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MITIGATION OF EARLY DELTAMETHRIN INDUCED HEPATOTOXICITY IN MALE MICE OF SWISS STRAIN BY ALLIUM SATIVUM

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ABSTRACT

The importance of Allium sativum as herbal remedy in the system of Indian traditional medicine and its prime position in Ayurveda with other great herbs is unquestionable. Though extensive research has been carried out by number of researchers on its medicinal properties, there is still paucity regarding its ameliorative efficacy. Hence, the present study focuses to evaluate the potential ameliorative effects of Allium sativum (AS) against Deltamethrin (DLM) induced toxicity in hepatic tissues of male mice of Swiss strain (Mus musculus). Animals were divided into seven different experimental groups viz. Control, Vehicle treated (peanut oil), DLM low dose treated (3 mg/kg b.wt.), DLM high dose treated (6 mg/kg b.wt), control + AS, DLM low dose + AS and DLM high dose + AS. Various bio-chemical indices and gravimetric parameters were assessed at durations of 14 and 21 days. Results revealed that in experimental (DLM treated) groups parameters like total body weight, liver weight, protein content as well as the activity of enzymes like SDH, ALKpase, ACPase and ATPase were found to be reduced in a dose and duration dependent manner, whereas cholesterol level in the hepatic tissue was found to be elevated. Moreover, these altered parameters were significantly recovered when AS (200mg/kg body weight) was supplemented along with DLM and the observed recovery was comparable to control values.

KEYWORDS: Deltamethrin, Allium sativum, Hepatotoxicity, Amelioration.

INTRODUCTION

Pesticides are extensively used throughout the world to protect variety of crops. They are of foremost value in our society as they yield good quality food and other plant products of economic importance. Moreover they are also being used to combat against vector borne diseases like malaria. However they have been reported to contaminate our environment as their residues accumulate in air, soil, water, human and animal tissue samples globally. Studies regarding pesticides and their toxicity are considered important in order to decrease pesticide risks and help to improve public health policies.[1]

Organophosphates and chlorinated pesticides then got replaced by pyrethroids which are organic compounds similar to the natural pyrethrins produced by the flowers of pyrethrums (Chrysanthemum cinerariefolium and Chrysanthemum coccineum). The first generation pyrethroids, developed in the 1960s, are more active than the natural pyrethrum but unstable in sunlight. Later in 1974, second generation pyrethroids were developed that have more resistance to degradation by light and air, thus making them suitable for use in agriculture. Further the pyrethroids now constitute a major part of the commercial house hold inscticides.[2] Deltamethrin was one of such pyrethroids developed then, and is still widely used throughout the globe and is considered to be safe.[3] Deltamethrin though thought to be a "safe pesticide'' raises multiple questions due to controversial reports in the literature in recent times where its potential toxicity has been reported. This necessitates the need to reconfirm the safety issues of the said pyrethroid "Deltamethrin".

Moreover, hepatic tissue is a major detoxifying and excretory unit for many endo and exogenous substances, thereby it helps in maintaining good health.[4,5] Further it plays a major role in metabolic activities as well as structural stability of the body. Any type of injury due to systemic drug, food, preservatives, agrochemicals and addiction to alcohol or impairment of its function leads to metabolic complications.[6] It can be considered a target organ for multiple toxicities and in fact the lesions observed are assessed on the basis of substance involved, multiple route exposures and absorption as well as the duration of exposure.[7] After an acute exposure, the most frequently observed types of damage are steatotic, necrotic or hepato-biliary dysfunction. On the other hand, chronic exposure is usually followed by a cirrhotic or neoplastic damage.[8,9]

The present study therefore, has been focused towards investigating the toxic effects of deltamethrin and its mitigation on important bio-chemical indices of liver in mouse model, in view of the fact that hepatic tissue is the principal target site for pesticide accumulation[4]. Thus evaluation of biochemical alterations of in dose and time dependent manner in hepatic tissue DLM treated mice was essential to guage the toxicity. Furthermore, in the present investigation Allium saitivum has been used to ameliorate such hepatotoxicity induced by Deltamethrin. Various applications of A. sativum have been indicated in the ancient scriptures,as well as modern literature favours the efficacy of the herb to different extent. A. sativum prevents high blood pressure and cholesterol level and exhibits anticoagulant effect, thereby reducing the chances of heart disease.[10,11] The antioxidant properties of A. sativum is due to biologically active lipophilic sulphur-bearing compounds such as allicin, S-allyl-cystein, diallyl-disulphide and diallyl sulphide. Additionally, A. sativum also contains a high concentration of selenium, which is responsible in part for antioxidant and cancer preventive effects.

