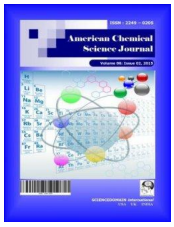

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Potential Effect of Vitamin C and Curcumin on Oxidative Stress and Skin Lesion Induced by Dermal Intoxication with Cypermethrin

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAN designed the study, performed the statistical analysis, author MEAE wrote the protocol and wrote the first draft of the manuscript. Authors RMZ and HEAF managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Dermal exposure to pyrethroids produces adverse health effect on different body systems. This study examined the effect of simultaneous oral supplementation of curcumin and vitamin C on systemic and skin injuries induced by dermal intoxication with cypermethrin.
Experimental Design: Four groups of rats treated for 28 days, -ve control, +ve control (curcumin 200 mg/Kg) and vitamin C (100 mg/Kg), Pesticide (cypermethrin (1/25 LD50 = 200 mg/Kg) on shaved skin of rats and pesticide and antioxidant (rats dermally intoxicated with cypermethrin (1/25 LD50 = 200 mg/Kg) and supplemented orally with (curcumin 200 mg/Kg) and vitamin C (100 mg/Kg).
Results: Dermal intoxicated rats with cypermethrin (1/25 LD50 = 200 mg/Kg) showed Inhibition in

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ATPase enzyme in serum, liver and brain associated with Induction of oxidative stress biomarkers malodialdehyde (MDA) and protein carbonyl in serum, liver but not in brain, concomitant with significant reduction in defense system serum SH-protein and reduced glutathione in liver and brain. As well as significant decrease in antioxidant enzymes activities superoxide dismutase (SOD), glutathione -S- transferees (GST) and catalase in serum, liver and brain. Penetration of cypermethrin through skin of treated rats induced necrosis and scales formation in the superficial layer of the epidermis of animals associated with inflammatory cells.

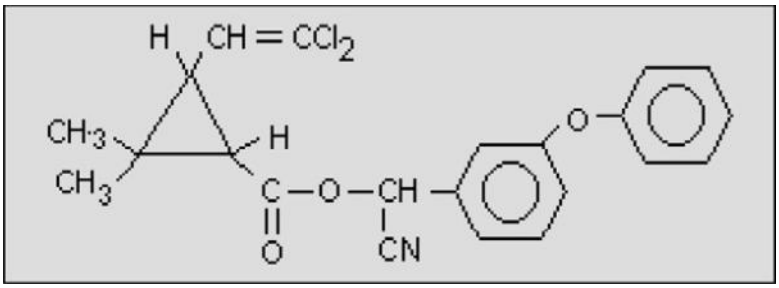
Conclusion: Simultaneous Supplementation with curcumin and vitamin C orally improved the alteration in the above mentioned parameters. However it failed to counteract the injures in the skin. Finally dermal exposure to pyrethroids threat the health of exposed subjects and needs long time in retrieve their health.

Keywords: Dermal toxicity; Pyrethroids; oxidative stress; liver; brain; skin.

1. INTRODUCTION

Skin is a complex tissue with a large surface area whose primary function is to protect the body from physical or chemical insult. Percutaneous absorption is reported as the possible route of entry in 65-85% of all cases of occupational exposure with pesticides [1]. Dermally reached pesticides due to Spraying or dusting was about 20-1700 times the amount deposited in the respiratory tract [2]. Cutaneous exposure to pesticide have been reported for non occupational uses like that epidemics induced by contamination of infant clothing to talcum powder with pesticides [3]. Pyrethroids are class of synthetic pesticides that have applications in agriculture, medicine, forestry, horticulture, and veterinary practices [4]. Inhalation and dermal exposure to pyrethroids is most common during their occupational or residential use [5]. Pyrethroids are neurotoxicants and act by blocking the voltage-sensitive sodium channel and possibly other ion channels [6,7]. Cypermethrin, a synthetic pyrethroid insecticide has been extensively used in the last two decades in many of the developing countries, especially Saudi Arabia and Egypt; for combating agricultural pests and insects of veterinary as well as human concern. Animals exposed to pyrethroids exhibited changes in their physiological activities beside other pathological features [8].

