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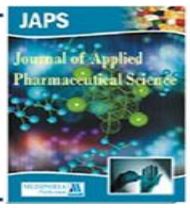

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Individual and combined biochemical and histological effect of Cypermethrin and Carbendazim in male albino rats

V. Muthuviveganandavel, P. Muthuraman, S. Muthu and K. Srikumar

V. Muthuviveganandavel
Dept of Zoology, Tagoore Arts and Science College, Puducherry, India.

P. Muthuraman
Dept. of Animal Science, Chonbuk National University, Jeonju, South Korea.

S. Muthu
Dept. of Zoology, Arignar Anna Arts and Science College, Karaikal, India.

K. Srikumar
Dept. of Biochemistry and Molecular Biology, Pondicherry University, India

For Correspondence
Prof. K. Srikumar
Dept. of Biochemistry and Molecular Biology, Pondicherry University, India.

ABSTRACT

Cypermethrin (CYP) and Carbendazim (CAR) are synthetic pesticides that have been in use for more than a decade. A study of a low-dose exposure of these pesticides in rat administered different doses of each of these compounds, individually and in combination, followed by investigation of the biochemical and histological status of the rat brain, will highlight early enzymatic and histological changes that generally accompanied a toxic response in the animal. Thus, even though CYP was used at 5, 10, 25 and 50mM, for durations of 12 and 24hr in the different groups of rats, results of the study indicate that simultaneous with changes in enzyme activity, histological sections of specific rat brain yielded observations suggestive of degenerating tissue morphology and architecture even at the low-dose levels.. Tissue specific changes in the activities of these marker enzymes were suggestive of the lack of tissue resistance to pyrethroids or carbamates.

Keywords: Carbendazim; Cypermethrin; Enzymes; Histology; Rats.

INTRODUCTION

Pesticides are compounds that kill pests, and include insecticides, rodenticides, herbicides (Butterfield and Lauderback, 2002) fungicides and fumigants (Ellenhorn et al., 1977). The introduction of novel, more toxic and rapidly disseminating pesticides into the environment has necessitated an accurate identification of their potential hazards to human health. Although these toxic chemicals have become an integral part of the ecosystem, many of them remain extremely toxic to mammals and other non-target creatures. The implication of pesticide residues in human health however is yet to be comprehensively understood. Free radicals are known to play an important role in the toxicity due to pesticides and of environmental chemicals. Pesticides may induce oxidative stress, leading to the generation of free radicals, resulting in changes in the status of the antioxidant, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation (Etemadi-Aleagha et al., 2002).

The main target organ for short-term toxicity of pyrethroids is the nervous system. Pyrethroid related-sensory irritation in the respiratory tract was studied (Pauluhn, 1996) by nose-only exposure studies in mice and rats. The guinea- pigs received 0.1 ml of a 0.01, 0.1, or 1.0% solution of CYP in ethanol or a 1, 10 or 20% solution of CYP (w/v) in corn oil on the skin. Sensory stimulation was quantified by counting the number of times each animal turned to lick or bite its treated flank in preference to the untreated flank. Skin stimulation was observed during a 2hr period at all dose levels except the lowest (Dewar, 1971). The results of the study concluded that CYP was not a skin sensitizer in guinea-pigs (Hend, 1983).

Elevated levels of plasma malonialdehyde (MDA), indicative of lipid peroxidation, were observed (El-Gohary, 1999). CYP in DMSO (20%) administered to wistar rats as a single oral dose of 40 mg/kg body weight, with an exposure time of 6 hr, failed to produce any detectable DNA strand damage in the DNA. (Wooder, 1982).

CYP applied dermally, especially in high dose (250mg/kg bwt), for 28 days caused slight histological changes in the brain (Luty et al., 2003). Changes in the brain of female wistar rats were noted after one and four weeks when a high dose of chlorpyrifos and CYP were administered on the tail skin. These changes manifested due to pycnosis of neurocytes in the various areas of the brain and in the cells of stratum granulosum and stratum pyramidale hippocampi (Jadwiga et al., 2001). Slight histopathological changes such as increased density of cytoplasm in the neurocytes of both experimental groups 3 weeks post exposure to CYP were also observed (Jadwiga et al., 2003).

Acute toxicity effect of single oral LD_{50} (140mg/kg in DMSO) of CYP in rats produced the meninges and cerebellum were observed (Manna et al., 2004). Repeated toxicity dose studies of deltamethrin at 1/10 LD_{50} produced mild to moderate histological changes as observed in the cerebellum of the rats (Manna et al., 2005). The most frequently noted changes were cerebro-organic disorders, like reduced intellectual performance, personality disorders, sensory and motor polyneuropathy frequently associated with the lower legs and vegetative disorders (Muller-Mohnssen, 1999).

CAR is an important member of the benzimidazole group with broad- spectrum nature. CAR is an International Standardization Organization (ISO) approved common name for methyl 2-benzimidazole carbamate, a systemically active benzimidazole compound that is widely used as an agricultural and horticultural fungicide/pesticide around the world (Dreisbach, 1983). The time and dose-dependent effect of CYP, CAR and their combinations in male albino rat brain is presented here

MATERIALS AND METHODS

Materials

CYP (95.6% pure) and CAR (98.3% pure) was a gift from Gharda Chemicals Ltd., Mumbai, India. All chemicals used in this study were of analytical grade. Glass distilled water was used for the preparation of all reagents. Male Wistar strain-albino rats weighing 150–200 g were used for the investigation. The animals were housed under controlled temperature and hygiene conditions with 12 hours of light and dark cycle throughout the experimental period. Commercial rat chow with free access to drinking water ad libitum was provided for the animals.