MATERIALS AND METHODS

Animals and Chemicals

Healthy, adult, pathogen free, colony bred male albino mice (Mus musculus) of Swiss strain weighing between 30 - 40gm obtained from IAEC recognised supplier Cadila Healthcare and Pharmaceutical, Ahmedabad, Gujarat (India) were used for the experiments. Approval of the experiment and the justification of animals required for the experiment was obtained from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India, under the Ministry of Social Justice and Empowerment, Government of India, New Delhi. All the animals were acclimatized for seven days prior to the commencement of experiment. The animals were housed in an air-conditioned animal house at a temperature of 26±2^{o}C and exposed to 10-12 hours of day light and relative humidity of 40-50%. Animals were arranged into control and treated groups and were caged separately. Standard chow (obtained from Amrut laboratory, Baroda, India) and water was provided ad libitum. Test chemical Deltamethrin (technical grade) of 98.11% purity was generously gifted from Meghmani Organics Limited, Ahmedabad (India). All the other chemicals used were procured from Himedia Laboratories, India and Sigma Aldrich (UK). All the chemicals used were of analytical grade.

Dose Preparation

Deltamethrin was administered via oral gavage dissolved in peanut oil at two different dose concentrations, low dose (3mg/kg body weight) and high dose (6 mg/kg body weight). All the doses were determined on the basis of LD_{50} of deltamethrin in peanut oil i.e. 30 mg/kg body weight (EMEA 2001).[12] Crude extract of Allium sativumof the single clove variety was prepared from bulbs purchased in bulk from the market. The cloves were sliced into pieces ground into a fine paste. Working solution was then prepared by dissolving 5 gm of this paste in 100 ml of deionized water, where 1 ml of the extract contained 50 mg of crude Allium sativum. Freshly prepared Allium sativum extract was then administered to mice at a dose level of 200 mg/kg body weight accordingly. Dose of the garlic was based on previous studies (El-Kott et al., 2012).[13]

Experimental Design

Group I: Control (given distilled water only)

Group II: Vehicle Control (given only peanut oil)

Group III: Low Dose (given 3 mg/kg body weight deltamethrin)

Group IV: High Dose (given 6 mg/kg body weight deltamethrin).

Group V: Control + Allium sativum(200 mg/kg body weight)

Group VI: Low Dose Deltamethrin + Allium sativum

Group VII: High Dose Deltamethrin + Allium sativum

All the groups were treated for 14 and 21 days and at the end of experiment animals were weighed and sacrificed using light ether anaesthesia.

Tissue collection

At the end of experiment, mice were dissected as per institutional ethical committee norms. Hepatic tissue was blotted free of blood, weighed, and homogenates were prepared.

Protein estimation

Protein estimation was done using standard protocol of Lowry et al. (1951).[14] Colour development was read at 540 nm in Systronics Digital Spectrophotometer 167 against blank.

Cholesterol estimation

Cholesterol estimation was done by the method of Zlatkis et al. (1953).[15] The absorbance was read at 540 nm.

Enzymatic assays

Succinate Dehydrogenase (SDH)

SDH activity was measured by the method of Beatty et al. (1966).[16] The electrons released by the enzyme SDH from the substrate are taken up by an electron acceptor INT which is reduced to red coloured formazan. After extracting it in ethyl acetate the colour intensity was measured at 420 nm against blank. SDH activity was expressed as µg formazan formed/15 minutes/mg tissue weight.

Alkaline Phosphatase (ALPase)

Alkaline Phosphatase (ALPase) activity was determined by the method of Bessey et al. (1946).[17] The enzyme ALPase hydrolyses the substrate p-nitrophenyl phosphate into inorganic phosphate and p-nitrophenol. The quantity of p-nitrophenol released under standardised condition was measured at 410 nm. Enzyme activity was expressed as µ moles p-nitrophenol released/30 minutes/mg protein.

