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# Effect of concurrent application of heat, swim stress and repeated dermal application of chlorpyrifos on the hippocampal neurons in mice

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**Abstract**

*Dermal absorption of chlorpyrifos (CPF), an organophosphate (OP) pesticide, is important because of its popular use. Stress has been reported to exacerbate neurotoxic effects of certain OP pesticides; however, quantitative studies to corroborate this are not reported. This study correlates the changes in acetylcholinesterase (AChE) levels and neuronal counts in areas of the hippocampus to consecutive exposure of stress, heat and CPF. Male mice (60 days) were segregated into six groups: one control, one stress control, and four treated groups (n=10). CPF was applied in doses of 1/2 and 1/5 of dermal $LD_{50}$ (E1 and E2) over the tail of mice under occlusive bandages for 3 weeks. Stress control [(s) C] mice were subjected to swim stress at 38°C (6 mins/day, 3 weeks). (s) E1 and (s) E2 were subjected to swim stress before CPF application. Blood and brain AChE levels were estimated using a spectrofluorometric method (Amplex Red). Pyramidal neurons of the cornu ammonis of the hippocampus under Nissl stain from histological sections were counted per unit area of section and analyzed statistically using one way ANOVA. Swim stress at 38°C aggravated reduction of serum AChE by dermal exposure to CPF by 19.7%. Neurons of CA3 and CA1 regions of the hippocampus showed significant reduction in neuronal counts in (s) E1 and (s) E2 groups compared to E1 and E2 groups. Whereas application of CPF 1/2 dermal $LD_{50}$ (E1) showed significant reduction of neuronal counts only in the CA3 area.*

***Key words:*** *Dermal toxicity, chlorpyrifos, swim stress, heat, cholinesterase inhibition, hippocampus, histomorphometry.*

## Introduction

Chlorpyrifos (CPF), an organophosphate pesticide, is extensively used in agriculture and is also applied to pastures and woodlands. An estimated 11 million pounds are used in houses and commercial applications whereas another 13 million pounds are used as pesticide in agriculture annually [25]. CPF is also used in insect sprays, to control household pests such as mosquitoes and cockroaches. Exposure to chlorpyrifos (CPF) occurs usually via oral, inhalation and dermal routes. The group of people most susceptible to direct contact with CPF comprises workers in the agricultural or pesticide manufacturing sector.

Dermal exposure to CPF tended to be highest among mixers and loaders, where there is risk of

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hand exposure to concentrated solutions. Workers applying CPF by ground boom received higher dermal exposures relative to aerial, handheld sprayers or planters [8]. A study on human volunteers reported that following single dermal application of ethanol diluted CPF for 4 hours, 4.3% of the applied dose was absorbed and chlorpyrifos was retained by the skin with mean elimination half-life of 41 hrs [14]. Importantly, organophosphates such as CPF are known to have an inhibitory effect on the enzyme acetylcholinesterase (AChE), with negative neurophysiological and neurobehavioural effects [5].

Hence an issue of concern is that the toxic effect of environmental neurotoxicants like CPF may be incremented by stress factors such as high temperature and exercise [9]. The potential for heat stress is influenced by factors such as ambient temperature, humidity and type of personal protective equipment (PPE) [26].

Therefore health concerns for those most susceptible to CPF are reasonable considering the conditions of application, i.e. under heat stress. For example, the neurobehavioral performance of Hispanic immigrant farm workers was found to be lower than that observed in a non-agricultural Hispanic immigrant population, and a positive correlation was found between urinary organophosphate metabolite levels and poor performance in some neurobehavioral tests [17]. It has also been shown based on human data that pyridostigmine (an acetylcholinesterase inhibitor) crosses the blood brain barrier more readily in stressed (Gulf war experience) individuals compared to non-stressed individuals [6]. Animal studies have documented a possible role of stress in enhancing central effects of anti-cholinesterase chemicals [3,13].