Cypermethrin (Scheme 1) has a wide spectrum effect on moths, pests of cotton, fruit and vegetable crops. It has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries and brain due to lipophilic nature. It causes free radical- mediated tissue damage in brain and liver *In vitro* and *in vivo* studies [10]. Cytochrome P-450 enzymes is the metabolic pathway for cypermethrin and other pyrethroids in liver. Reactive oxygen species (ROS) responsible for oxidative stress in mammals were generated through hydrolytic ester cleavage [11]. Pesticides may induce oxidative stress by generating increased free radicals and decreasing antioxidant levels and activities of free-radical scavenging enzymes [12]. Vitamin C (ascorbic acid) is a very important and powerful antioxidant. Vitamin C protects membranes against oxidative stress [13]. It is considered to be one of the most prevalent ant oxidative components of fruit and vegetables. Vitamin C induced chemo preventive effects without toxicity at doses higher than the recommended dietary allowance of 60 mg/day [14]. A positive correlation has been established between dietary supplementation with certain vegetables and plant products and the reduction of toxic effects of various toxicants and environmental contaminants. Turmeric contains a wide variety of phytochemicals, including curcumin, Curcumin is the phytochemical that gives a yellow color to turmeric and is now recognized as being responsible for most of the therapeutic effects. It is estimated that 2–5% of turmericis curcumin. Curcumin was first isolated from turmeric in 1815 [15]. Curcumin has anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, antifertility, hepatoprotective and cardio protective biological actions [16-18]. CMN is a potent scavenger of a variety of ROS including superoxide anion radicals and hydroxyl radicals. CMN also prevent the free radical formation induced lung injury in rats intoxicated with paraquat [17]. The objective of this study was to investigate if dermal intoxication with cypermethrin could be reduced by oral simultaneous supplementation with vitamin C and curcumin.



Scheme 1. Cypermethrin with this structure formula [9]

2. MATERIALS AND METHODS

2.1 Insecticide

Cypermethrin belongs to type-II pyrethroids ‘‘cyanopyrethroids” and has the chemical name [a-Cyano-(3-phenoxyphenyl)methyl(±)-cis/trans-3-(2,2-dichlorovinyl)2,2-dimethyl cyclopropanecarboxylate]. The insecticide was supplied as an emulsifiable concentrate (50% EC) formulation by the Mammalian and Aquatic Toxicology Department, Central Agricultural of Pesticides Laboratory, Dokki, Cairo, Egypt. Acute dermal toxicity of the formulation for rats is 5000 mg/kg b.w. Cypermethrin for dermal application was used in a dose 1/25 LD50 (200 mg/kg).

2.2 L-ascorbic Acid (Vitamin C)

A pure form of L (+) ascorbic acid was supplied as pure crystals (Sigma code: AX 1776-1) by E. Merck Science, a division of EM industries Inc., Darmstadt, West Germany. A freshly prepared aqueous solution of L-ascorbic acid was orally administered to the treated rats throughout the experiments, in a dose level of 100 mg/kg b.wt. for 5 successive days/week for four successive weeks [19].

2.3 Curcuma Longa (Curcumin)

The plant material was obtained from the local market of spices in Egypt ground into powder. One liter of double distilled water was mixed with 200 g of powdered C. longa rhizome, filtered with nitrocellulose membrane and the extracted liquid was subjected to water bath evaporation to remove the water. For water bath evaporation, liquid extract material was then placed into a beaker and subjected to water bath evaporation at 70°C temperature for 7-10 h daily for 2-3 days until a semisolid state of extracted liquid is obtained. The semisolid extract produced was kept in the deep freezer at -20°C overnight and then subjected to freeze drying. Extract obtained by this method was be then weighed and stored at 22°C in desiccators until further use in a dose 200 mg /kg b.w. [20].

2.4 Animals

Forty male rats *Rattus norvegicus* (3–4) month’s age, weighing between 160 -180 g were used. Animals were supplied by the breeding unit of NODCAR, Egypt. The animals were housed in plastic cages, fed *ad libitum* and allowed to adjust to the new environment for two weeks before starting the experiment. The rats were housed at 23±2°C dark/light cycles. All animals were treated according to the [21] guidelines repeated dose dermal toxicity study in rodents.

2.5 Experimental Design

Twenty-four hours before the experiment, rats were anesthetized by diethyl ether. Hair on the dorsal surface was removed from these animals with electric clippers (Oster Corp., Model A2, Milwaukee, WI, USA) using a size 40 blade. The clipped area was washed with distilled water to remove extraneous matter. Rats were randomly divided into four groups (10 animals each) the groups were treated as follow:

Group (C): Animals were orally treated with distilled water, act as (-ve control).