Acid Phosphatase (ACPase)

Activity of ACPase was determined by the method of Bessey et al. (1946).[17] ACPase catalyzes hydrolysis of p-nitrophenol nitrate at pH 4.8, liberating paranitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with NaOH to form a yellow colored complex which is measured at 420 nm and is directly proportional to the enzyme activity. Enzyme activity was expressed as µ moles of p-nitrophenol released/30 minutes/mg protein.

Adenosine Triphosphatase (ATPase)

The ATPase activity in liver of control and all treated groups of animals was assayed by the method of Quinn and White (1968)[18]; while inorganic phosphate liberated was estimated using the method of Fiske and Subbarow (1925)[19]. Readings were taken at 660 nm on a Systronics Digital Spectrophotometer 167.

Statistical Analysis

All the data are expressed as Mean±SEM. Statistical analysis was performed using the trial version of SPSS software package version 16.0 (USA). Comparison between groups was made by one-way analysis of variance (ANOVA) taking significance at $p<0.05$ followed by Student's t-test. Tukey's honestly significance difference (HSD) post hoc test was used for comparison among different treatment groups ($p<0.05$).

RESULTS

Gravimetric Indices

Terminal Body weight: The body weight of mice decreased non-significantlyonly after 14 days low dose Deltamethrin treatment in group-III. While the decline was significant to an order of $p<0.01$ after 14 days of treatment in group-IV (high dose) and 21 days of treatmentin group-III(low dose) and in group-IV-(High Dose ($p<0.005$)) in comparison to that of control group. Results of ameliorative studies clearly indicated the beneficial effect of antidote used in the present study. Allium sativum (200 mg/kg body weight), when administered alone(Group-V) and along with lowdose (Group-VI) and high dose (Group-VII) of Deltamethrin during both the time intervals, recorded non-significant reduction in body weight as compared to control group. (Table 1, 2).

Liver weight: Liver weight of mice decreased non-significantly after 14 days of deltamethrin treatment ingroup III (Low Dose) while group IV (High Dose) showed significant decrease ($p<0.01$).21 days of treatment revealed a significant decline in hepatic tissue weight in both group-III (low dose ($p<0.01$)) and group-IV-(High Dose ($p<0.005$)) in comparison to that of control group. Allium sativum (200 mg/kg body weight) when administered alone(Group V) and along with low dose (Group VI) and high dose (group-VII) of Deltamethrin for 21 days, registered non-significant decline in liver weight as compared to control group (Table 1,2).

Bio-chemical indices

Total protein

Total Protein content exhibited non-significant decline in both Group III (Low Dose) and Group IV (High Dose) of deltamethrin treated mice after 14 days and decreased significantly in group-III(low dose ($p<0.005$)) and group-IV(high dose ($p<0.005$)) after 21 days of treatment in comparison to that of control group. Allium sativum (200 mg/kg body weight) alone (Group-V) and along with low (Group VI) and high dose (Group VII) of DM for both the duration registered non-significant decline in protein content of liver and obtained values were comparable to control group (Table 3,4)

Cholesterol

A non-significant increase of cholesterol content in group-III (Low Dose), while significant increase was noted in group-IV (High Dose ($p<0.005$)) after 14 days of treatment. While significant increase was also noted in both group-III (low dose ($p<0.005$)) and group-IV (high dose ($p<0.005$)) after 21 days of treatment. Allium sativum (200 mg/kg body weight) (Group

V) alone and along with low dose (Group VI) and high dose (Group VII) of Deltamethrin during both the time intervals, registered non-significant upsurge in cholesterol content of liver and obtained values were comparable to control group (Table 3, 4).

Succinate Dehydrogenase

Activity of SDH showed a significant decline in group-III (low dose ($p<0.01$)) and group-IV(high dose ($p<0.005$)) in liver after 14 days of treatment and significant decrease in both group-III (low dose ($p<0.005$))and group-IV (high dose ($p<0.005$)) after 21 days of treatment compare to control.Allium sativum (200 mg/kg body weight) alone (group V)and along with (low dose (group VI) and (high dose (group VII) of Deltamethrin during both the time intervals, registered non-significant decline in SDH activity of liver and obtained values were comparable to control group (Table 3,4).