The hippocampus plays a vital role not only in learning, memory and visceral functions, but also in regulating negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis, which is the endocrine component of the stress response [11,27]. Stress can have an effect on the hippocampus, for example chronic swim stress can result in shortening of the total length of the apical dendrite of the hippocampal CA3 pyramidal neuron [28]. The hippocampus is therefore a suitable site to examine the effects of stress and CPF toxicity.

Very few reports have been found in the literature regarding quantitative estimation of neuronal damage in the areas of the central nervous system, affected by repeated sub-toxic dermal exposure to anti-cholinesterase pesticides along with stress and enhanced temperature in adult animals. This study evaluates the effect of concurrent swim stress at 38°C and repeated dermal application of CPF in sub-toxic doses in adult Swiss albino mice on acetylcholinesterase levels of blood and brain and neuronal density in the cornu ammonis pyramidal neurons of the hippocampus. Findings from this study suggest that dermal exposure to sub-toxic doses of CPF with stress has negative neurophysiological effects and is a health concern.

## Materials and Methods

### Chlorpyrifos (CPF)

A commercial preparation of CPF (O, O-diethyl-3, 5, 6-trichloro-2-pyridyl phosphorothioate), Zespest, produced by Zeenex Agro Science Sdn Bhd, Kuala Lumpur, Malaysia, was used in this study. The preparation contained 38.7% W/W of CPF in the organic solvent xylene. The commercial preparation was further diluted with xylene to prepare solutions containing 1/2 $LD_{50}$ (101 mg/kg body weight CPF in 1 ml) and 1/5 $LD_{50}$ (40.4 mg/kg body weight CPF in 1 ml) doses [7].

### Animals and groupings

Male Swiss albino mice (species: Balb/c), 60 days old (20-25 g) were used in this study. All animals were in good condition without any macroscopic changes in the skin and tail. The mice were fed with standard pellet feed and water ad libitum. They were housed in plastic cages (five in a cage) and were exposed to a natural 12-hour light-dark cycle. Animal experiments adhered to the principles stated in the guide-book of laboratory animal care and user committee of the International Medical University and in accordance with the declaration of Helsinki. The animals were divided into six groups (n=10). Xylene was applied over the tails of the control group (C) of mice whereas CPF in the dose regimen of 1/2 $LD_{50}$ (E1 group) and 1/5 $LD_{50}$ (E2 group) was applied over the tails of experimental groups of mice. Swim stress followed by application of xylene over the tails was done in control group s(C), whereas swim stress followed by application of CPF in the dose regimen of 1/2 $LD_{50}$ [s (E1) group] and 1/5 $LD_{50}$ [s (E2) group] was done over the tails of experimental mice.

## Dermal application of chlorpyrifos

The CPF solution in xylene was applied over the tail skin of the mice. The applications were done for 3 weeks except Sundays (total application of 18 days). An absorptive fabric containing 1 ml of either xylene or CPF at specific concentrations was wrapped around the tail. This was followed by wrapping of layers of plaster and aluminium foil as barriers to prevent the solution from evaporating. Time of exposure was 6 hours daily. After removal of the fabrics, the remaining solution over the tail was swabbed off with wet gauze.

## Swim stress at 38°C and application of chlorpyrifos

A Plexiglas container filled with water up to the 30 cm water level was used. The water was heated up to 38°C and the mice were placed in the water for a swimming session of six minutes. After the forced-swimming session, the mice were dried and allowed to rest for approximately 15 minutes. They were then subjected to dermal CPF or xylene application as previously described.

Body weights of all the animals were measured twice a week. After 3 weeks, the animals were anaesthetized with intraperitoneal administration of pentobarbitone. Blood and brain samples were collected for acetylcholinesterase assay and histomorphometric studies.