Group (P): Animals were dermally treated with cypermethrin at a dose level 200 mg/Kg b.w (1/25 LD50) daily (5 days /week) for 28 days.

Group (+ve C): animals were orally treated with aqueous extract of curcuma longa (200mg /Kg b.w) and vitamin C (100 mg / Kg b.w) daily (5 days /week) for 28 days.

Group (P+ Vit C. and CMN): Animals were dermally treated with cypermethrin at the dosage level 200 mg/Kg b.w. and orally supplemented with aqueous extract curcuma longa (200 mg/Kg

b.w) and vitamin C (100 mg/Kg b.w) daily (5 days /week) for 28 days. Animal experimental procedure was approved by the animal research ethical guidelines in Central Agricultural of Pesticides Laboratory, Agriculture Research Center, Egypt.

2.6 Sampling

At the end of experiment, rats were fasted overnight and blood samples were collected from retro orbital plexus vein into non-heparinized tubes. Blood samples were centrifuged at 2000 g for 15 min to obtain serum. Serum were separated and kept in deep-freezer at -20ºC till the assays were carried out.

2.6.1 Tissue preparation

Liver and brain were removed from rats at the end of experiment (28days) under ether anesthesia and washed with cold saline buffer. Washed tissues were immediately stored at -80ºC. For determination enzymatic activities, tissues were homogenized in ice cold 50mM sodium phosphate buffer (pH: 7) containing 0.1 mM ethylendiaminetetraacetic acid (EDTA) yielding 10% (W/V) homogenate. The homogenates were centrifuged at 12.000g for 30 min at 4ºC. The supernatant was used for investigation of enzymes activities.

2.6.2 Biochemical assay

Biomarkers of the oxidative stress were measured in serum, liver and brain. Lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (malondialdehyde) [22]. Protein carbonyl content was assayed by the described method [23], Where Soluble protein (1.0 mL) was reacted with 10 mM dinitrophenylhydrazin (DNPH) in 2N hydrochloric acid. After incubation at room temperature for 1 h in dark, 0.5 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing SDS 3.0%), 2.0 mL of heptane (99.5%) and 2.0 mL of ethanol (99.8%) were added sequentially vortexed for 40s and centrifuged at 10,000 g for 15 min. Then, the protein isolated from the interface was washed twice by suspension in ethanol / ethyl acetate (1:1) and suspended in 1 mL of denaturing buffer and the carbonyl protein content was measured spectrophotometric ally at 370 nm .Assay was performed in duplicate and two tubes blank incubated with 2NHCl without DNPH were included for each sample. The total carbonated protein was calculated using a molar extinction coefficient of 22,000 M/cm. Total thiol proteins were determined in serum the method based on the development of a yellow color when 5,5-dithiobis (2-nitrobenzoic acid) DTNB is added to serum [24]. Activity of superoxide dismutase (SOD) and catalase (CAT) and glutathione s-transferees (GST) were measured [25-27]. Total adenosine triphosphates (ATPase) activity was determined [28] based on the determination of the liberated inorganic phosphorus from substrate. Total glutathione content in liver and brain tissues was measured [29].

2.6.3 Histopathological examination

Samples were taken from the skin of rats in different groups and fixed in 10% formalin saline for twenty four hours and decalcification was occurred on formic acid. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin & eosin stain and examined by light microscopy [30].

2.7 Statistical Analysis

The data obtained from the biochemical analysis of different groups are represented in tables as Mean±Standard error (mean±SE). The significance of the difference between the groups was calculated by one-way analysis of variance (ANOVA) followed by Duncan's test at $P < 0.05$ using the SPSS-PC computer software package version 10.

3. RESULTS

3.1 Biomarker Changes in Serum

The depicted data in Table 1 revealed that dermal application of 1/25LD50 of cypermethrin induced significant inhibition in serum ATPase enzyme activity, biomarker of pyrethroid intoxication, versus control at $p<0.05$. However, co administration of curcumin and vitamin C in the presence of cypermthrin application elicit the activity of the enzyme significant versus all other treated groups. Concurrent to the principal