Methods

The experimental approach aimed to investigate the effect of the insecticide CYP and the fungicide CAR on the biochemistry and histopathology of male albino rat brain. Control and treated groups of rats contained six animals each. Dose levels of each compound used were 5, 10, 25 and 50mM, individually and in combined doses. These dose levels corresponded to 60.45, 120.9, 302.25 and 604.5 µg of CYP / kg.b,wt, and 477.5, 955, 2387.5 and 4775 µg of CAR/ kg.b,wt of the animal. The compounds were instantly dissolved in 0.1 ml ethanol and were administered intradermally. The time intervals selected for investigation were 12 and 24 hrs following administration of each compound in all the studies.

Preparation of tissue homogenate as crude enzyme source

Rat brain was surgically removed, immediately rinsed in ice cold 1.15% KCL solution, pre-cut into small pieces and taken for homogenization employing several strokes in a potter-Elvejham homogenizer using a Teflon pestle, in the appropriate buffer, to obtain 10% (w/v) tissue homogenate. Throughout the homogenization process, the tissue homogenate was maintained on crushed ice in an ice bucket. The tissue homogenate was then centrifuged in a refrigerated high-speed centrifuged at 4^oC and at 10,000 x g for 20 minutes. The clear supernatant obtained from homogenate was used an enzyme source for the investigations.

Biochemical Parameters

Protein content was measured by the method of Lowry et al (Lowry et al., 1951). Alanine transaminase (ALT) & aspartate transaminase (AST) enzyme activities were measured by Reitman and Frankel (1957). Alkaline phosphatase (ALP) enzyme activity was measured by Bessey et al., (1946). Acid phosphatase (ACP) activity was measured by Tennis wood et al. (1976). γ- Glutamyl transpeptidase (GGT) activity was measured by the method of Volohonsky et al., (2002). Amylase (AMY) enzyme activity was measured by the method of Bernfeld (1955). Hexokinase (HK) enzyme activity was measured by the method of Mc Lean and Brown (1993). Malondialdehyde (MDA) content was determined by the method of Ohkawa et al (1979). DNA content was measured by the method of Burton (1956). RNA content was measured by the method of Rawal et al (1977). Cholesterol content was measured by Zak's et al. (1954). Estimation of Triglycerides content was measured by the method of Bucolo et al. (1973). Urea content was measured by the method of Natelson et al. (1951). Uric acid content was measured by the method of Caraway, (1963).

Histopathology

Histological examinations were done by the method of Humason (1972).

Statistical Analysis

All values expressed were as mean ± SEM. Statistical analysis was done using SPSS 14 program. The statistical significance of differences between the two means was assessed by one way ANOVA. P values < 0.05 were considered to be significant.

RESULTS

This study used CYP and CAR, two different pesticide compounds at lower doses individually and in combination to

determine their metabolic impact in male albino wistar strain rat brain tissues as a mammalian model. The study estimated selected marker enzyme activities and total protein in rat brain. Peroxidative damage of lipids was assessed monitoring the MDA formed in the thiobarbituric acid assay, in control as well as for the pesticide affected rat brain. Additionally, brain tissue content of DNA, RNA, cholesterol, triglyceride, urea and uric acid were also determined. The biochemical investigations were followed with histological study of the rat brain subject to each pesticide effect.

Control protein values varied between 60-100mg/g tissue. CYP increased brain tissue protein at 12hr by 15% at the highest dose, while CAR and the combination reduced brain tissue protein content to the extent of 50% at 12hr. The 24hr tissue response was however different, with CYP increasing the tissue protein content by 50%, while CAR and the combination maintaining the protein levels below control values (Table 1).

Table 1: Rat brain total protein (mg/g) in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	61.0 ± 2.87	65.0 ± 2.58
CYP -5mM	54.0 ± 4.16	60.0 ± 2.67
CYP-10mM	62.8 ± 4.46	80.0 ± 3.19
CYP-25mM	70.0 ± 4.19	71.0 ± 3.04
CYP-50mM	54.0 ± 3.75	90.0 ± 3.15
CAR -5mM	50.0 ± 4.23	60.0 ± 2.20
CAR-10mM	38.6 ± 3.21	61.0 ± 3.75
CAR-25mM	30.0 ± 2.63*	50.0 ± 3.36
CAR-50mM	62.0 ± 4.06	70.0 ± 5.16
CYP+CAR - 5mM(each)	34.0 ± 3.57	50.0 ± 3.43
CYP+CAR-10mM(each)	40.0 ± 3.01	51.0 ± 2.35
CYP+CAR-25mM(each)	39.3 ± 1.64	53.0 ± 3.11
CYP+CAR-50mM(each)	30.0 ± 3.94*	50.0 ± 3.18

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05.