## Estimation of serum acetylcholinesterase (AChE)

Serum AChE concentration was estimated by using Amplex Red acetylcholinesterase assay kit from Molecular Probes Inc. USA (Invitrogen Detection Technologies) as recommended by the manufacturer. This kit provides an ultra-sensitive method for measuring AChE concentration in serum samples. After preparing the stock solutions (as per manufacturer's protocol), the AChE estimation was conducted using serum samples pooled within the various experimental and control groups of mice. A working solution of 400 µM Amplex Red reagent containing 2 U/ml HRP, 0.2 U/ml choline oxidase and 100 µM ACh was prepared from the stock solutions. The reaction began when 100 µL of the working solution was added to each well containing 100 µL of the serum samples and controls. Serum samples and controls were tested in triplicate. The fluorescence emitted from the samples and the controls was measured in the Tecan microplate reader using excitation in the range of 560 nm and emission detection at 590 nm. For each point, background fluorescence was corrected by subtracting the values derived from the negative control.

The $Log_{10}$ of the mean fluorescence readings were then plotted against the $Log_{10}$ of the AChE concentration of the positive controls. A linear regression was obtained, indicating that the AChE activity in a sample increases exponentially with increasing concentration of a sample. The $Log_{10}$ of AChE concentration of the experimental samples thus could be calculated. Following this, the AChE concentration of the undiluted experimental samples can be estimated in pooled serum samples of C, E1, E2, s(C), s (E1) and s (E2) groups.

## Estimation of brain acetylcholinesterase (AChE)

The portion of the brain containing cerebral hemisphere along with cerebellum was removed from half of the animals in each group. After collection, the brain samples were weighed and stored in PBS solution at -80°C temperature until further use. The brain samples were homogenized in PBS solution at 4°C and were used for AChE estimation. A procedure for determining the protein concentration of each brain homogenate sample was carried out using Bio-Rad Lab's Quickstart™ Bradford dye reagent, with bovine serum albumin (Sigma-Aldrich) as a standard. This procedure allowed estimation of AChE concentration, in terms of U/mg protein in sample. This calculation was crucial as the amount of brain samples used to make the homogenate may vary between each animal.

Estimation of AChE concentration in brain homogenate samples was performed using the same materials, equipments and protocol used in estimation of serum AChE. However, the dilutions of the brain homogenate samples and positive controls were performed using PBS solution instead of reaction buffer. The negative control was also the PBS solution.

## Histological and histomorphometric studies

Perfusion of brains was carried out in half of the animals by using 10% formal saline and areas of fo-

rebrain between optic chiasma and infundibulum showing hippocampus and iso-cortex were further dissected. 8 micron thick coronal serial sections of dissected area processed with paraffin were stained with Nissl stain (0.2% thionin in acetate buffer). Qualitative observations of stratum pyramidalis of CA 1, CA 2 & CA 3 areas of hippocampus were done. Every 10$^{th}$ section (5 slides in each animal) containing hippocampal area was chosen from each animal. Using brightfield compound Nikon microscope YS100 (attached with Nikon camera), the slides were examined and photographed under the 400X objective. For each slide, two areas of CA1, one area of CA2, and two areas of CA3 were randomly selected. Using Image-Pro Express software, count of neurons with prominent nucleolus within a measured rectangular area was performed in the selected regions. Random measurements of neuronal cell diameter were also taken for each region. The absolute neuronal density (P) per unit area of section was estimated using the Abercrombie formula P = A. M / L+M [2]; M= section thickness in microns (8 microns); L = mean nuclear diameter of respective area; A= crude neuronal count per sq. mm of section.

### Statistical analysis

The absolute neuronal count (per mm$^2$) was subjected to statistical analysis using SPSS 11.5. One way ANOVA was performed on the counts of each area (CA1, CA2 and CA3) to determine if there were any statistically significant differences in absolute neuronal density between the treatment groups in that area.