Alkaline Phosphatase

After 14 days of treatment, ALKpase activity does not show any significantchange in group-III (Low Dose) whereas the enzyme activity was significantly increased activity in group-IV (High Dose ($p <0.01$)).Results for alkaline phosphatase activity after 21 days of treatment shows significant increase in both groups (group-III (low dose ($p<0.01$)) and group-IV (high dose ($p<0.005$)) in hepatic tissue(Table 3, 4). Allium sativum (200 mg/kg body weight) (Group V) aloneand along with low dose (group VI) and high dose (group VII) of Deltamethrin treated miceduring both the time intervals, registered non-significant increase in ALKpase activity of liver and values were comparable to control group (Table 3,4).

Acid Phosphatase

Deltamethrin treatment for 14 days brought about non-significant increase in ACPase activity in the liver of animals of Group III (low dose) but shows significant increase in Group IV (high dose ($p<0.01$)) as compared to the control Group. ACPase activity significantly increased in both group-III(low dose ($p<0.005$)) and group-IV(high dose ($p<0.001$)) after 21 days of treatment compare to control. Allium sativum (200 mg/kg body weight) (Group V) alone and along with low and high dose of Deltamethrin (Group VI and VII) during both the time intervals, registered non-significant change in ACPase activity of liver and obtained were comparable to control group (Table 3,4).

Adenosine triphosphatase

Activity of ATPase enzyme was declined significantly in Group III (low dose ($p<0.01$))as well asin group-IV(high dose($p<0.01$)) after 14 days of DM treatment. Further ATPase activity alsosignificantly decreased in both group-III(low dose ($p<0.01$)) and group-IV(high dose ($p<0.005$)) after 21 days of treatment. Allium sativum (200 mg/kg body weight) alone(Group V) and along with low and high dose of Deltamethrin (Group VI and Group VII) during both the time intervals, registered non-significant decline in ALKpase activity of liver and obtained values were comparable to control group (Table 3, 4)

Table 1: Showing body weight (gm) and weight of Liver (gm) of control, Deltamethrin and Allium sativum treated mice after 14 days.

Parameter	Control	Vehicle treated	Low Dose(LD)	High Dose (HD)	AS	LD + AS	HD + AS
Body Weight	41.12 ± 0.9	41.6 ± 0.8 NS	39.99 ± 0.87 NS	36.34 ± 1.21*	41.23 ± 0.67 NS	38.89 ± 0.89 NS	37.88 ± 0.75 NS
Liver Weight	2.17 ± 0.07	2.12 ± 0.07 NS	1.93 ± 0.05 NS	1.86 ± 0.04*	1.98 ± 0.06 NS	1.99 ± 0.04 NS	1.97 ± 0.05 NS

Values are represented as Mean ± S.E., *$p<0.01$, NS- Non-Significant, Analysis of Variance at $p<0.05$ level. LD= Low Dose, HD= High Dose and AS= Allium sativum treated

Table 2: Showing body weight (gm) and weight of Liver (gm) of control, Deltamethrin and Allium sativum treated mice after 21 days.

Parameter	Control	Vehicle treated	Low Dose(LD)	High Dose (HD)	AS	LD + AS	HD + AS
Body Weight	40.73 ± 0.58	42.99 ± 0.4 NS	37.34 ± 1.02*	35.45 ± 1.16**	41.88 ± 0.92 NS	39.32 ± 1.05 NS	37.89 ± 1.11 NS
Liver Weight	2.11 ± 0.07	2.12 ± 0.07 NS	1.90 ± 0.05*	1.78 ± 0.05**	2.01 ± 0.06 NS	1.93 ± 0.06 NS	1.89 ± 0.08 NS

Values are represented as Mean ± S.E., *$p<0.01$, **$p<0.005$, NS- Non-Significant, Analysis of Variance at $p<0.05$ level. LD= Low Dose, HD= High Dose and AS= Allium sativum treated

Table 3: Showing Total Protein, Cholesterol, SDH, ALPase, ACPase and ATPase activities in liver of control, Deltamethrin and Allium sativum treated mice for 14 days.

