Original Article

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Ameliorative Role of Ascorbic Acid against Monocrotophos Induced Toxicity in Albino Mice

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Abstract

Monocrotophos (MCP) is a organophosphate insecticide used for several years in agriculture. The objective of this study was to investigate the possible protection of Vitamin C as antidot against the toxic effect of 1/5 LD50 dose of monocrotophos in albino mice. Forty eight male albino mices divided into 4 groups of 6 animals in each group were used for this study. Group-I (control): received no treatment, Group-II: ascorbic acid(20 mg/Kg bw), Group-III: Ascorbic acid+MCP, Group-IV: treated with MCP (0.2mg/Kg bw). The regimen was administered orally for two periods (1 and 2 week). At the end of 1st and 2nd weeks of exposure hepatic tissues were collected to examine the toxicity of MCP on protein[5] ,ascorbic acid content[6] and Na^+-K^+-ATPase and Mg^{++}-ATPase.[7] In MCP treated mice (Group-IV) enzyme activity significantly increased in N^+-K^+-ATPase and Mg^{++}-ATPase after 1 week of exposure but after 2 week of MCP exposure enzyme activities significantly decreased in N^+-K^+-ATPase and Mg^{++}-ATPase. In Group-III and Group-IV animals, after 1 &2 week of exposure of MCP ascorbic acid contents and protein contents were reduced. While certain amelioration for these elevations were detected in the animals treated with vitamin C + Monocrotophos. In Group-II animals insignificant increase of all the biochemical parameters are seen. Biochemical results of this study revealed that the Ascorbic acid reduces the toxicity of MCP and ATPase act as biomarker to MCP contamination.

Keywords: Ascorbic acid; Liver; Mg^{++}-ATPase; Monocrotophos; Protein; N^+-K^+-ATPase.

Introduction

A serious problem with organophosphorus compounds has been their high acute toxicity to man and non-target organisms.[1] Organophosphorus compounds may induce oxidative stress leading to generation of free radicals and reactive oxygen species (ROS) scavenging enzyme.[2] Administration of ascorbic acid as an antioxidant against insecticide can significantly decrease the extent of damages induced by insecticides[3], especially in the hepatic toxicity as antioxidant agent and prevent the effect of free radicals for vital cells.[4] The present work the effects of monocrotophos on contents of liver such as protein, ascorbic acid and activity of Na^+-K^+-ATPase and Mg^{++}-ATPase has been investigated and also to evaluate an ameliorative role of ascorbic acid in modulating these toxic effects.

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Material and Methods

Swiss albino mice (*Mus musculus*) body weight range 15-33 g (approximate age 2-3 months) of both Sexes were procured from a commercial farm at Kolkata and were maintained at room temperature (30±2°C) provided with a balanced diet and water was provided *ad libitum*. A minimum acclimation period of 3 week was always allowed before the beginning of the experiment. After laboratory acclimation forty eight adult mice weighted 25-30 g were used for the study and

divided in to four groups, compromised 6 mice in each group and received their corresponding substance for 1 and 2 weeks, respectively.

Animal in all groups were distributed as follows:

Group I: Control, received no treatment (normal diet)

Group II: Ascorbic acid (20 mg/ kg body weight orally)

Group III: Monocrotophos (0.2mg/kg body weight orally).

Group IV: Ascorbic acid + Monocrotophos.

Chemicals

Monocrotophos of commercial grade 36% SL (Hilcron) of Hindustan Insecticides LTD was obtained locally.

Ascorbic acid (L-Ascorbic acid) was supplied by Hi Media, Mumbai, India. All other chemicals used for experiments were purchased from Merck or Hi Media and were of analytical grade.

Tissue processing

At the end of exposure period the animals were sacrificed by cervical decapitation. Liver were quickly dissected out in precooled Mammalian Ringer (Krebs's Ringer phosphate) and the adherent tissues were cleaned off. A part of liver was used for the estimation of protein content [5] and ascorbic acid content of the liver was estimated colorimetrically.[6] The rest of the tissues were blotted off in Whatman filter paper No.1 and weighted. A 5% homogenate of each tissue was prepared in 0.25 M sucrose solution using a REMI homogenizer (BOMBAY) at a medium speed for 1 minute. This homogenate was used for the assay of ATPase estimation calorimetrically.[7]

Statistical Analysis

The experimental data are expressed as MEAN ±SEM. The data were analyzed by the student t-test.[8,9] The differences were considered to be statistically significant when 'P' values of 0.05 level and below considered significant and above 0.10 level as not significant (NS) statistically.