## Results

### Body weight

The control group (C) and control swim stress group [s(C)] showed uniform weight gain at the end of the experiment (16.7% and 17.7% respectively). Mice receiving dermal application of 1/2 $LD_{50}$ CPF [E1] and swim stress + 1/2 $LD_{50}$ CPF [s (E1)] showed similar reduction in mean body weight at the end of the experiment (17.5% and 16.4% respectively). In contrast, the mice exposed to dermal application of 1/5 $LD_{50}$ CPF [E2] showed slight increase in mean body weight (3%), but the group with swim stress + 1/5 $LD_{50}$ CPF [s(E2)] showed 14.3% reduction in mean body weight.

### Changes in serum acetylcholinesterase (AChE)

Mean fluorescence readings of the serum samples after 3 weeks of exposure to CPF showed (Fig. 1) that AChE level was lower in group s(C) compared to group C by 17.8%. This indicates that swim stress at 38°C reduces serum AChE levels by 17%. Similarly, the mean serum AChE levels for groups E1, E2, s(E1) and s(E2) were significantly lower than both control groups C and s(C). Group s(E1) showed the lowest mean AChE levels at 0.12 U/ml followed by group E1 at 0.3 U/ml, group s(E2) at 0.47 U/ml and group E2 at 2.59 U/ml. The results show that dermal exposure to CPF can reduce mean AChE levels by as much as 76% at 1/5 $LD_{50}$, but with swim stress and CPF these levels were further reduced, to 95.7%. This indicates that swim stress at 38°C aggravated reduction of serum AChE by dermal exposure of CPF by 19.7%.

### Changes in brain acetylcholinesterase (AChE)

Mean brain AChE concentrations of swim stress + CPF exposed groups, group s (E1) and s (E2), were reduced uniformly (50%) compared to CPF exposed groups, E1and E2 (Fig. 2). Application of swim stress only reduced mean brain AChE in group s(C) by 28% compared to the control. Group E1 receiving 1/2 $LD_{50}$ of CPF showed 56% inhibition compared to the control.

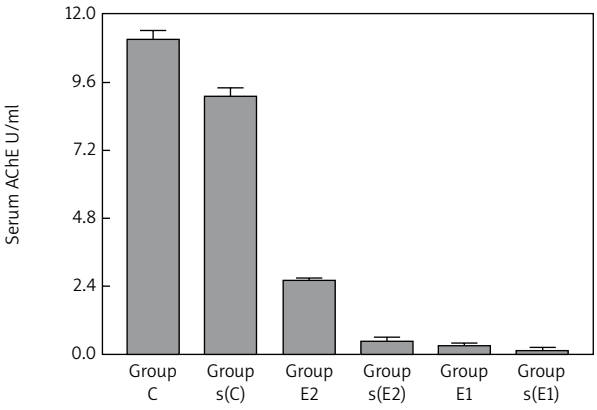


**Fig. 1.** Bar charts showing mean AChE concentration in pooled serum samples (from 10 mice) of each group at the end of 3 weeks, derived from 40X dilution of the samples

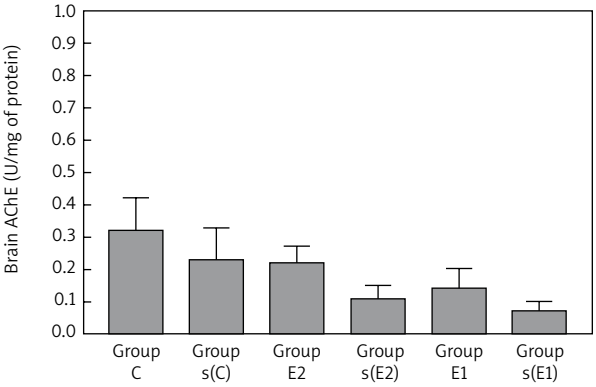


**Fig. 2.** Bar charts showing mean AChE concentration per mg of protein in brain samples of each group (n=5) derived from 40X dilution of the samples