Parameter	Control	Vehicle treated	Low Dose(LD)	High Dose (HD)	AS	LD + AS	HD + AS
Total protein	17.11±0.21	17.09±0.20 NS	16.36±0.16 NS	16.01±0.32 NS	16.91±0.25 NS	16.73±0.11 NS	16.59±0.21 NS
Cholesterol	1.20±0.03	1.22±0.04 NS	1.29±0.06 NS	1.34±0.04**	1.24±0.04 NS	1.21±0.07 NS	1.23±0.07 NS
SDH	17.26±0.44	17.25±0.34NS	15.75±0.32*	15.12±0.36**	16.56±0.30 NS	16.22±0.33NS	15.97±0.34 NS
ALKpase	0.47±0.02	0.48±0.02 NS	0.51±0.02NS	0.53±0.01*	0.49±0.03NS	0.49±0.02 NS	0.52±0.02 NS
ACPase	0.93±0.04	0.92±0.01 NS	0.98±0.02NS	1.06±0.04*	0.95±0.03NS	0.95±0.07 NS	1.04±0.06 NS
ATPase	3.59±0.06	3.58±0.12 NS	3.39±0.06*	3.29±0.09*	3.42± 0.12NS	3.44±0.17 NS	3.31±0.11 NS

Values are represented as Mean ± S.E., *p<0.01, **p<0.005, NS- Non-Significant, Analysis of Variance at p<0.05 level. LD= Low Dose, HD= High Dose and AS= Allium sativum treated

Table 4: Showing Total Protein, Cholesterol, SDH, ALPase, ACPase and ATPase activities in liver of control, Deltamethrin and Allium sativum treated mice for 21 days.

Parameter	Control	Vehicle treated	Low Dose(LD)	High Dose (HD)	AS	LD + AS	HD + AS
Total protein	17.19 ±0.17	17.19 ±0.31NS	16.23 ± 0.25**	15.9 ±0.27***	16.94 ±0.20 NS	16.71 ±0.28 NS	16.54 ±0.23 NS
Cholesterol	1.18 ± 0.05	1.24 ±0.03 NS	1.38 ± 0.04**	1.4 ±0.04**	1.27 ±0.07 NS	1.22 ±0.07 NS	1.24±0.09 NS
SDH	17.39±0.59	17.40±0.44NS	15.02±0.21**	14.81±0.31**	16.77±0.35 NS	16.20±0.43NS	15.97±0.29 NS
ALKpase	0.49±0.007	0.49±0.01 NS	0.54±0.02*	0.56±0.02**	0.48±0.02NS	0.48±0.02 NS	0.53±0.03 NS
ACPase	0.93±0.01	0.94±0.01 NS	1.03±0.03**	1.13±0.04***	0.97±0.04NS	0.97±0.04 NS	1.05±0.08 NS
ATPase	3.60±0.12	3.59±0.13 NS	3.25±0.04*	3.13±0.06**	3.46± 0.14NS	3.37±0.15 NS	3.24±0.16 NS

Values are represented as Mean ± S.E., *p<0.01, **p<0.005, ***p<0.001, NS- Non-Significant, Analysis of Variance at p<0.05 level. LD= Low Dose, HD= High Dose and AS= Allium sativum treate.

DISCUSSION

In past few decades, use of synthetic pyrethroids has increased due to its quick biodegradability and target oriented insecticidal action. A number of recent reports have been published on toxicity of Deltamethrin on animals[20-24] and human beings[25] though initially it was thought to be a safe insecticide. Deltamethrin is considered to be readily absorbed when given orally as all pyrethroids are lipophilic, absorption in the gastrointestinal tract is higher compared to other routes.[26] Hence oral route of administration was selected for the present study. Hepatic tissue was investigated as it is a vital target to be affected by xenobiotic.

