albino mice of Balb/C strain weighing 40-45 g were randomly divided into three groups. Each group comprised 10 mice. Control group A which received normal diet. Cypermethrin experimental group B received cypermethrin with normal diet experimental group C which received cypermethrin and vitamin C with normal diet. This process continued for 28 days. After this duration serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) values were determined.

Results: Serum ALT and AST levels were significantly increased in group B as compared to group A ($p < 0.001$). ALT levels of group A and group C were insignificant ($p = 0.473$). AST levels of group A and C were significantly different ($p < 0.01$).

Conclusion: Ascorbic acid can protect liver from Cypermethrin induced oxidative stress in mice.

Keywords: Ascorbic acid, Balb /C mice article, Cypermethrin, Oxidative stress.

INTRODUCTION

Rise in global food demand has increased use of pesticides in agriculture. Pesticide poisoning is a major cause of mortality and morbidity especially in the developing world. Every year there are 3 million cases of severe poisoning and 220,000 deaths; the majority of these poisonings and 99% of the resulting deaths occur in the third world[1]. Pyrethrin is obtained from chrysanthemum cinerarifolium flowers. Cypermethrin is a type 2 synthetic pyrethroid with potent insecticidal properties. It effectively control many pests and useful in household treatment. The absorption and excretion of cypermethrin takes a quick course. Cypermethrin, both cis and trans isomers are metabolized to phenoxybenzoic acid and cyclopropanecarboxylic acid[2]. The myth of mammalian bio-safety is proving wrong as new research is coming forth. Pyrethroid residues have been found in dairy items, meat, soft drinks and even in water and is reported to cause liver and kidney damage[3]. Insecticide Pyrethroid produces oxidative stress that can be confirmed histopathologically and biochemically[4]. DNA damage, brain toxicity, hepato-nephrotoxicity and anaemia are some deleterious effects which also have been reported due to this insecticide[5,6]. Cypermethrin intoxication initiates free radical generation that causes different complications in the body. Oxidative stress is a harmful process that can mediate damage to cell structures, including lipids, proteins, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in nervous tissue, kidneys and liver. The rise in levels of ALT and AST give a good overview of hepatic damage[7]. Oxidative stress (OS) is thought to be involved in the pathogenesis of eurodegenerative disorder and many chronic diseases in particular cardiovascular disease, cancer, cataract and aging by inducing oxidative changes to cellular lipid, protein and DNA. Excessive reactive oxygen species production can occur during

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normal aging process or following exposure to environmental toxicants[8]. Liver is a vital organ, which plays an essential role in health, disease and overall development and growth. It is associated with metabolism and elimination of toxic material from body so changes in its biochemical parameter are a clue to observe oxidative stress and its reversal by antioxidant. Vitamin C or ascorbic acid involved in synthesis of collagen, carnitine, and epinephrine, absorption of dietary iron and mobilization of storage iron for erythropoiesis. Vitamin C is non enzymatic antioxidant and is therefore potentially involved in protecting cells against oxidative stress[9]. It is water soluble and widely distributed in plants and animal tissues. Prolonged deficiency in man results in a condition known as scurvy. As vitamin C degrades to inactive constituents by irreversible oxidation so a large proportion of it is not beneficial for body while it is cooked, processed or stored[10]. In view of the potential hazardous effect of Cypermethrin on health, this study was planned in mice to determine the beneficial effects of ascorbic acid on oxidative stress in Cypermethrin expose mice. These exposed mice were supplemented with normal mice diet, Cypermethrin and Cypermethrin with ascorbic acid rich diet for 4 weeks. Almost all Cypermethrin research work is of western origin. So it is a new addition of work to prevent hepatic damage by adding natural resources in our daily life.

MATERIAL AND METHODS

This laborarty based randomized controlled trial was conducted at National Institute of Health (NIH), Islamabad in association with department of pathology, Army Medical College Rawalpindi. This study was conducted on 30 albino Balb/c mice weighing 30-40g. Mice were procured from National Institute of Health and experimental research continued for 28 days. The mice were randomly divided into three groups of 10 mice each using random number table: First group A (Control group): Ten mice in first group proposed as control. Mice were given routine laboratory diet according to requirement. National institute of Health has facility to prepare

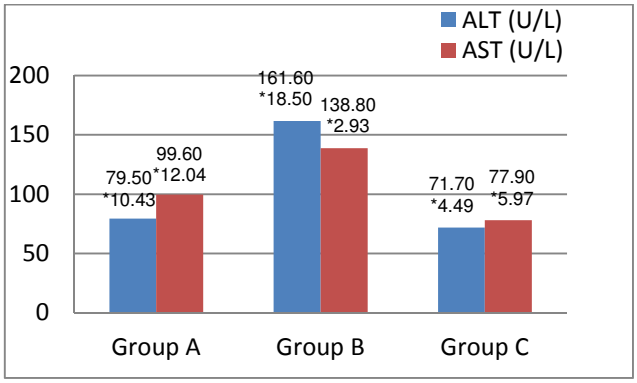

Figure-1: Comparison of serum ALT and AST among group-A, group-B and group-C, showing normal raised in group B (Cypermethrin experiment); Both levels were normal when co-administered with vitamin C in group C. *SD