CYP administered at 5 and 10 mM dose to the rat significantly (p≤0.01; p≤0.05) decreased ALT enzyme activity in the rat brain tissue. On the other hand, CAR fungicide when used at doses of 10, 25 and 50mM, significantly decreased (p≤0.01) ALT activity in the brain tissue. The degree of decrease was noted to be 30% at 5mM, 33% at 10mM, 38% at 25mM and 44% at 50mM. The combined application of CYP+CAR at different doses also significantly (p≤0.01) decreased, Surprisingly, 12hr following CYP (5 & 50mM) and CAR (10 & 25mM) treatment, the ALT activity was noted to increase (38 & 39% and 76 & 68%) significantly (p≤0.01). Increase in ALT activity was found to be 28% at 5mM, 34% at 10mM, 85% at 25mM and 75% at 50mM CYP+CAR combinations. In a similar manner, the 24hr effect on rat brain due to CYP (5 & 25mM) and CAR (10 & 25mM) was noted to be that they significantly increased (30&26% and 39&30%) the ALT activity. Combined treatment with the insecticide and the fungicide at 10mM dose led to a significant 32% increase in the ALT activity in rat brain (Table 2).

Table 2: Rat brain ALT specific activity (IU/mg protein/g $x10^{-2}$) in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	5.94 ± 0.399	5.29 ± 0.303
CYP -5mM	8.22 ± 0.511**	6.90 ± 0.592**
CYP-10mM	6.58 ± 0.429	5.25 ± 0.414
CYP-25mM	5.82 ± 0.487	6.70 ± 0.478**
CYP-50mM	8.24 ± 0.506**	3.85 ± 0.276**
CAR -5mM	6.60 ± 0.405	5.11 ± 0.307
CAR-10mM	10.5 ± 0.778**	7.40 ± 0.407**
CAR-25mM	10.0 ± 0.466**	6.90 ± 0.489**
CAR-50mM	5.16 ± 0.390	4.80 ± 0.401
CYP+CAR -5mM(each)	7.66 ± 0.689**	5.22 ± 0.531
CYP+CAR-10mM(each)	8.00 ± 0.405**	7.02 ± 0.320**
CYP+CAR-25mM(each)	11.0 ± 0.626**	5.30 ± 0.420
CYP+CAR-50mM(each)	10.4 ± 0.541**	5.40 ± 0.255

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 3: Rat brain AST specific activity (IU/mg protein/g $x10^{-2}$) in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	1.17 ± 0.177	1.48 ± 0.177
CYP -5mM	2.81 ± 0.208**	1.44 ± 0.180
CYP-10mM	2.54 ± 0.223**	1.39 ± 0.155*
CYP-25mM	2.55 ± 0.223**	1.40 ± 0.226*
CYP-50mM	2.89 ± 0.195**	1.41 ± 0.177
CAR -5mM	1.41 ± 0.128**	1.62 ± 0.151**
CAR-10mM	2.40 ± 0.179**	2.15 ± 0.197**
CAR-25mM	2.37 ± 0.187**	1.96 ± 0.137**
CAR-50mM	1.20 ± 0.12	1.87 ± 0.241**
CYP+CAR -5mM(each)	2.35 ± 0.129**	1.73 ± 0.215**
CYP+CAR-10mM(each)	1.77 ± 0.119**	1.56 ± 0.166*
CYP+CAR-25mM(each)	2.41 ± 0.173**	1.51 ± 0.191
CYP+CAR-50mM(each)	2.44 ± 0.129**	1.54 ± 0.191*

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 4: Rat brain ALP specific activity (IU/mg protein/g x 10^{-2}) in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	2.16 ± 0.227	2.06 ± 0.152
CYP -5mM	2.36 ± 0.283	2.41 ± 0.258**
CYP-10mM	1.81 ± 0.268*	2.15 ± 0.187
CYP-25mM	1.64 ± 0.252**	2.33 ± 0.231**
CYP-50mM	2.37 ± 0.268	2.46 ± 0.280**
CAR -5mM	4.30 ± 0.499**	3.20 ± 0.274**
CAR-10mM	4.46 ± 0.339**	3.50 ± 0.229**
CAR-25mM	6.66 ± 0.380**	4.06 ± 0.267**
CAR-50mM	3.31 ± 0.266**	2.70 ± 0.327**
CYP+CAR -5mM(each)	4.80 ± 0.333**	1.80 ± 0.146**
CYP+CAR-10mM(each)	3.50 ± 0.242**	2.20 ± 0.212
CYP+CAR-25mM(each)	4.86 ± 0.339**	1.94 ± 0.256
CYP+CAR-50mM(each)	5.20 ± 0.287**	1.64 ± 0.261**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Similar to studies on ALT, AST activity of the rat brain was also studied as control verses treated using the two pesticides at different times. On the contrary, the brain AST specific activity remained enhanced (146% by CYP, 105% by CAR and 109% by CYP+CAR) at 12hr also, while it reduced nearly 50% by 24hr. Surprisingly CAR alone enhanced this enzyme specific activity above control at 12 and 24hr but showed enhancement of specific activity using the combination only at 12hr (Table 3). ALP enzyme activity in the brain tissue was enhanced (208% and 87%) by CAR at 12 & 24hr, and by the combination (141%) at 12hr. CYP was found to be ineffective in increasing the rat brain ALP activity (Table 4).

ACP activity was elevated by CYP and CAR but at different times. While the CYP effect was noted 118%-166% and 38-54% sustained its effects through 12 and 24hr, the CAR effect (53%-66%) was noted only by the 12th hr and seemed to decline (7%) by 24hr (Table 5). GGT activity in brain seemed to remain suppressed both for the control as well as for the different doses of these compounds administered to the animal at 12 and 24hr. Elevation of brain GGT specific activity was found (341%) very pronounced due to CAR than (114%) due to α-CYP (Table 6). CYP or CAR did not exceed more than two fold in most tissues excepting in rat brain, whereas CAR alone showed elevation (119%-812%) of amylase specific activity in the brain at 12hr. Mild elevation (71%-631%) due to CAR at 24hr was noted in the brain (Table 7).