Results

The membrane bound Na^+-K^+-ATPase activity of liver exposed to monocrotophos (Group-III) for 1 week were significantly increased ($p<0.001$) but decreased significantly($p<0.001$) in animals exposed for 2 week as compared to their respective control. Results indicate that short-term (1 week) exposure of low concentrated MCP stimulates the enzyme activity and long term exposure (2week) inhibits the enzyme activity. Vitamin C treatment to MCP (C+MCP) normalizes the enzyme activity in both the cases. Na^+-K^+-ATPase decrease significantly ($p<0.001$) in 1 week exposure animal and increase significantly ($p<0.001$) in 2 week exposure

Table 1: ***In vivo*** **effect of monocrotophos (0.02mg/kg body) on ATPase activity (µg pi/gm tissue /hr) of liver in different groups of mice**

Treatment	Na^+-K^+-ATPase	
	1st week	2nd week
(Group-I) Control	104.5±1.88	106.21±0.159
(Group-II)Vitamin C	110.01±2.12	120.13±0.33
(Group-III)MCP	174.45±1.77*	71.03±1.63*
(Group-IV)Vitamin C+MCP	114.16±1.64*	100±0.766*

*P≤0.001, **P≤0.01, ***≤0.05, N.S Non significant. (Student's ' *t* ' test).N=6. (Mean ± SEM).
Group-III (MCP) is compared with Group-I (Control) and Group-IV (Vitamin C +MCP).

Table 2: *In vivo* effect of monocrotophos (0.02mg/kg body) on ATPase activity (µg pi/gm tissue /hr) of liver in different group of mice

Treatment	Mg^{++}ATPase	
	1st week	2nd week
(Group-I) Control	90.68±1.03	93.23±0.373
(Group-II)Vitamin C	98.36±0.56	112.73±2.6
(Group-III)MCP	162.98±1.52**	53.53±0.80*
(Group-IV)Vitamin C+MCP	101.73±1.67**	82.61±0.56*

*P≤0.001,**P≤0.01,*** ≤0.05, N.S Non significant. (Student's 't ' test).N=6(Mean ±SEM).
Group-III (MCP) is compared with Group-I (Control) and Group-IV (vitamin C+MCP).

Table 3: *In vivo* effect of monocrotophos (0.02mg/kg body) on for ascorbic acid content (mg/100 gm of tissues) of liver in different group of mice

Treatment	Ascorbic acid	
	1st week	2nd week
(Group-I) Control	78.3±0.44	80.29±0.445
(Group-II)Vitamin C	83.88±1.36	85.03±1.03
(Group-III)MCP	41.43±1.25*	34.39±0.43*
(Group-IV)Vitamin C+MCP	70.45±1.008***	55.01±0.63**

*P≤0.001, **P≤0.01, *** ≤0.05, N.S Non significant. (Student's't ' test).N=6(Mean±SEM).
Group-III (MCP) is compared with Group-I (Control) and Group-IV (vitamin C+MCP).

Table 4: *In vivo* effect of monocrotophos (0.02mg/kg body) on protein contents (mg/gm tissue wet wt.) of liver in different group of mice

Treatment	Protein content	
	1st week	2nd week
(Group-I) Control	59.35±0.57	60.75±0.315
(Group-II)Vitamin C	66.23±1.77	71.76±0.704
(Group-III)MCP	33.4±1.06**	27.7±0.40*
(Group-IV)Vitamin C+MCP	57.31±1.65*	52.86±0.32***

*P≤0.001, **P≤0.01, ***≤0.05, N.S Non significant. (Student's ' *t* ' test).N=6(Mean±SEM).
Group-III (MCP) is compared with Group-I (Control) and Group-IV (vitamin C+MCP).

animal as compared to the animals exposed to monocrotophos (MCP) alone. These results indicate an ameliorative role of vitamin C on the membrane bound ATPase to normalize the activity but could not come up to control. (Table 1).

The membrane bound Mg^{++}-ATPase activity of liver exposed to monocrotophos (Group-III) for 1 week were significantly increased ($p<0.01$) but decreased significantly($p<0.001$) in animals exposed for 2 week as compared to their respective control. Results indicate that short-term (1 week) exposure of low concentrated MCP stimulates the enzyme activity and long term exposure (2 week) inhibits the enzyme activity. Vitamin C treatment to MCP (C+MCP) normalizes the enzyme activity in both the cases. Mg^{++}-ATPase decrease significantly ($p<0.01$) in 1 week exposure animal and increase significantly ($p<0.001$) in 2 week exposure animal as compared to the animals exposed to monocrotophos (MCP) alone (Table 2).

In the present study ascorbic acid content in Group-III animals for both the period of exposure decrease significantly ($p<0.001$) as compared to their respective control.Vitamin C treatment to MCP (C+MCP) exposed animals (Group-IV) in both the exposure periods ascorbic acid content increase significantly in 1 week exposure ($p<0.05$) and 2 week exposure ($p<0.01$) as compared to the

animals exposed to monocrotophos (MCP) alone (Table 3).

In Group-III animals treated for 1week and 2 week shows significantly decreased protein content ($p<0.01$) and ($p<0.001$) respectively. However treatment with vitamin C to MCP (C+MCP) exposed animals (Group-IV) showed a significant increase in the levels of protein ($p<0.001$) in 1 week exposure and ($p<0.05$) in 2 week exposure animals as compared to animals exposed to MCP alone. These results indicate a protective effect of vitamin C on the membrane bound ATPase activity by virtue of its antioxidant property but could not come up to control (Table 4).