## Changes in histological and histomorphometric studies

Under qualitative observations, the CA3, CA2 and CA1 hippocampal pyramidal neurons in the s(C) group receiving only swim stress showed evidence of neuronal damage in the form of pyknosis of nuclei and vacuolation of neuropil around the damaged neurons (Fig. 3B). The pyknosis of nuclei was more commonly seen in CA3 and CA1 regions compared to the CA2 region. The hippocampal pyramidal neurons in the E2 group receiving dermal application of 1/5 $LD_{50}$ of CPF showed dissolution of Nissl granules and discontinuity of nuclear membrane in a few neurons but no pyknosis (Fig. 3C). In the s(E2) group receiving swim stress at 38°C followed by application of 1/5 LD50 of CPF, a few pyknosed neurons were observed in CA 3, CA2 and CA1 regions (Fig. 3D). The E1 group receiving dermal application of 1/2 $LD_{50}$ of CPF showed comparatively more pyknosed neurons in CA3, CA2 and CA1 regions but the s(E1) group receiving swim stress + dermal application of 1/2 LD50 of CPF showed presence of many pyknosed neurons surrounded by vacuolated neuropil in all 3 cornu ammonis areas (Figs. 3E,F). Under quantitative histomorphometric study, the density of pyramidal neurons as evidenced by the absolute neuronal count per sq. mm area of section in the control group of mice was found to be more in the CA1 region (6285.9/sq. mm) compared to that in CA3 (5726.9/sq. mm) and CA2 regions (5603.9/sq. mm). The mean absolute neuronal count was reduced in both mice groups E1 and E2 compared to the mean count of the control group in CA3, CA2 and CA1 regions. After performing one way ANOVA, no statistically significant difference was found between the C, E1 and E2 groups in terms of CA1 and CA2 area neuronal counts. A statistically significant difference in mean absolute neuronal density between the three groups (control, E1 and E2) was found only in the CA3 region of the hippocampus (Table I). In a post-hoc statistical test (Bonferroni), the mean absolute neuronal count per sq. mm area in the E1 group of mice receiving dermal application of 1/2 $LD_{50}$ of CPF was reduced significantly ($p<0.05$) compared to the count in the control group. Application of swim stress at 38°C reduced neuronal density by 16%, 10% and 22% in CA3, CA2 and CA1 areas of the hippocampus compared to the control group C. Post-hoc Bonferroni analysis showed a statistically significant ($p<0.001$) reduction in neuronal density in

**Table I.** Mean (±SD) neuronal count per sq. mm of sections in regions of hippocampus in different treatment groups (n=5)

| Examined groups | CA3 region | CA2 region | CA1 region |
|---|---|---|---|
| Control | 5726.9 ± 1282.5 | 5603.9 ± 1352.3 | 6285.9 ± 1345.6 |
| Control Stress | 4808.5 ± 535.8**# | 5015.3 ± 458.1 | 4883.61 ± 640.8** |
| E2 | 5388.2 ± 845.8 | 5472.7 ± 809.8 | 5742.8 ± 1296.3 |
| s(E2) | ^4395.6 ± 812.9## | ^4522 ± 929.4 | ^4875.8 ± 841.9## |
| E1 | 5114.6 ± 1281.2* | 5098.2 ± 1285.1 | 6040.5 ± 1177.6 |
| s(E1) | ^3741.7 ± 829.3$ | ^4469.2 ± 950.4 | ^4226.2 ± 1027.8$ |

*** Mean difference from control group significant (≤0.001) post-hoc Bonferroni test after one way ANOVA. # Mean difference from E2 (1/5 LD50 CPF) group significant (≤0.05) post-hoc Bonferroni test after one way ANOVA. ## Mean difference from E2 (1/5 LD50 CPF) group significant (≤0.05) post-hoc Bonferroni test after one way ANOVA. ^ Mean difference from control group significant (≤0.001) post-hoc Bonferroni test after one way ANOVA. * Mean difference from control group significant (≤0.05) post-hoc Bonferroni test after one way ANOVA. $ Mean difference from E1 (1/2 LD50 CPF) group significant (≤0.05) post-hoc Bonferroni test after one way ANOVA.*