Table 5: Rat brain ACP specific activity (IU/mg protein/g x10^{-2}) in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	8.00 ± 0.693	9.64 ± 0.821
CYP -5mM	17.42 ± 0.958**	14.86 ± 1.29**
CYP-10mM	18.73 ± 1.01**	13.28 ± 0.740**
CYP-25mM	21.25 ± 1.30**	9.44 ± 0.513
CYP-50mM	19.53 ± 0.676**	6.10 ± 0.513**
CAR -5mM	9.58 ± 0.989	10.26 ± 1.13
CAR-10mM	12.20 ± 0.801**	10.51 ± 0.555
CAR-25mM	13.26 ± 1.31**	9.25 ± 0.973
CAR-50mM	9.62 ± 0.417	9.00 ± 0.741
CYP+CAR -5mM(each)	16.10 ± 0.769**	8.66 ± 0.707
CYP+CAR-10mM(each)	12.89 ± 0.766**	13.42 ± 0.844**
CYP+CAR-25mM(each)	13.58 ± 0.907**	11.54 ± 0.846
CYP+CAR-50mM(each)	18.66 ± 0.941**	10.45 ± 0.877

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 6: Rat brain GGT specific activity (IU/mg protein/g x10^{-2}) in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	0.350 ± 0.025	0.327 ± 0.046
CYP -5mM	0.440 ± 0.015**	0.350 ± 0.015**
CYP-10mM	0.420 ± 0.014**	0.287 ± 0.010**
CYP-25mM	0.400 ± 0.014**	0.616 ± 0.008**
CYP-50mM	0.380 ± 0.008**	0.455 ± 0.030**
CAR -5mM	0.580 ± 0.008**	0.390 ± 0.020**
CAR-10mM	0.366 ± 0.009**	0.184 ± 0.041**
CAR-25mM	0.466 ± 0.018**	0.300 ± 0.012**
CAR-50mM	0.300 ± 0.008**	0.140 ± 0.017**
CYP+CAR -5mM(each)	0.466 ± 0.019**	0.200 ± 0.015**
CYP+CAR-10mM(each)	0.660 ± 0.030**	0.320 ± 0.009**
CYP+CAR-25mM(each)	0.520 ± 0.008**	0.240 ± 0.018**
CYP+CAR-50mM(each)	0.366 ± 0.009**	0.160 ± 0.010**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01

Table 7: Rat brain Amylase specific activity (IU/mg protein/g x10^{-2}) in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	0.073 ± 0.008	0.041± 0.004
CYP -5mM	0.11 ± 0.009**	0.083 ± 0.01**
CYP-10mM	0.116 ± 0.017**	0.062 ± 0.006**
CYP-25mM	0.077 ± 0.009**	0.056 ± 0.002**
CYP-50mM	0.13 ± 0.02**	0.033 ± 0.002**
CAR -5mM	0.16 ± 0.022**	0.07 ± 0.006**
CAR-10mM	0.666 ± 0.058**	0.3 ± 0.052**
CAR-25mM	0.5 ± 0.05**	0.2 ± 0.015**
CAR-50mM	0.2 ± 0.025**	0.08 ± 0.011**
CYP+CAR -5mM(each)	0.133 ± 0.017**	0.1 ± 0.011**
CYP+CAR-10mM(each)	0.08 ± 0.005**	0.04 ± 0.007**
CYP+CAR-25mM(each)	0.1 ± 0.012**	0.06 ± 0.009**
CYP+CAR-50mM(each)	0.124 ± 0.018**	0.1 ± 0.01**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 8: Rat brain Hexokinase specific activity (IU/mg protein/g x10^{-2}) in control and treated samples. CYP), CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	1.35 ± 0.115	1.26 ± 0.129
CYP -5mM	3.65 ± 0.183**	2.87 ± 0.193**
CYP-10mM	4.03 ± 0.127**	3.65 ± 0.174**
CYP-25mM	4.71 ± 0.201**	3.82 ± 0.110**
CYP-50mM	3.14 ± 0.170**	2.74 ± 0.151**
CAR -5mM	1.37 ± 0.123	1.12 ± 0.113**
CAR-10mM	2.14 ± 0.147**	1.93 ± 0.145**
CAR-25mM	2.42 ± 0.216**	2.16 ± 0.138**
CAR-50mM	1.93 ± 0.127**	1.76 ± 0.128**
CYP+CAR -5mM(each)	0.742 ± 0.071**	0.651 ± 0.093**
CYP+CAR-10mM(each)	1.67 ± 0.136**	1.35 ± 0.098**
CYP+CAR-25mM(each)	1.25 ± 0.112**	1.04 ± 0.102**
CYP+CAR-50mM(each)	1.03 ± 0.099**	0.721 ± 0.086**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01

Interestingly the HK enzyme specific activity in the rat tissues brain was noted to increase with different doses of CYP, while CAR and the combination exhibited only moderate changes with respect to control (Table 8). MDA as the marker employing the thiobarbituric acid reactive substances (TBARS) established control values to compare MDA level in the range 0.5-10 nmol/mg protein. Against this, CYP was noted to increase the MDA level in the brain at all time points, in a dose dependent manner (Table 9). The DNA content of the brain increased in 12hr, in response to CYP. Brain tissues did not respond to CAR. In fact, these tissues registered DNA content below control level. In a similar manner, CAR effect on tissue DNA content was almost null at the 24hr time point. However, the combination of CYP+CAR seemed to increase DNA content (10-112) of brain (Table 10).