mechanism of pyrethroids, significant elevation in oxidative stress markers malondialdehyde (MDA) and protein carbonyl was recorded in serum of dermal intoxicated animals significant versus control and +ve control (curcumin + vitaminC) groups at ($p<0.05$) Table 1. Significant reduction in SH-protein the defense line in biological system was recorded in serum of dermal intoxicated animals versus control groups at ($P<0.05$). Supplementation with curcumin and vitamin C to dermal intoxicated group enhanced the level of SH-protein to be (172.5%) from control. However, slight reduction was recorded in stress markers MDA and protein carbonyl significant versus control and non supplemented group at ($P<0.05$). Regarding antioxidant and detoxifying enzymes activities remarkable significant reduction in catalase and glutathione – S- transferees (GST) activities versus control at $p<0.05$ was recorded in dermal intoxicated animals with percent change (-22.58%) and (-6.75%) from control respectively. On the other hand, improvement in Catalase and GST activities was recorded in dermal intoxicated group supplemented with curcumin and vitamin C significant versus control at ($p<0.05$). No changes were recorded in superoxide dismutase (SOD) Table 1.

3.2 Biomarker Changes in Liver

Dermal intoxication with cypermethrin induced significant reduction in ATPase activity in liver tissue versus control and +ve control groups at ($P<0.05$). Concomitant with significant elevation in oxidative stress markers MDA and protein carbonyl versus control and +ve control at ($p<0.05$), with 61.90% and 35.18% from control. Co supplementation with curcumin and vitamin C to dermally intoxicated animals improved the activity of ATPase and the level of biomarker stress in liver tissue to be nearly reached the control level significant versus control and non-supplemented groups at ($p<0.05$). Enzymatic antioxidants (catalase, SOD and GST) and non-enzymatic defense system reduced glutathione (GSH) recorded significant reduction in their activities and levels in dermal intoxicated group versus control at $p<0.05$. on the other hand, intoxicated animals supplemented with curcumin and vitamin C slightly improved the activity of catalase and GST enzymes as well as the level of GSH as demonstrated in Table 2. However, it failed to counteract the toxic effect on SOD enzyme where a remarkable significant reduction versus other groups was recorded with percent change (-25.17%) from control Table 2.

3.3 Biomarker Changes in Brain

Regarding alteration of biomarkers in brain of dermal intoxicated animals, a significant reduction in ATPase activity (21.35%) was recorded. The previous mechanism exist in the same time with elevation in stress marker MDA (28.48%) from control. While, no changes in protein carbonyl level was recorded. Co supplementation with curcumin and vitamin C improved the above changes in ATPase and MDA nearly reached the control level. Meanwhile, remarkable significant decrease in GSH, Catalase, SOD and GST was observed in dermal intoxicated group with (-67.76%, -36.55%, -1.62% and -23.32%) from control respectively. On the other hand, supplementation with curcumin and vitamin C slightly improved GSH and GST levels to be (-35.19% and -14.54%) from control. However, statistical analysis declared significant negative effect on catalase and SOD) versus control and intoxicated groups at $p<0.05$ Table 3.

3.4 Histopathological Examination of Skin

Fig. 1 depicted Skin of rats in control and +ve control groups showed no histopathological alteration and the normal histological structure of the epidermis with different layers and the underlying dermis and subcutaneous adipose tissue were recorded in photomicrograph (a & b). Dermal application of 1/25 LD50 of cypermethrin showed that necrosis and scales formation were noticed in the superficial layer of the epidermis of skin of animals associated with inflammatory cells infiltration (d) and congestion of the blood vessels ,as well as focal hemorrhage ,in the deep layer of the dermis and subcutaneous tissue. Mature and advanced birds' nests formation (squamous cell carcinoma) was observed in the dermal layer associated with acanthosis in the prickle cell layer of the epidermis photomicrograph (d). On the other hand, co supplementation with curcuma and vitamin C to animals dermally intoxicated animals with CYP showed that Mild acanthosis was detected in the prickle cell layer of the epidermis associated with formation of early and immature birds' nests (squamous cell carcinoma) in the dermis with massive number of inflammatory cells infiltration in the surrounding area photomicrograph (c).

