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ALPHA CYPERMETHRIN TOXICITY IN LIVER OF MICE AND ITS AMELIORATION BY RESVERATROL, A BIOCHEMICAL STUDY

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ABSTRACT

The present study was an attempt to evaluate alpha cypermethrin toxicity in liver of mice and its amelioration by resveratrol. For these, mice were treated with high dose of alpha cypermethrin along with untreated control and vehicle control groups. In another sets of experiments, animals were treated with high dose of alpha cypermethrin along with different doses of resveratrol to see its ameliorative effect. All treatments were carried out orally for 30 days. Results revealed significant reduction in protein and glycogen content as compared to vehicle control when treated with high dose of alpha cypermethrin. In addition, lipid and cholesterol contents were significantly increased in liver of alpha cypermethrin treated mice. On the other hand, oral administration of resveratrol doses along with alpha cypermethrin restored all toxic changes in biochemical constituents significantly as compared to high dose alpha cypermethrin treated groups. The effect was dose dependent. It is concluded that resveratrol ameliorates alpha cypermethrin induced biochemical toxicity in liver of mice.

KEY WORDS

Alpha cypermethrin, Resveratrol, Protein, Glycogen, Lipid and Cholesterol

INTRODUCTION

Alpha cypermethrin, a synthetic pyrethroid, is a kind of insecticide used in the crop fields to kill pests. Alpha-Cypermethrin contains more than 90% of the insecticidally most active enantiomer pair of the four cis isomers of cypermethrin as a racemic mixture. The metabolism of alpha cypermethrin is quite rapid, and during its metabolism reactive oxygen species (ROS) are generated. [01] These free radicals, which are most active, cause oxidative stress through peroxidation of the lipid membrane. Damage may occur in certain tissues and organs, due to either the free radicals [02] that are generated or the direct effect of the pesticides on biological membrane [03] In a study conducted alpha cypermethrin treatment caused histological changes in liver, kidney, lung and brain in rats. [04] Resveratrol (3, 5, 4'-trihydroxystilbene) is a naturally occurring polyphenolic compound found largely in the skin of grapes. It is also a part of several herbal preparations. Drakshasava, an Ayurvedic formulation prepared from red grapes is used in treatment of several diseases affecting gastro-intestinal system.[05] Resveratrol attracted little interest until 1992, when it was first postulated to explain some of the cardioprotective effects of red wine.[06] In 2008, research by Markus and his colleagues [07] showed that resveratrol is useful in prevention of aging and enhances life span.

The present study is an attempt to evaluate alpha cypermethrin toxicity in liver of mice and its amelioration by resveratrol in mice. Toxicity is evaluated in terms biochemical constituents viz., protein, glycogen, lipid and cholesterol level in different experimental groups.

MATERIALS AND METHODS:

In present study, Swiss strain female albino mice (*Mus musculus*) were used as the test animal. Animals were kept at Animal House of Department of Zoology, Gujarat

University, Ahmedabad. These animals were procured from Cadila Research Centre, Dholka, Ahmedabad, India. Animal were kept in an animal house of zoology department with 12 hrs of light and dark period. Animal were kept at $25^0C \pm 2^0C$ temperature with relative humidity 50-55% and provided standard mice pellet diet prepared by Amrut Feeds, Pranav Industries Ltd, Pune. All chemicals were of analytical grade from HiMedia limited. and Sigma-aldrich limited. Alpha cypermethrin 99.5% was gifted from local pesticide manufacturing company. Animals were fed with alpha cypermethrin and resveratrol diluted in corn oil through oral feeding gauge. The experimental groups were as mentioned in Table 1.