The RNA content determined in brain tissue did not exceed a maximum value of 5mg/g of wet tissue, for the control. Significant increase in RNA content was noted in specific instances with specific doses and at different time points. CAR and combination were noted to increase brain tissue RNA content (Table 11).

Table 9: Rat brain Lipid peroxidation (nmol/mg protein) in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	7.62 ± 0.734	11.1 ± 0.427
CYP -5mM	6.34 ± 0.598*	15.9 ± 0.818**
CYP-10mM	7.15 ± 0.64	19.3 ± 1.17**
CYP-25mM	8.72 ± 0.988	24.2 ± 0.928**
CYP-50mM	6.81 ± 0.626	14.7 ± 0.818**
CAR -5mM	8.04 ± 0.841	15.7 ± 0.987**
CAR-10mM	19.1 ± 0.958**	13.2 ± 0.771
CAR-25mM	11.4 ± 0.573**	12.3 ± 0.649
CAR-50mM	8.41 ± 0.741	7.32 ± 0.683**
CYP+CAR -5mM(each)	13.7 ± 0.496**	11.5 ± 0.923
CYP+CAR-10mM(each)	11.4 ± 0.504**	18.3 ± 0.758**
CYP+CAR-25mM(each)	14.1 ± 0.733**	20.0 ± 1.1**
CYP+CAR-50mM(each)	13.7 ± 0.558**	10.8 ± 0.772

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 10: Rat brain DNA **(**mg/g) content in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	1.43 ± 0.109	0.898 ± 0.065
CYP -5mM	1.46 ± 0.098*	0.923 ± 0.075**
CYP-10mM	1.51 ± 0.105**	1.02 ± 0.094**
CYP-25mM	1.72 ± 0.073**	1.20 ± 0.105**
CYP-50mM	1.86 ± 0.114**	1.25 ± 0.103**
CAR -5mM	1.30 ± 0.103**	0.732 ± 0.087**
CAR-10mM	1.15 ± 0.066**	0.791 ± 0.067**
CAR-25mM	1.15 ± 0.081**	0.840 ± 0.051**
CAR-50mM	1.12 ± 0.078**	0.754 ± 0.063**
CYP+CAR -5mM(each)	1.25 ± 0.074**	1.155 ± 0.106**
CYP+CAR-10mM(each)	1.61 ± 0.101**	1.181 ± 0.066**
CYP+CAR-25mM(each)	2.46 ± 0.122**	1.90 ± 0.095**
CYP+CAR-50mM(each)	1.33 ± 0.075**	0.988 ± 0.076**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 11: Rat brain RNA **(**mg/g) content in control and treated samples. CYP), CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	1.52 ± 0.166	1.23 ± 0.155
CYP -5mM	1.74 ± 0.137**	1.37 ± 0.176**
CYP-10mM	1.65 ± 0.134**	1.26 ± 0.134
CYP-25mM	1.58 ± 0.160*	1.13 ± 0.156**
CYP-50mM	1.67 ± 0.172**	1.28 ± 0.144
CAR -5mM	1.62 ± 0.114**	1.43 ± 0.189**
CAR-10mM	1.55 ± 0.123	1.27 ± 0.124
CAR-25mM	1.52 ± 0.173	1.34 ± 0.102**
CAR-50mM	2.18 ± 0.114**	1.82 ± 0.193**
CYP+CAR -5mM(each)	1.51 ± 0.166	1.39 ± 0.180**
CYP+CAR-10mM(each)	1.58 ± 0.141*	1.46 ± 0.147**
CYP+CAR-25mM(each)	2.13 ± 0.205**	1.63 ± 0.142**
CYP+CAR-50mM(each)	1.51 ± 0.150	0.714 ± 0.093**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Variation in cholesterol content in the brain tissue was noted at 12 and 24hr. The change in cholesterol content was in response to CAR at the three time points. However, CYP effect was noted at 24hr when the cholesterol content (40-109mg %) increased 1.5-2.5 folds. The combination of CYP+CAR induced increase in cholesterol level (56-117mg %) when used at 25 and 50mM each for the brain tissue (Table 12).

The control triglyceride level in the brain tissue did not exceed the value of 6mg/g tissue weight. The brain triglyceride content at 12 and 24hr the triglyceride elevation was only due to the combination effect (Table 13). Urea level showed increase over the respective control at 12 and 24hr in brain tissue, such an elevation was only moderate (Table 14). Changes in uric acid level (33mg% and 36mg %) were noted for the brain tissue with 10mM CYP+CAR at 12hr and due to 50mM CYP at 24hr (Table 15).

Histophathological changes were observed in the brain tissue of male rats in all treatment groups (CYP, CAR and CYP+CAR (each 50mM doses) compared to control group. Histopathological examination of control rat brain tissue changes showed normal blood vessels, cytoplasm and nucleus in neurons. Figure 1 and 2 showed cerebral edema, dialated blood vessels and necrosis with hemorrhage due to 50mM CYP and CAR administration at all time points (12 and 24hr). Relative astrocytosis and gliosis were also seen to be formed at all times by 50mM CYP+CAR combination in the rat brain tissue.