Table 1. Effect of Co administration of curcuma longa (Curc) and vitamin C (Vit C) on (ATPase, MDA, Protein carbonyl, SH-protein, catalase, SOD and GST) in serum of rats dermally intoxicated with cypermethrin (Cyprm)

Groups and Parameters	ATPase (µmol Piliberated / min/mg protein)	MDA (mmol/ml)	Protein carbonyl (mmol/ml)	SH-protein (µmol/dl)	Catalase (U/mg protein)	SOD (U/mg protein)	GST (mmol / min / mg protein)
Control	86.29±0.53	98.4±6.83	96.57±3.3	231.16±7.54	5.89±0.21	12.28±0.1	61.52±2.41
Curc+VitC	89.42±0.9 (3.63%)	102.00±6.29[a] (3.66%)	108.4±9.67 (12.25%)	349.79±11.93[a] (51.32%)	6.09±0.18 (3.40%)	12.29±0.19 (0.081)	66.73±1.54 (8.47%)
Cyperm.	79.61±1.73 (-7.67%)	136.52±0.36[a,b] (38.74%)	161.94±2.99[a,b] (67.69%)	107.13±2.54[a,b] (-53.66%)	4.56±0.18[a] (-22.58%)	12.28±0.18 (0)	57.37±1.57[a] (-6.75%)
Curc+VitC+Cyperm.	96.65±2.55[a] (12.00%)	130.83±4.69[a] (32.96%)	151.15± .99[a,c] (56.52%)	629.9±8.52[b,c] (172.50 %)	6.48±0.34[b] (10.01%)	12.59±0.18 (2.44%)	59.3±2.29[a] (-3.74%)

All data are expressed as means ± SE five rats; % changes from control; a Significant differences versus control at p < 0.05; b Significant difference versus Curc+VitC Group at p < 0.05; c Significant difference versus Cyperm; Groups at p < 0.05

Table 2. Effect of coadministration of curcuma longa (Curc) and vitamin C (Vit C) on (ATPase, MDA, Protein carbonyl, GSH, catalase, SOD and GST) levels in liver tissues of rats dermally intoxicated with cypermethrin (Cyperm)

Groups and Parameters	ATPase (µmol Piliberated / min / mg protein)	MDA (mmol/ml)	Protein carbonyl (mmol/ml)	SH-protein (µmol/dl)	Catalase (U/mg protein)	SOD (U/mg protein)	GST (mmol/min/mg protein)
Control	72.05±3.65	41.37±2.24	100.14±3.64	10.46±0.81	85.19±2.82	5.68±0.08	25.35±1.23
Curc+VitC	73.74±0.91 (2.35%)	46.58±1.07[a] (12.59%)	105.24±2.99 (5.10%)	10.58±0.51 (1.15%)	79.82±4.64 (-6.30%)	4.67±0.06[a] (17.78%)	24.23±0.61 (-4.41%)
Cyperm.	61.66±1.84[a,b] (-14.42%)	66.98±0.36[a,b] (61.90%)	135.37±0.85[a,b] (35.18%)	4.42±0.31[a,b] (-57.74%)	78.16±3.64 (-8.25%)	4.99±0.17[a] (-12.2%)	18.77±0.75[a,b] (-25.95%)
Curc+VitC+Cyperm	72.23±2.5[a,c] (0.25%)	49.01±1.34[a,c] (18.47%)	110.54±2.49[a] (10.39%)	8.52±0.19[a,b,c] (-22.80%)	85.17±2.7 (-0.23%)	4.25±0.6[a,b,c] (-25.2%)	22.84±0.76[b,c] (-9.78%)

All data are expressed as means ± SE five rats; % changes from control; a Significant differences versus control at p < 0.05; b Significant difference versus Curc+VitC Group at p < 0.05; c Significant difference versus Cyperm. Groups at p < 0.05

Table 3. Effect of coadministration of Curcuma longa (Curc) and vitamin C (Vit C) on (ATPase, MDA, Protein carbonyl, GSH, catalase, SOD and GST levels in Brain Tissues of rats dermally intoxicated with cypermethrin (Cyperm)

Groups and parameters	ATPase (µmol Piliberated / min / mg protein)	MDA (mmol/ml)	Protein carbonyl (mmol/ml)	SH-protein (µmol/dl)	Catalase (U/mg protein)	SOD (U/mg protein)	GST (mmol/min/mg protein)
Control	42.39±1.06	15.98±0.45	18.76±0.32	8.1±0.5	4.46±0.25	15.43±0.08	24.62±0.75
Curc+VitC	41.93±2.33 (-1.08%)	14.04±0.78[a] (-12.14%)	18.81±0.26 (0- 026%)	7.81±0.30 (-3.58%)	4.14±0.31 (-7.17%)	15.13±0.1[a] (-1.94%)	22.48±0.49 (0.57%)
Cyperm.	33.34±0.61[a,b] (-21.35%)	20.53±1.23[a,b] (28.48%)	18.54±0.41 (-0.018%)	2.53±0.26[a,b] (68.76%)	2.83±0.09[a,c] (36.55%)	15.18±0.08[a] (-1.62%)	18.88±1.00 (-23.32%)
Curc+VitC+Cyperm	42.98±0.81[b,c] (1.37%)	19.00±0.34[a] (18.90%)	18.73±1.28 (-0.005%)	5.25±0.04[a,b,c] (35.19%)	2.76±0.14[a,c] (38.16%)	14.84±0.01[a,b,c] (-3.82%)	21.04±0.39 (-14.54%)