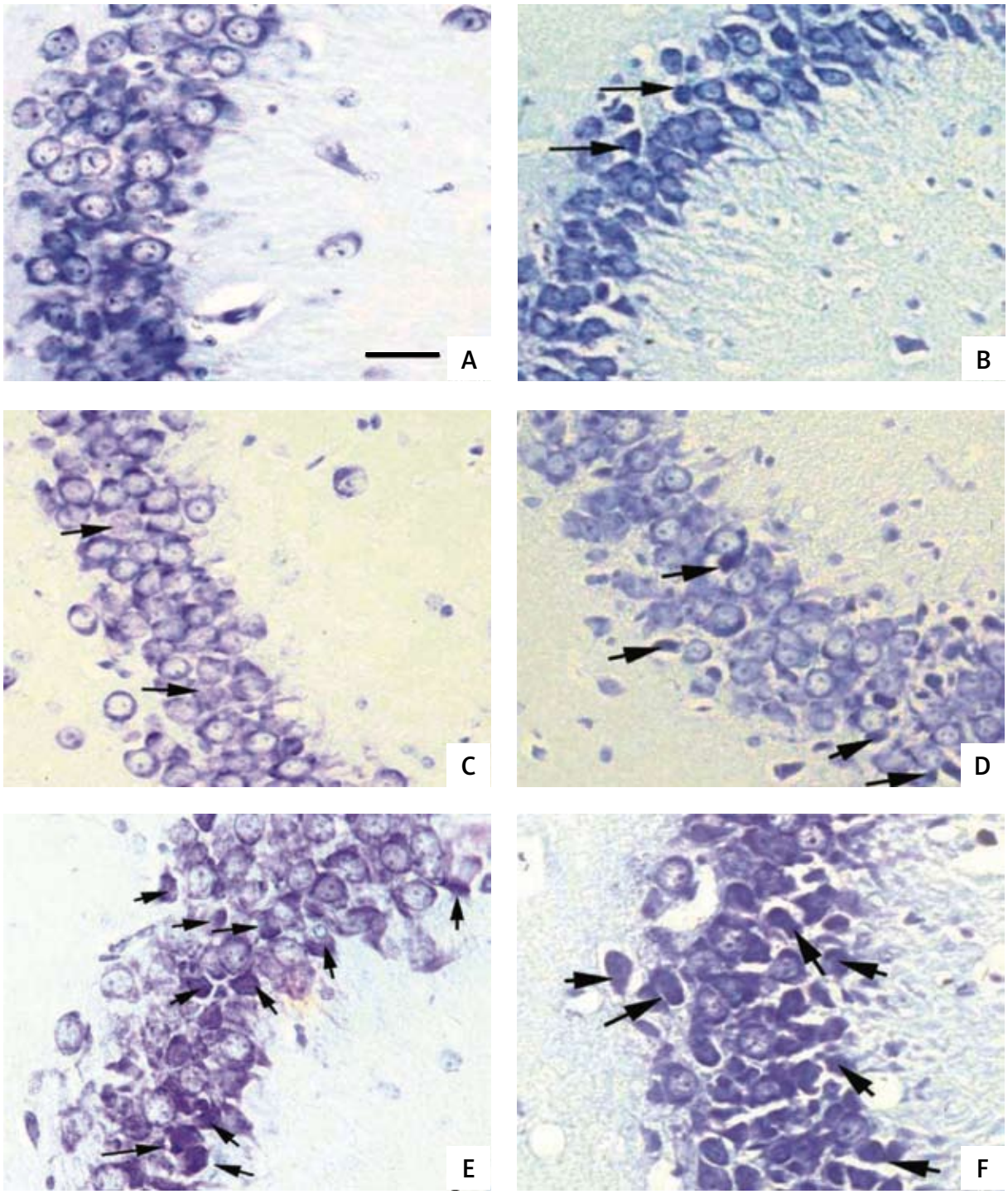


**Fig. 3.** Microphotograph of hippocampal CA3 neurons in different groups of mice. A. Group C; perinuclear Nissl granules; B. Group s(C); pyknosis of few nuclei with vacuolation of neuropil shown by arrows, density thinner; C. Group E2; dissolution of Nissl granules, discontinuity of nuclear membrane shown by arrows; D. Group s(E2); pyknosis of nuclei shown by arrows; E. Group E1; numerous pyknosed neurons shown by arrows. F. Group s(E1); many pyknosed neurons surrounded by vacuolated neuropil shown by arrows (thionin stain, ×400, Bar 8 µ)

CA1 and CA3 areas only (Table I) in this group compared to the control group.

However, the E2 group receiving dermal application of 1/5 $LD_{50}$ of CPF did not show a significant reduction of neuronal counts at CA3, CA2 and CA1 in hippocampal regions (6%, 2.3% and 8.6% reductions). Application of swim stress at 38°C with dermal application of 1/5 $LD_{50}$ of CPF increased the reduction of neuronal density in s(E2) groups by 23%, 19% and 22.4% in CA3, CA2 and CA1 regions compared to group C. The percentage reduction in neuronal density in swim stress + CPF application groups compared to CPF application only groups was higher in mice groups s(E1) receiving 1/2 $LD_{50}$ of CPF (30% in CA1, 12% in CA2 and 26.7% in CA3 areas). One way ANOVA analysis followed by post-hoc Bonferroni test found a statistically significant difference ($p<0.05$) between the swim stress + CPF application groups and CPF application only groups in terms of CA1 and CA3 hippocampal area neuronal count (Table I). A dose-dependent difference in neuronal density reduction was found only in the CA3 area between swim stress + 1/5 $LD_{50}$ of CPF and swim stress + 1/2 $LD_{50}$ of CPF groups. Post-hoc Bonferroni test showed a statistically significant reduction in swim stress + 1/2 $LD_{50}$ of CPF groups.