Table 12: Rat brain Cholesterol **(**mg/g) content in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	2.0 ± 0.129	2.2 ± 0.093
CYP -5mM	2.1 ± 0.123**	1.3 ± 0.089**
CYP-10mM	2.0 ± 0.057	2.1 ± 0.159
CYP-25mM	2.1 ± 0.089**	3.1 ± 0.081**
CYP-50mM	2.3 ± 0.077**	4.6 ± 0.194**
CAR -5mM	2.3 ± 0.106**	2.8 ± 0.129**
CAR-10mM	3.5 ± 0.129**	3.7 ± 0.073**
CAR-25mM	3.0 ± 0.106**	3.3 ± 0.112**
CAR-50mM	2.0 ± 0.096	2.8 ± 0.106**
CYP+CAR -5mM(each)	1.8 ± 0.123**	3.1 ± 0.121**
CYP+CAR-10mM(each)	2.0 ± 0.150	3.2 ± 0.178**
CYP+CAR-25mM(each)	2.5 ± 0.123**	4.1 ± 0.096**
CYP+CAR-50mM(each)	3.8 ± 0.240**	4.0 ± 0.224**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 13: Rat brain Triglyceride (mg/g) content in control and treated samples. CYP, CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	3.0 ± 0.196	2.8 ± 0.150
CYP -5mM	2.5 ± 0.247**	2.4 ± 0.2**
CYP-10mM	2.7 ± 0.165**	2.5 ± 0.152**
CYP-25mM	3.2 ± 0.222**	2.5 ± 0.146**
CYP-50mM	2.6 ± 0.123**	2.55 ± 0.172**
CAR -5mM	2.3 ± 0.204**	2.2 ± 0.141**
CAR-10mM	1.9 ± 0.165**	2.1 ± 0.123**
CAR-25mM	3.0 ± 0.220	2.4 ± 0.191**
CAR-50mM	3.3 ± 0.217**	2.6 ± 0.203**
CYP+CAR -5mM(each)	5.0 ± 0.208**	3.5 ± 0.131**
CYP+CAR-10mM(each)	4.8 ± 0.326**	3.3 ± 0.141**
CYP+CAR-25mM(each)	2.7 ± 0.148**	2.5 ± 0.194**
CYP+CAR-50mM(each)	2.1 ± 0.115**	2.0 ± 0.129**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 14: Rat brain Urea (mg/g) content in control and treated samples. CYP), CAR and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	3.2 ± 0.269	3.8 ± 0.389
CYP -5mM	3.6 ± 0.453**	4.0 ± 0.293**
CYP-10mM	4.0 ± 0.317**	4.4 ± 0.36**
CYP-25mM	3.9 ± 0.484**	4.41 ± 0.306**
CYP-50mM	3.8 ± 0.309**	4.4 ± 0.286**
CAR -5mM	4.1 ± 0.194**	4.0 ± 0.27**
CAR-10mM	3.5 ± 0.232**	4.0 ± 0.123**
CAR-25mM	3.9 ± 0.276**	4.2 ± 0.212**
CAR-50mM	2.9 ± 0.267**	3.2 ± 0.157**
CYP+CAR -5mM(each)	4.0 ± 0.313**	3.1 ± 0.161**
CYP+CAR-10mM(each)	1.9 ± 0.182**	1.7 ± 0.194**
CYP+CAR-25mM(each)	3.3 ± 0.216	3.0 ± 0.226**
CYP+CAR-50mM(each)	4.1 ± 0.335**	3.6 ± 0.274**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

Table 15: Rat brain Uric acid (mg/g) content in control and treated samples. CYP= (α-Cypermethrin), CAR= (Carbendazim) and CYP+CAR (each) at 12 & 24 hr duration.

TREATMENT	12 hr	24 hr
CONTROL	0.3 ± 0.03	0.28 ± 0.04
CYP -5mM	0.32 ± 0.022**	0.24 ± 0.027**
CYP-10mM	0.31 ± 0.04**	0.25 ± 0.015**
CYP-25mM	0.31 ± 0.02**	0.21 ± 0.029**
CYP-50mM	0.32 ± 0.031**	0.38 ± 0.028**
CAR -5mM	0.05 ± 0.006**	0.17 ± 0.014**
CAR-10mM	0.1 ± 0.009**	0.19 ± 0.015**
CAR-25mM	0.105 ± 0.014**	0.21 ± 0.016**
CAR-50mM	0.2 ± 0.022**	0.25 ± 0.024**
CYP+CAR -5mM(each)	0.2 ± 0.017**	0.3 ± 0.031**
CYP+CAR-10mM(each)	0.4 ± 0.035**	0.29 ± 0.024**
CYP+CAR-25mM(each)	0.11 ± 0.012**	0.26 ± 0.017**
CYP+CAR-50mM(each)	0.1 ± 0.012**	0.3 ± 0.018**

Values are mean ± SEM from 6 rats in each group. Statistically significant at *p≤0.05 and **p≤0.01.