Significant decline in body and the hepatic tissue weight were noted after high dose deltamethrin treatment in both the intervals studied whereas with low dose the decline was significant in 21 days treatment corresponding decline in total protein level was probably attributed to loss of appetite and reduced food intake which in turn could be the reason of weight loss. Previous reports have also indicated a decline in body weight due to administration of deltamethrin in experimental animals such as rats and rabbits.[27] In fact, it has been reported that an increase or decrease in an absolute or terminal organ weight after administration of any chemical is an indicator of the toxic effect of that particular drug/chemical.[28] Therefore evaluation of body or organ weight is an important indicator of toxicity. Further the decrease in protein content[29] of hepatic tissue after deltamethrin treatment shows dose and time dependent pattern suggestive of liver disease and poor nutrition. The data available in present investigation also points to either reduced protein synthesis or increased protein breakdown.

Further the significant upsurge in cholesterol content recorded in liver may be relative to the effect of deltamethrin on lipid metabolism and permeability of intoxicated cells.[29,30] This raised cholesterol level may be due to decreased utilisation of cholesterol under stress. Remai et al. (2008)[31] have also suggested that the elevation of cholesterol levels may be attributed to enhanced cholesterol and triglyceride synthesis and/or reduced cholesterol catabolism or cholesterol accumulation or impaired turn-over of cholesterol.

Significantly declined SDH activity in liver (Table 3, 4) as compared to control, might have been due to the stress induced shifting the metabolic pathway towards anaerobic pathway to meet the increased and immediate energy demand. Venkateshwarlu and Shanmugam (2005)[32] have made similar observations suggesting a decrease in oxidative pathway

enzymes, which supports the results of present study. Moreover the probable accumulation of Deltamethrin might have intoxicated the hepatic cells, causing inhibition inoxidative metabolism and a decline in the SDH activity levels of the tissues.[33]

A wide range of environmental xenobiotics are known to cause adverse effects on phosphatase activities. The present study revealed elevated levels of Acid and Alkaline phosphatase activity post deltamethrin treatment in the hepatic tissue. It is suggested that this elevation may be due to glycogenolysis as a result of toxicity and enhanced breakdown of phosphate to release energy in impaired ATPase system during metal toxicity.[34] Thus this may be also true for pesticide toxicity. The increased Alkaline phosphatase maybe suggestive of bile duct obstruction.[30]

ATPase along with SDH reflects the energy metabolic status of cell. In the present study the ATPase activity in liver decreased significantly in both low and high dosed treated mice as compared to control after 14 and 21 days in present study it implies towards inadequate utilization of ATP produced in the cell. It is known that enzymatic hydrolysis of ATP by ATPase is a ubiquitous property of cells which is important for intracellular transfer of energy. Reductions in ATPase activity due to various classes of toxicants have also been documented.[35-36] The possible reasons for the decrease in the activity of the ATPase may be due to changes in active sites which causes the phosphorylation and dephosphorylation mechanisms of ATPase reaction. Thus, reduced aerobic oxidation and ATP generation could be responsible for the observed reduction in ATPase activity.

Moreover, the Deltamethrin toxicity at lower dose was non-significant in majority of bio-chemical parameters and gravimetric readings irrespective of the time duration. At higher dosage, the toxicity is visible as early as 14 days of treatment and becomes more significant as time interval increases.[29.37,38] This suggests that DM exposure for longer period and at higher concentration can be deleterious for animal health.

Further, the amelioration with Allium sativum has been effective in curbing the DM induced toxicity. The antioxidant potential of AS was noticed in both the dosages of DM. Although the data obtained is suggestive of usage of higher AS concentration to achieve excellent protective shield against DM toxicity especially for higher toxic doses and longer exposures, the use of AS at lower dose DM exposures could turn out to be excellent protective agent.

CONCLUSION

Results obtained from the present study reveal that oral administration of deltamethrin resulted in hepatotoxicity and caused gravimetric and biochemical changes in the treated animals. Moreover, these effects can be manifested in a dose and duration dependent manner, since highly significant changes were observed with high dose deltamethrin treated animals for 21 days. It can be further concluded from the results obtained that the use of these insecticides should be done under strict supervision and irresponsible use can cause many undesirable effects in non-target organisms. Further Allium sativumhas come out as a good ameliorative agent in the current study so that those who are being affected by this pesticide can be given Allium sativum as a food supplement.

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