Table 1 showing different experimental groups

Groups	Treatment	No.of Animal	Duration of treatment	Day of Autopsy
I	Untreated control	10	30 days	31st
II	Vehicle Control (0.2 ml corn oil /animal/day)	10	30 days	31st
III	Resveratrol control (150 mg/kg body weight/day)	10	30 days	31st
IV.	High dose alpha cypermethrin (HD ACP) (7mg/kg body weight (b.w) /day)	10	30 days	31st
V.	High dose alpha cypermethrin (HD ACP) (7 mg / kg b.w./ day) + Low dose resveratrol (LD RES) (50 mg / kg b.w./ day)	10	30 days	31st
VI.	High dose alpha cypermethrin (HD ACP) (7mg/kg b.w./day) + Mid dose resveratrol (MD RES) (100 mg / kg b.w./ day)	10	30 days	31st
VII.	High dose alpha cypermethrin (7mg/kg b.w/day) (HD ACP) + High dose resveratrol (HD RES) (150 mg / kg b.w./day)	10	30 days	31st

On 31st day mice were humanly sacrificed, and liver tissue was isolated, blotted free of blood and used for the estimation of protein, total lipid, cholesterol and glycogen. The protein level was estimated by the method of Lowry *et al.* (1951).[08] Reaction of protein with Folin Ciocalteau reagent produces blue colour due to interaction of alkaline copper sulphate reaction with peptide bonds; the other reaction counts for blue colour development is phosphomolybdic and phosphotungstic acids reduction by aromatic amino acids present in the protein. Resulting blue colour was measured at 540 nm. The protein contents were expressed as mg /100 mg fresh tissue weight. The glycogen content in the liver was estimated by the method of Seifter *et al.* (1950). [09] The glycogen present in tissue is converted to glucose, which reacts with anthrone reagent and gives a green coloured product which was read at 620 nm. The glycogen content was expressed as µg/100 mg tissue weight. Total lipid content in the liver was estimated according to the method of Fringes *et al.* (1972) [10] using olive oil as a standard. Lipid when boiled with sulphuric acid along with vanillin and phosphoric acid yields a pink colour whose optical density is measured at 530 nm. The total lipid content was expressed as mg /100 mg of tissue weight. The level of cholesterol was estimated in the liver by the method of Zlatki *et al.* (1953) [11] Cholesterol forms a coloured complex with FeCl3 along with concentrated sulphuric acid and glacial acetic acid and optical density of colour compound was measured at 540 nm. The cholesterol content was expressed as mg/100 mg tissue weight in liver and mg/dL. The mean value of each parameter of each group was calculated and then it was followed by measuring standard deviation and standard error. This calculation was done by Microsoft Excel,2010. Significance difference among groups at $p< 0.05$ was carried out by Analysis of Variance followed by One-way Tukey's Test by SPSS, 16 software.

RESULTS:

Table-2: Showing effect of alpha cypermethrin on major bio constituents in liver of mice and its amelioration by resveratrol.

Parameters	EXPERIMENTAL GROUPS						
	I	II	III	IV	V	VI	VII
	Untreated Control	**Vehicle control**	**RES control**	**HD ACP**	**HD ACP + LD RES**	**HD ACP + MD RES**	**HD ACP + HD RES**
Protein mg/100 mg tissue weight)	20.43 ± 0.19	20.38± 0.06	20.43± 0.14	9.50 ± 0.12^{acde} *(53.39)*	12.39± 0.11^{abde} **(26.5)**	15.29± 0.78^{abce} **(53.17)**	18.49 ± 0.09^{abcd} **(82.61)**
Glycogen µg/100 mg tissue weight)	1453 ± 11.4	1461 ±7.74	1470 ±9.41	545 ± 6.18^{acde} *(62.69)*	874 ± 5.10^{abde} **35.92**	1135 ± 9.63^{abce} **(64.41)**	1352 ± 9.49^{abcd} **(88.01)**
Total Lipid content (mg/100 mg tissue weight)	3.37 ± 0.09	3.31 ± 0.06	3.65 ± 0.09	9.32 ± 0.03^{acde} *(181.57)*	6.26 ± 0.05^{abcd} **(50.92)**	5.32 ± 0.07^{abce} **(66.55)**	4.15 ± 0.11^{abcd} **(86.02)**
Cholesterol content (mg/100 mg tissue weight)	1.09 ± 0.02	1.13 ± 0.02	1.10 ± 0.03	2.15 ± 0.02^{acde} *(90.26)*	1.84 ± 0.01^{abcd} **(30.39)**	1.62 ± 0.01^{abce} **(51.96)**	1.36 ± 0.02^{abcd} **(77.45)**