mice diet according to standards set internationally. This whole process continued for 28 days. Second group B (Cypermethrin experimental group): Ten mice of second group received diet and Cypermethrin. Cypermethrin 15 mg/kg body weight mixed in mice diet, prepared at NIH Islamabad for 04 weeks[11]. Group C (Ascorbic acid experimental group): Ten mice in group C fed with Cypermethrin and vitamin C. One gram vitamin C dissolved in one litre water was given to mice as sole supply of water for whole day[12]. Five mice were placed in one iron cage. Every day animals were exposed to 12 hour dark period and 12 hour light period at 23 ± 5^0C. Mice were given free accessibility to water and food. At the end of 30 days mice were anesthetized in chloroform chamber and blood samples taken through intracardiac route. These samples were put in gel seperater tubes, to measure serum ALT and AST. These enzymes were measured by ALT UV kit and AST diagnostic kit by Selectra auto analyser in department of pathalogy, Army Medical College Rawalpindi.

Statistical analysis

Data was analyzed using SPSS version 15. Descriptive statistics were used to describe the results. One way analysis of variance (ANOVA) was applied for comparison of quantitative variables between the groups followed by post-hoc tukey test. A *p*-value <0.05 was considered as significant.

RESULTS

There was significant difference among group-A, group-B and group-C mice in serum ALT levels ($p < 0.001$) and serum AST levels ($p < 0.001$) (Fig-1).

ALT and AST level were significantly serum raised in group-B as compared to group-A and group C. The difference in ALT level of group-A and group-C was insignificant ($p > 0.05$) while difference in AST level of group-A and group-C was significant (Tables-1,2).

DISCUSSION

Insecticide toxicity is a slow process which inflicts damage to human body and its effects reveal in later life. In this case free radicals produce in abundance as their half life is extremely less so these damage protein, lipid and nucleic acids. Antioxidants can scavenge free radical. Increase in serum ALT and AST showed oxidative damage to hepatocytes membranes. Cypermethrin produced oxidative stress which increased liver enzymes level in group–B in comparison with control. While in group-C ALT levels were decreased to normal levels. AST levels were also reduced but below than normal in comparison with control. It means vitamin C had antioxidant property which effectively scavenged free radicals while Cypermethrin had potential to produce oxidative stress. Our research outcome exactly match with Hussain et al and Manal et al which gave evidence about antioxidant property of vitamin C[13,14]. Our study correlates with multiple series of research work carried by Sohini et al and Soujanya et al in which they found that vitamin C quench ROS under natural and chemically simulated oxidative stress conditions[15,16]. Ebuehi et al has shown from his research that vitamin C has antioxidant effect so it reduces oxidative stress induced in brain cells which is according to our results[17]. Similar findings published by Assayed et al who gave evidence about decreasing teratogenic effect of Cypermethrin by vitamin C[18]. It ameliorates antioxidant effect of vitamin E, selenium, polyphenol and lipoic acid as indicated by

Table-1: Post-hoc Comparisons of (ANOVA followed by Tukey HSD) serum ALT levels between the groups showing significantly raised serum ALT levels in group B (Cypermethrin experiment) as compared to group A (control) whereas significantly lower than group C (Cypermethrin and vitamin C). No significant difference between group A and B.

Paired on Post-Hock comparison	Mean differene	*p*-value
Group A versus group B	82.10	<0.001*
Group A versus group C	7.80	.473[NS]
Group B versus group C	89.9	<0.001*

*Siginicant difference ($p < 0.05$), [NS] insignificant difference ($p > 0.05$)

Table-2: Comparison of groups (ANOVA followed by Tukey HSD) serum AST levels between the groups showing significantly raised serum AST levels in group B (Cypermethrin experiment) as compared to group A (control) whereas significantly lower than group C (Cypermethrin and vitamin C). AST levels significantly lower in group C as compared to group.

Paired on Post-Hock comparison	Mean difference	*p*-value
Group A versus group B	39.2	<0.001*
Group A versus group C	21.7	<0.001*
Group B versus group C	60.9	<0.001*

*Significant difference ($p < 0.05$), [NS]insignificant difference ($p > 0.05$)

Akmans et al, Sokmen et al and Kopec et al[19-21]. Cypermethrin act as a stomach and contact insecticide. It is widely used for storage of cereals, vegetable and fruit, in household treatment and in animal husbandry. Its residual effects are also creating health problems for human beings. Walia et al studied the clearance of residual amount of Cypermethrin by different methods[22]. The processed samples were analyzed by gas chromatography. Dislodging of Cypermethrin residue was observed more in grilling (50.12%), followed by cooking in oil (45.2%), cooking in water (41.4%), and microwave cooking (40.89%) after first day of the treatment. Reduction of residue after washing treatment was minimal. Our study correlates well with research work of Sinan et al and Sankar et al which showed that Cypermethrin damage hepatic tissue and produce oxidative stress in hepatic cells which raised ALT and AST in serum[23,24]. Our study also correlates with research work carried by Hussain et al and Sekhar et al in which Cypermethrin given with aflatoxin and sodium fluoride respectively enhance Cypermethrin induce oxidative stress in cells[25,26].

CONCLUSION

Present study substantiates the observation that vitamin C has antioxidant effect against Cypermethrin induce hepatotoxicity, vitamin C intakes in natural sources can scavange free radicals and decrease oxidative stress.

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