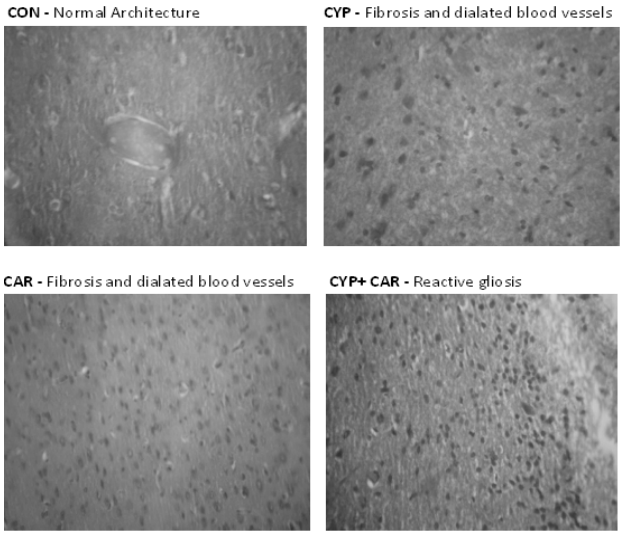

Fig 1: Section of rat brain following pesticide administration (12hr) of α-cypermethrin (50mM), Carbendazim (50mM) and α-cypermethrin + Cabendazim (each 50mM).

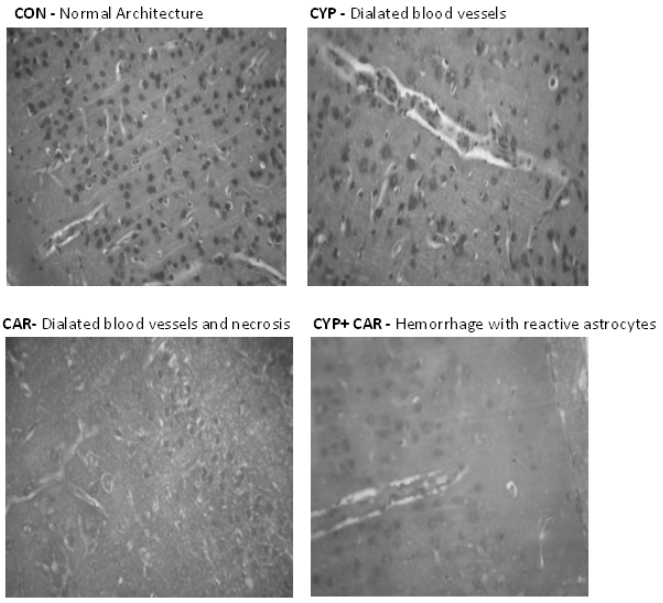

Fig 2: Section of rat brain following pesticide administration (24hr) of α-cypermethrin (50mM), Carbendazim (50mM) and α-cypermethrin + Cabendazim (each 50mM).

DISCUSSION

CYP is known to undergo metabolism through the cytochrome P450 microsomal system resulting in oxidative stress. Consequently CYP led to depletion of glycogen level, CAT and superoxide dismutase activity (Manna et al., 2004). The acute LD_{50} value for α-cypermethrin in DMSO is reported to be 145mg/kg bwt. Increased total protein in the developing muscle in newly hatched chick (Gallus domesticus) was reported as due to the use of sublethal dose of permethrin (Khurshid Anwar, 2003) in the studies. It was considered that the increase in protein content could be due to an increase in the rate of translation of protein. There is also claim that the cypermethrin had no significant effect on the total protein content of a tissue (Altug et al., 2006). There has been claim that only amylase and ALP enzyme activities were affected by CYP, whereas other enzyme activities remained unaltered (Khurshid Anwar, 2003).

In our studies, a increase in ALT activity was observed that signified increased transamination reaction within the cells of the brain tissues, whereas decreased ALT activity signified a reduction in the cellular transamination process. Since brain is enriched in amino compounds it is possible that the enhanced transamination activity within the brain tissue served to neutralize the biochemical and biological responses that resulted due to the amino compounds in response to the insecticide and fungicide effects. On the other hand, pyruvate and α-keto glutarate are the most significant keto substrates that accepted amino groups from transamination reaction. The resulting products, alanine and glutamate amino acids shall either accumulate in the brain tissue are be utilized for the formation of serine and glutamine also amino acids, by further transamination. Both amino compounds are constituents of protein and hence may be utilized in the protein synthetic process in the brain cells. The experimental observation

supporting increase in protein content in most tissues following treatment of the animal with these pesticides was suggestive of such an occurrence.

The CYP induced increase in specific activity of brain AST is indicative of an augmented process of oxalicacetate formation from aspartate. CAR and the combination reduced the AST specific activity and thereby reduced oxaloacetate formation within brain cells. While the former was suggestive of an induced process for the generation of pyruvate from oxaloacetate, the reduction in oxaloacetate formation from aspartate due to CAR was indicative of aspartate utilization by brain cells for either protein synthesis or as a brain function modulator, since aspartate can be utilized for the formation of N-Methyl D-aspartate (NMDA), a neuro signaling molecule that utilized the NMDA receptor for function. Increase in ALT and AST and BUN (Blood Urea Nitrogen) level in rats treated with 520, 560, 600mg/kg benomyl for seven days, were reported as equivalent to CAR initiated increase in these enzyme activities (Selmanoglu et al., 2001).

It is well known the ALP enzyme activity dephosphorylated, phosphorylated organic substrates in the animal tissues. ALP is a hydrolase and a transphosphorylase in function associated with cell membranes (Onikienko et al., 1963). Increase in the specific activity of this enzyme therefore suggested the existence of a greater depohsphorylation potential within the animal cell. In the case of rat brain ALP, the use α-cypermethrin was not found to be effective in increasing in the brain ALP specific activity.

In a similar manner, increase in the specific activity of ACP in the rat tissues, only confirmed the increase in the dephosphorylation potential within the rat cells due to the pesticides. The lack of phosphorylated compounds within the animal cells probably led to reduction in stored phosphate, the phosphate depletion affecting the calcium:phosphorous ratio within each cell, eventually resulting in membrane damage and lack of energy compounds. Decrease in ALP activity was taken as an index for parenchymal damage (Meister et al., 1973).