Data from the same tables showed insignificant increase in vitamin C received groups (Group-II) as compared to their respective control groups throughout the experimental periods.

Discussion

There has been a sharp increase in the use of insecticides and other chemical agents in agriculture since the past two decades. Monocrotophos insecticide represents one group of pesticides that is widely used and has been shown to have toxic effects in human and animals. The great hazards caused by pesticides on the live stocks are due to their accidental exposure to these pesticides either by ingestion or inhalation.[10-13] The present study is concerned with the effect of monocrotophos on some biochemical parameters. The role of vitamin C (as antidote) was also studied. Liver was taken as the target organ for this investigation. It is the organ of immense importance due to its peculiar anatomy and metabolic functions which are exactly meant for efficient removal of the toxins. Liver is the largest exocrine gland in the body making up about 2 percent of the body weight of adult mice and of an adult human.

The activity of the energy enzyme ATPase showed highly significant increase in mice treated with monocrotophos for 1 week. The finding in the present investigation reveals that enough energy is consumed during metabolism due to biochemical alterations in the liver. Studies on the phosphorus metabolism of the fly *Calliphora erthrocephala* (Meig.) state that probably there would be inter conversion in the ATPase activity i.e., when ATPase activity increased, amount of ATPase decreased and vice-versa.[14] In the absence of sufficient experimental evidences, it may be interpreted that the amount of phosphorus decreases in the body due to consumption of energy which is evident from the increase of ATPase activity.[15]

In the present study it has also been recorded that increase in the duration of exposure for 2 week of monocrotophos caused decrease in the activity of Na^+-K^+-ATPase and Mg^{++}-ATPase, in the liver of mice. The inhibition of ATPases by pesticides disrupt ATP utilization within the synaptic area and alter the energy metabolism of the nerve terminated by secondarily altering the activities of other enzymes for which ATP or ADP may be allosteric effects.[16] Thus, ATPases are very sensitive to chemical interaction and can be used as reliable biomarker for the mechanistic toxicity studies of pesticides. In the present study, it has also been found that increasing duration of monocrotophos exposure caused decreased activity of ATPases in liver. This could be due to pesticide induced effect on cell membrane because of their strong affinity for interaction with member lipids causing inhibition of membrane bound ATPase enzymes activity by affecting enzyme complex.[17-20] Cell membrane is believed to be the site of action of insecticides by altering structural and functional integrity of cell membrane and also affects membrane bound enzymes such as Na^+-K^+-ATPase and Mg^{++}-ATPase.[21-22] Recently[23] have reported an OP herbicide anilofos causes inhibition of total ATPase, Na^+-K^+-ATPase, Mg^{2+}- ATPase in RBCs, brain and liver of rats.

In the present study it was revealed that during both the exposure period of

monocrotophos decreased level of protein in the liver of mice was observed. This might be due to catabolism of protein and or malfunction of liver.[24] It has been suggested that acute treatment with monocrotophos showed tissue specific inhibition of microsomal cyt-p-450 in hepatic and extrahepatic tissues resulting in the loss of haemoprotein in rats.[25] It has also reported that the decrease in total proteins and soluble proteins indicate their metabolic utilization.[26] The increase in the activity of proteases correlated with the decrease of soluble and total protein. The increasing duration of exposure of monocrotophos caused decrease in the level of glycogen in the liver of mice. The changes in the levels of protein and glycogen is either due to an increased catabolism of the biomolecules to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function.[27] It shows utilization of protein in energy production. The decline in protein level suggests the mobilization of amino acids during insecticide stress to meet the energy demands and in the various catabolic reactions. The amount of protein becomes reduced when the concentration and the period of exposure increases.

A significant decrease in ascorbic acid contents during both the experimental condition was observed. This might be due to the depletion of endogenous ascorbic acid level and its constant use in scavenging the ROS, thereby protecting the organ from potential injury. The observed protective effect may be due to the an-toxicant property of vitamin C. It has been reported to modify response to oxygen radicals and known to be an excellent antioxidant[28,29] along with its nucleophilic character.[30] It might be due to detoxification or incapacitation of the toxin molecules via chemical processes mediated by various detoxification enzymes[31] and elimination of abnormal cells by apoptosis.[32] In the liver, the ascorbic acid content decreased; it may be due to shifting of ascorbic acid to other tissues due to increase demand of energy in the liver of mice in both exposed duration.

Conclusion

From the above results it was obvious that monocrotophos insecticide has a marked and severe toxic effect on male albino mice. Finally, it is recommended that the use of monocrotophos insecticide must be limited due to its hazardous effect to the non-target species including the farmers and the farm birds even the technicians who exposed to it and also the residue in the agricultural products. Also, it is well recommended to use vitamin C as antioxidant to prevent or alleviate the toxicity induced by monocrotophos insecticide.

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