Abbreviations: LD=Low dose, MD=Mid dose, HD=High dose,
ACP: alpha cypermethrin, RES=Resveratrol
'a' represents significant difference (p<0.05) when compared to vehicle control, i.e group II
'b' represents significant difference (p< 0.05) when compared to Group IV
'c' represents significant difference (p<0.05) when compared to Group V
'd' represents significant difference (p<0.05) when compared to Group VI
'e' represents significant difference (p<0.05) when compared to Group VII
Values in italics within parenthesis indicate percentage change from vehicle control value,
Values in bold within parenthesis indicate hepatoprotective index based on Group IV values.

No significant difference was observed amongst different control groups of animals. Oral administration of alpha cypermethrin caused significant reduction in protein and glycogen content in liver of mice where as it increased lipid and cholesterol content. However, co administration of resveratrol along with alpha cypermethrin caused significant amelioration in all parameters in all co treatment groups (Groups V to VII). Resveratrol co treatment increased protein and glycogen content where as it depleted lipid and cholesterol content significantly p<0.05 compared to high dose alpha cypermethrin treated groups. The effects were dose dependent. Maximum amelioration was with high dose resveratrol for all parameters (Table 2). In terms of hepatoprotective index it is more than 77 for all the parameters.

DISCUSSION

Toxicity of alpha cypermethrin is confirmed from the significant (p<0.05) difference in values of protein, total lipid, glycogen and cholesterol from vehicle control. It is evident from the result that alpha cypermethrin alters the biochemical constituent's level significantly from the vehicle control groups. In a study conducted by Bengum G. (2005), [12] it was found that the alpha cypermethrin treatment for 10 days reduces protein level in liver of the test animals. Bhusan B. et al., (2013) [13] showed that cypermethrin treatment to rat leads to decrease in glycogen and protein level significantly. This decrease in protein content is because of breakdown of structural protein. Pyrethroid and its toxic metabolites lead to hepatocellular membrane damage which in turn leads to protein depletion. [14]

Cypermethrin metabolized in the liver via the hydrolytic ester cleavage and an oxidative pathway by the cytochrome P-450 microsomal enzyme system, which caused oxidative stress and reduces glycogen level, leading to hepatic degeneration and necrosis.[15] The depletion in protein can be due to fact that free radicals are generated due to alpha cypermethrin treatment.[16] Free primary oxygen radicals produced in cells and their secondary lipid radical intermediates can modify and fragment proteins; the products are often more susceptible to enzymatic hydrolysis and so radical fluxes may accelerate proteolysis inside and outside cells.[17] Proteins can be oxidatively modified in three ways: oxidative modification of specific amino acid, free radical mediated peptide cleavage, and formation of