All data are expressed as means ± SE five rats; % changes from control; a Significant differences versus control at p < 0.05; b Significant difference versus Curc+VitC Group at p < 0.05; c Significant difference versus Cyperm; Groups at p < 0.05.

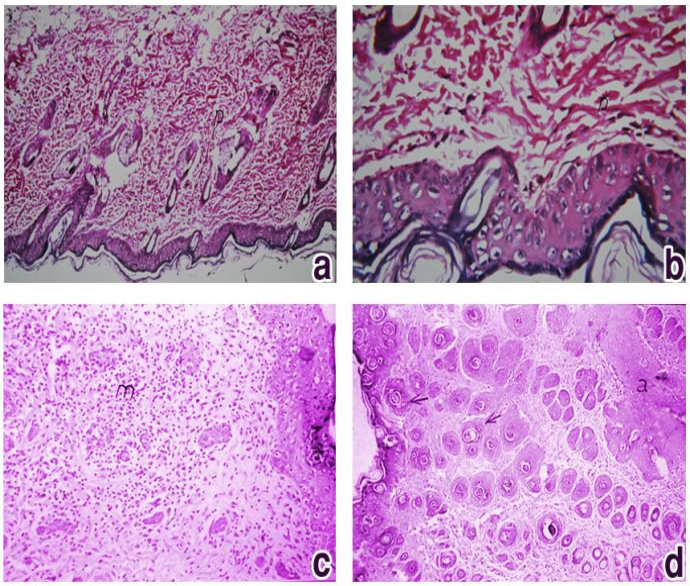


Fig. 1. Normal histological structure of the epidermis with different layers and the under lying dermis and subcutaneous adipose tissue photomicrograph (a, b) (HxE40). Skin of cypermethrin treated group showing acanthosis of the prickle cells layer of the epidermis (a) with birds' nests formation (squamous cells carcinoma) in the dermis photomicrograph (d) (HxE40). Skin of cypermethrin intoxicated animals supplemented with Curcumin and vitamin C showing massive number of inflammatory cells infiltration in the dermis (m) photomicrograph (C). (HxE40)

4. DISCUSSION

Hazards dermal exposure to Pesticides should be assessed. Because handlers (e.g., mixers, loaders, applicators, flaggers) of most pesticides and field workers (e.g., harvesters) obtain a majority of internal dose from dermal contact with pesticides. Dermal exposure is the most significant route of absorption for agricultural applicators and sprayers [31]. The penetration of pyrethroids into the skin is slow and may cause a typical local paraesthesia (tingling and burning), which may persist for several hours [32]. Several studies confirmed that the nervous system (CNS) is the principle target of synthetic pyrethroids [33]. Na+, K+-ATPase, a membrane bound lipid dependent enzyme catalyzes the active transport of Na+ and K+ in CNS that necessary to maintain cellular homeostasis and the ionic gradient for neuronal excitability. Also, Na+, K+-ATPase is a key enzyme implicated in neural excitability, metabolic energy production and uptake and release of catecholamine and serotonin [34]. Inhibition in ATPase enzyme activity is a well-known mechanism of pyrethroids. In the present study dermally exposed rats to cypermethrin have a depletion in activity of serum, brain and liver ATPase these results are in consistent with that of [35,36] who recorded depletion in brain, kidney and liver ATPase activities in fish *Labeo rohita* organs exposed to 0.139 ppm of cypermethrin for 45 days. Also, Recent reports suggest that the activities of ATPase were inhibited by deltamethrin in human erythrocytes [37]. Brain total ATPase activity has been found to be significantly decreased after acute and sub-acute treatments of rats with beta- cyfluthrin [38,39]. In the present study significant elevation in oxidative stress marker malondialdehyde (MDA) was recorded in serum, liver and brain of dermally intoxicated rats. While, protein carbonyl elevated in serum and liver only. cypermethrin was found to produce significant elevation in oxidative stress in cerebral and hepatic tissues of rats, was evident by the elevation of the level of thiobarbituric acid reactive substances (TBARS) in both tissues [10] MDA is the indicator of lipid peroxidation increased in brain, liver and kidney of cypermethrin-treated Rats increased production of ROS and oxidative stress damage lipids and induce oxidative modification of proteins as confirmed from significant increased in the brain MDA and protein carbonyl (PC) levels [40]. Main while, the enhanced