GGT catalyzed the transfer of a glutamyl moiety between peptide donors and amino acid / peptide acceptors (Meister et al., 1973). GGT was also involved in the transfer of amino acid across the cell membrane, Further, GGT had a role in glutathione metabolism transferring the glutamyl moiety to various acceptor molecule including water, L-amino acids and peptides. Such a process results in the retention of the cysteinyl glycine that was considered to preserve intracellular homeostasis during oxidative stress. GGT is an enzyme activity that is also implicated as a lymphoid cell surface marker and in blastogenesis and differentiation. It is also implicated in the synthesis of glucotrine D'4.

Amylase acted on a complex carbohydrate like starch to yield individual glucose units for energy metabolism within the plant cells. Rat brain amylase specific activity were extraordinarily enhanced over their control by CYP and CAR indicating that carbohydrate stores of these two tissues will be under the hydrolytic influence of amylase.

Most surprisingly the HK enzyme specific activity in the various rat tissues increased as a consequence of different doses of CYP, though moderate changes were noted with CAT and the combination. Increase in HK activity was indicative of greater glucose phosphorylation in the cells of the various rat tissues, and therefore greater glucose utilization for energy yield.

The influence of these pesticides to generate peroxidative damage in various tissues was found significant. Generations of free radicals and reactive oxygen species (ROS) as a result of increased cerebral lipid peroxidation have been observed in the rat brain by cypermethrin intoxication (Giray et al., 2001).

It was surprising to note that the DNA content increased in the various tissues of rat by 12hr as a result of these pesticide administration. Giri et al (Giri et al., 2003) had observed that cypermethrin induced sister chromatid exchanges in bone marrow cells in a murine test system in vivo. Increase in DNA content at 200ppm of cypermethrin might have caused some mutations in young chick embryo (31). A comparatively higher amount of CAR was required to effect change in tissue DNA content. However interestingly, brain remained unaffected by CAR. The CYP+CAR combination increased DNA content of brain. The results seem to indicate that most cells in each tissue is driven into a proliferative phase with a resultant increase in the DNA content in the S-phase. The histology of various tissues seems to indicate this. The biological consequences of short term increase in DNA content increase remains unexplored.

Similar to DNA the total RNA content in the various tissues was also noted to increase following pesticide administration. No discriminative study was carried out to identify the nature and type of RNA that was increased by the pesticide effect. Since total protein content of various tissues were noted to increase due to the pesticide effect, the increase in RNA content might have occurred, to synthesize proteins under cypermethrin induced stress condition (Khurshid, 2003). It was suggestive of the fact that the increase in RNA content will more likely involve rRNA and mRNA species. It is also suggestive of the fact that the transcriptional activation of certain genes were occurring while excess RNA synthesis could reflect gradual cellular degeneration due to accumulation of RNA to eventual toxic level indicative of cells stress probably similar to the reports on the use of tobacco leading to cells stress and resulting in the excessive production of SINE RNA (Kumura et al., 2001).

Differences in cholesterol content between rat tissue preparations were noted. Although CAR effected change in brain tissue cholesterol content at all time points studied, the α-CYP effect was noted only at 24hr for a two fold increase in brain tissue cholesterol content. The pesticide combination also increased the brain tissue cholesterol content. Combination of CYP+CAR returned the serum cholesterol content to its normal level. The triglyceride levels also registered an increase in the brain due to the cypermethrin effect.

Temporal changes in urea content were noted in a tissue specific manner following the use of these pesticides in the rat. Yousef et al, (2003) had reported that there was increase in blood urea due to CYP. Increase in urea content is indicative of an increase in tissue ammonia generation. It was cited earlier that these pesticides enhanced the tissue transamination processes mediated by ALT & AST enzyme activities. It is therefore possible that elevation of urea content is the consequence of metabolic changes in rat cells brought about by these pesticides. Further, it was also reported earlier in the discussion that the total protein content of the tissues increased due to this pesticide. Therefore the increase in protein synthesis and cellular increase in transamination activity contributed to tissue over load of ammonia that required elimination through urea formation as part of the tissue function.

Increase in uric acid content in the tissue is an indication of an increase in purine metabolism that remains targeted by these pesticides. Khurshid (2003) had reported elevation in uric acid content due to sub lethal dose of permethrin and CYP in the chick. Although elevation of uric acid was noted within a time duration of 24hr.

Tissue histology reflected the true and early responses in the rat due to each of the pesticides employed for the study even though the pesticides were used at low dose. Cerebral edema, hemorrhage, loss of lobar architecture, coagulation necrosis, congestion of blood vessels, fibrosis, necrosis, sclerosis of seminiferous tubules were some of the significant histologic changes observed in the normal tissues that responded to either CYP or CAR treatment, clearly indicating that CYP and CAR are anatomic and biological toxin in the animal, independent of dose levels. Pesticide consumption through agricultural use of these compounds therefore has severe repereussions, as much argument in favor of low dose tolerance of these compounds shall not withstand experimental scrutiny as noted in this work. It is also reported that oral exposure to CYP introduced significant histopathological alterations in the brain of rats (Manna et al., 2004). However, pyrethroid insecticides are claimed to exhibit low toxicity for human and animals (Lakkawar et al., 2004). This study presents experimental data questioning the validity of such a claim.

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