protein cross-linkage due to reaction with lipid peroxidation products. Protein containing amino acids such as methionine, cystein, arginine, and histidine seem to be the most vulnerable to oxidation; Protein oxidation affects the alteration of signal transduction mechanism, enzyme activity, heat stability, and proteolysis. [18] This finding supports present finding of depletion in protein level when treated with alpha cypermethrin. Alpha cypermethrin treatment in rat caused lipid peroxidation and generation of free radicals in liver. [19] These findings explain the significant depletion ($p<0.5$) in protein content in alpha cypermethrin treated group compare to vehicle control in present study. Sub lethal dose of pyrethroid treatment for 21 days caused depletion in protein and glycogen content in muscles and liver in the test animals in present study. In a study [12], the test animals showed an increased rate of lipogenesis in liver, muscle, kidney and ovary during the exposure period when kept at 1/3 LD_{50} for 96 hrs. In present study also increased in lipid level is seen due to alpha cypermethrin treatment to mice. As shown in result cholesterol level increased significantly in alpha cypermethrin treated groups. In a study [20] it was found that exposure to acute dose of alpha cypermethrin to male, guppy fish in liver, brain and gill caused increased in cholesterol level. In a study on rabbit [21] it was found that, cypermethrin (24mg/kg Body weight) caused a significant ($P<0.05$) increase in the levels of plasma total lipids (TL), cholesterol, triglyceride (TG), low density lipoprotein (LDL) and very low-density lipoprotein (VLDL). On other hand resveratrol co treatment in three different doses was given to alpha cypermethrin treated mice as mentioned earlier. Resveratrol has decreased lipid level significantly ($p<0.05$) in all three doses compared to alpha cypermethrin group alone, restoration of lipid level is quiet high in high dose resveratrol co treatment. In terms of hepatoprotective index, it was 86.02. In a study it was found that fatty liver was induced by high fat diet was ameliorated by resveratrol treatment [22] and liver lipid level was reduced significantly. This finding corroborates our findings where alpha cypermethrin treatment caused lipid depositions in liver. Resveratrol, a dietary polyphenol, has been identified as a potent activator for both sirtulin 1 (SIRT 1) and AMPK (AMP- activated kinases (AMPK). These both are signaling molecules for lipid metabolism pathway. Resveratrol treatment increases SIRT1 expression levels and stimulated AMPK activity in liver of mice [23] and this fact explain depletion of lipid after resveratrol treatment as resveratrol enhances lipid metabolism. Protein depletion is found due to treatment of alpha cypermethrin, this depleted protein restored in each resveratrol co treated group significantly from alpha cypermethrin treated group alone. Resveratrol protects against the reactive oxygen species induced protein damage [24] which may be the reason of restoration of protein by resveratrol treatment. In a study [25] resveratrol reduced liver oxidative stress and protect the liver from Non-alcoholic fatty liver disease (NAFLD) by reducing fatty acid availability in rat when feed on resveratrol with dose of 15mg/k.g. body weight and 45mg/k.g. body weight for 6 weeks. This finding also corroborates present finding where resveratrol co treatment along with alpha cypermethrin reduces lipid and cholesterol significantly. In a study [26] resveratrol inhibited deposition of triglyceride and cholesterol in the liver of rats fed with corn oil-cholesterol-cholic acid mixture and in same study it had been noted that oral administration of resveratrol reduced triglyceride synthesis from ^{14}C-palmitate in the liver of experimental animals. In present study also, resveratrol co treatment has decreased the lipid and cholesterol significantly from alpha cypermethrin treated groups. In present study it was found that resveratrol treatment increases glycogen level significantly ($p<0.05$) compare to alpha cypermethrin treated group alone. Resveratrol has anti diabetic properties and it has impact on carbohydrate metabolism; The daily oral administration of resveratrol (5 mg/kg body weight) to diabetic rat for 30 days demonstrated a significant ($p<0.05$) decrease in blood glucose and glycosylated haemoglobin levels and a significant ($p<0.05$) increase in plasma insulin level.[27] Insulin is the hormone that is responsible for glucose to glycogen conversion.[28] Results for glycogen restoration by resveratrol co treatment to alpha cypermethrin treated mice are in accordance with previous two facts.

CONCLUSIONS:

From present study it is evident that alpha cypermethrin causes significant alteration in liver constituents like protein, glycogen, lipid and cholesterol significantly from vehicle control group. These changes can be considered as morbid changes and various researches supports these findings. On other hand resveratrol at three different doses had restored these constituents

significantly ($p<0.05$) comparatively from high dose alpha cypermethrin treated groups alone. Thus, it can be concluded that resveratrol has potential as antidote against chronic or sub chronic exposure to alpha cypermethrin.

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