susceptibility of brain membranes to LPO can lead to loss of membrane bound ATPase activity that modulates the cell functions [41]. Moreover, it was established that the primary effect of pyrethroids occurs on the voltage sensitive sodium channels; disrupting the functioning of the CNS both in insects and mammals [42]. This membrane bound enzyme requires phospholipid for its activity and is highly vulnerable to oxidative insult. The mechanism of inactivation under such conditions also involves disruption of phospholipid microenvironment of the enzyme or direct damage to enzyme protein by ROS or LPO products that associated alteration of fluidity or other membrane properties [43,34]. Oxidative stress occurs usually with excessive generation of free radicals parallel with depletion of antioxidant enzymes like superoxide (SOD) ,catalase, and glutathione –S-transferees (GST) as well as SH - proteins and reduced glutathione provide the major defense against ROS induced cellular damage [44]. These findings are in consistent with our results where reduction in defense mechanism and detoxifying enzymes was recorded. Brain GST activity has been found to be decreased after acute and sub-acute treatment of beta-cyfluthrin [41]. A decrease in brain GST activity because of action of pesticide, that alters detoxification metabolism observed in the of rats after malathion and deltamethrin toxicity [45,46]. Changes in the above mentioned parameters supported by severe injuries in skin of cypermethrin treated rats similarly; cypermethrin (pyrethroid) can penetrate through the skin of animals and can exert its systemic effects [47]. Dermal exposure to permethrin (another pyrethroid) has often been considered a mitigating factor in Gulf War Syndrome [48]. Dermal absorption is the major source of pesticide exposure in occupational, agricultural and veterinary settings [49]. Since most of the pesticides are highly lipophilic, their penetration and accumulation in skin with resulting local dermal irritation, and/or mild to severe systemic toxic effects in the exposed animals cannot be overlooked. Occupationally, skin is the main route of pyrethroid absorption. The major toxic effect of dermal exposure is paresthesia, presumably due to hyperactivity of coetaneous sensory nerve fibers [50]. It should be noted here that supplementation with combined curcumin and vitamin C improved the activity of ATPase enzyme in all investigated organs this may be due to the counteracting effect on oxidative stress biomarkers due to improving defacing system and antioxidant enzymes. Curcumin is known to exhibit a strong antioxidant activity and is a potent scavenger of free radicals such as superoxide anion radicals, hydroxyl radicals and nitrogen dioxide radicals [51]. Curcumin treatment improved the levels of antioxidant enzymes, mainly GSH, SOD and catalase [52]. The beneficial effects of Curcuma longa have been postulated to be due to the phenolic yellow pigments of turmeric, curcuminoids, along with the major compound curcumin [53]. Vitamin C (ascorbic acid) is a very important and powerful antioxidant. Vitamin C ameliorates sensor motor and cognitive changes induced by acute chlopyrifos exposure in Wister rats [54]. Combined antioxidants more effective because each one may act in a way that enhances the ameliorative effect of the others [55]. This hypothesis was clearly reported in our results. The combined treatment with curcumin along with vitamin C before Cd intoxication was more effective than that with either of them alone in reducing such changes and reverses the changes induced by Cadmium toxicity almost similar to that of control. Combined administration of vitamin E and curcumin can act as potential agents against diazinon induced oxidative stress [56].

5. CONCLUSION

Dermal intoxication with pyrethroids represented by cypermthrin exhibited reduction in ATPase enzyme in each of serum, liver and brain concurrent with eliciting oxidative stress and drawback of defense system and antioxidant enzymes. Synchronous with the previous findings sever skin injury was found in dermally treated rats. Animals supplemented with combined curcumin and vitamin c improved the investigated parameters but failed to counteract the toxic effect of cypermethrin on the skin it may need long time in supplementation after withdrawing the pesticide.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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