## Discussion

Observations from this study suggest that forced swim stress and dermal application of CPF caused more irritability and excessive movements in the mice compared to forced swim stress only. After swim stress only, most mice showed tiredness and minimal locomotive activity. Similarly, the mean body weight of mice exposed to both swim stress and sub-toxic dermal exposure of CPF was lower compared to the stress only or CPF only groups of mice. Biochemically the effect of swim stress and sub-toxic dermal exposure of CPF was observed as levels of both serum and brain AChE were reduced in s(E1) and s(E2) compared to E1 and E2.

These observations support previous studies which investigated the effects of stress on AChE activity. For example, signs of cholinergic toxicity following pyridostigmine (PYR) were significantly increased in rats forced to run on treadmills prior to dosing. AChE levels in whole blood and diaphragm were significantly reduced in stressed rats than non-stressed rats [20]. No significant effects of treadmill running on PYR-induced AChE inhibition in brain regions were noted. The observation unique to this study is that the inhibitory effect of sub-toxic doses of dermal CPF exposure on AChE can be further exacerbated by stress, putting into perspective the possible risks posed by CPF when exposure is under stress. Cholinergic neurotransmission is intimately associated with mammalian stress responses. Inhibition of AChE by organophosphates, as well as by stress, elevates the levels of acetylcholine in the short term, and both conditions induce some common long-lasting behavioural symptoms [22]. Our study found that only application of stress produced a significant ($p<0.05$) reduction in neuronal counts in the CA3 hippocampal region when compared to dermal application of 1/5 $LD_{50}$ of CPF.

As stress involves a complex series of mechanisms, there are several possibilities of how stress can exacerbate CPF-induced neurotoxicity. Increase in blood brain barrier permeability caused by stress has been observed and this can in turn cause increased penetration of CPF into the brain [1,6]. The study by Sharma et al., 1992, shows that exposure to a temperature of 38°C for four hours in rodents, enhancing the core temperature of the body by 3.6°C, increases blood-brain permeability to tracers [21]. Recent studies on the effect of CPF and stress by Pung et al., 2006 and Hancock et al., 2007, however, state that brain AChE inhibition is absent in rodents exposed to prolonged swim stress (up to 6 weeks) and CPF exposure [10,16]. Furthermore, both these studies had subcutaneous application of CPF compared to dermal exposure as in this study. One major difference in our study compared to those recently reported is that dermal exposure to CPF although sub-toxic was repetitive, instead of a single dose as performed by the two reported studies. Repetitive daily exposure at sub-toxic doses was designed in this study to mimic situations in agricultural and pesticide industries where workers are probably exposed to CPF more frequently.

Qualitative examination of the photomicrographs taken from the hippocampus also revealed exacerbating effects of stress on CPF-induced neurotoxicity. Neuronal changes manifested by discontinuation of nuclear membrane, pyknosis of neurocytes, vacuolation of neuropil and loss of Nissl granules were noted in both s(E1) and s(E2) groups. The changes seen here are more apparent compared to E1 and E2 groups respectively. Latuszynska et al., 2001, admini-

stered a mixture of CPF (27.8 mg/cm$^2$) and cypermethrin (2.7 mg/cm$^2$) dermally over the tails of rats for four weeks and observed pyknosis of neuronal cells in the stratum granulosum of the dentate area and CA1 region of the hippocampus [12].

Quantitatively, this study shows that statistically significant differences exist between mean absolute neuronal counts of Control, s(E1) and s(E2) groups in all three areas of the hippocampus (CA1, CA2, CA3) (Table I). Roy et al., 2005, found a significant reduction in the number of neurons in CA1 and CA3 regions of the hippocampus of juvenile rats (postnatal days 15 and 20) following subcutaneous administration of 5 mg/kg of chlorpyrifos on postnatal days 11 to 14 [18].

Afferent input to the hippocampus from entorhinal cortex projects to the dentate gyrus, from where it passes to CA 3 neurons. Dendrites of CA 3 neurons project to CA 1 neurons. The efferent pathway passes from CA 1, then the subiculum, alveus and fimbria, and ultimately forms the fornix. Hence the CA3 region of the cornu ammonis occupies a central position and plays an important role in the process of consolidation of short-term memory. The reported deficits in several cognitive tests of memory, abstraction, word-finding and concentration [19,23] following occupational exposure to CPF may be extrapolated to be caused by reduction in neuronal count in CA 3 pyramidal neurons of the hippocampus, as evidenced by this study, which found a statistically significant reduction in neuronal count in the CA 3 region of adult mice following repeated dermal exposure to chlorpyrifos. Toxicity in the mammalian system by AChE inhibitors such as chlorpyrifos involves alteration in antioxidant activity and the scavenging system, leading to free radical-mediated injury to neurons. During the course of inhibition of AChE, a high rate of ATP consumption, coupled with the inhibition of oxidative phosphorylation, compromises the cell's ability to maintain its energy levels [15]. Stress might have increased the oxidative injury in hippocampal neurons produced by the CPF. 12-week treadmill exercise in mice showed hyperchromatic and shrunken nerve cells with nuclear pyknosis in the hippocampus. Many destroyed mitochondria were also observed in dark neurons by electron micrograph [24]. One study found parenchymal vascular damage accompanied by haemorrhages and loss of some nerve cells in the brain stem after immobilization stress in rats [4]. The present study found a significant reduction in neuronal density per sq. mm of section in CA1 and CA3 regions of the hippocampus in mice treated concurrently with swim stress and dermal application of CPF compared to only dermal application of CPF.

## Conclusions

This study proves that stress at elevated temperature can exacerbate the neurotoxicity in the hippocampal region produced by dermal application of a sub-toxic dose of chlorpyrifos. Agricultural workers with stress are more likely to develop behavioural changes following dermal exposure to chlorpyrifos-containing pesticides.

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