Ascorbic Acid Ameliorates Toxic Effects of Chlopyrifos on Testicular Functions of Albino Rats

Dr. Kolawole Victor Olorunshola, L.N. Achie and M.L. Akpomienie
Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria

Abstract: Chlorpyrifos (CPF) is a widely used organophosphate insecticide for both agricultural and domestic purposes with attendant human exposures. Many authors have documented the toxic effects of CPF on the central nervous system. This study was designed to study the effect of CPF and the influence of co-administration of ascorbic acid (AA) on the testicular functions of albino rats. Twenty five 2 months old male albino wistar rats were divided into 5 groups of 5 rats each (Group A-E). A (control) received vegetable oil, B received 16.3 mg/kg CPF, C received 32.6 mg/kg CPF, D received 16.3 mg/kg CPF + AA 100 mg/kg and E received 32.6 mg/kg CPF + AA 100 mg/kg. Treatment was orally for a duration of 21 days. Thereafter, body weight, serum testosterone, testicular, epididymal and seminal vesicle weight, epididymal sperm concentration, sperm motility and histopathology of the testis, epididymis and seminal vesicles were determined using standard methods. CPF caused a statistically significant change (p<0.05) in body weight, testicular weight, epididymal weight, sperm concentration, sperm motility and serum testosterone concentration. Seminal vesicle weight was not affected. Histopathological studies revealed reduced sperm reserve, fibrosis and fatty infiltration in the epididymis, seminiferous tubules and seminal vesicles respectively. Co-administration of AA significantly caused improvement in all the parameters measured. It is concluded that CPF caused testicular toxicity by possible oxidative stress which was reversed with co-administration of AA.

Key words: Ameliorative effects, atrophy, chlopyrifos toxicity, epididymis, insecticide, rats, seminal vesicle, serum testosterone, testicular functions and vitamin C

INTRODUCTION

The need to improve agricultural yield and public health standards has necessitated the widespread use of pesticides. Chlorpyrifos is one of the most widely used organophosphate insecticides. It is used for both agricultural and residential purposes to control termites, mosquitoes and pet cullers in a variety of buildings including residential, commercial, schools, restaurants, hospitals and hotels (EPA-US, 2002). Human exposure is believed to be primarily in-door, due to household use, pesticide exterminator application and dietary exposure (Buckley et al., 1997). A study of 386 pregnant women in East Harlem indicated that 42% of the women had detectable levels of chlorpyrifos metabolite in their urine (Berkowitz et al., 2003).

Chlorpyrifos’ toxicity is typically manifested in the central and peripheral nervous system where it inhibits acetylcholinesterase by its active metabolite (Chlorpyrifos - oxon) leading to cholinergic hyper stimulation. The administration of paraoxonase (a known detoxification enzyme for chlorpyrifos) both before and after administration of chlorpyrifos has been found to offer significant protection against chlorpyrifos toxicity (Li et al., 1995). Another associated mechanism implicated in the induced toxicosis includes the induction of oxidative stress by the organophosphate compounds (Qiao et al., 2005).

It is also believed to cause neuronal damage in the developing brain through other cellular mechanisms such as inhibition of adenyl cyclase and through oxidative stress (Song et al., 1997; Dam et al., 1998; Montine et al., 2002; Abdullahi et al., 2004 and Qiao et al., 2003, 2004, 2005).

Chlorpyrifos has been reported to cause developmental and neurobehavioural toxicity (Deacon et al., 1980; Whitney et al., 1995; Garcia et al., 2003; Qiao et al., 2001a, b., 2002, 2003, 2004; Salazar et al., 2009; Slotkin et al., 2009 and Sledge et al., 2009), hepatotoxicity (Goel et al., 2000, 2005) and immunotoxicity (Li et al., 2009). Reduced testicular weight and epididymal sperm concentration with degenerative lesions were observed by (Joshi et al., 2007). Testicular injury is assumed to result from direct tissue accumulation or due to lipid peroxidation; reported to be highly toxic to spermatozoa and causing irreversible arrest of sperm function. (Aitken et al., 1993; Verma and Srivastava, 2003). Ascorbic acid (AA) or vitamin C, is a potent antioxidant used as a therapeutic agent against many diseases (Balz, 2003) and in the prevention of the
adverse effects of stress factors in laboratory animals and humans when the body's AA is exhausted (Altan et al., 2003; Tauler et al., 2003). AA is reported to donate a free molecule of hydrogen that detoxifies the harmful reactive oxygen species generated by the body especially when the body’s natural anti-oxidants are overwhelmed and exhausted. AA is also known to potentiate gamma-aminobutyric acid (GABA) which reduces neurotransmission, including release of corticosteroids (Koshebekov, 1991; Altan et al., 2003). Co-administration of ascorbic acid with Arochlor 1254(a polychlorinated biphenol) protected epididymal sperm of rats from oxidative damage (Krishnamoorthy et al., 2007). Ambali et al. (2007) has demonstrated that co-administration of ascorbic acid protected Red Blood Cells (RBC) and white blood cells (WBC) from the effect of chlorpyrifos in mice.

The study was designed to examine the possible ameliorative effect of co-administration of Ascorbic acid (vitamin C) on the testicular functions of albino Wistar rats.

MATERIALS AND METHODS

This research was conducted in the laboratory of Human Physiology Department, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria in the month of May 2011.

Experimental design: Twenty five (25) two months old male albino Wistar rats weighing 112-193 g were purchased, allowed 2 weeks to acclimatize and then randomly divided into 5 groups as follows:

- (Control) received 2 mL of vegetable oil orally daily for 21 days
- Received 16.3 mg/kg body weight (bw) of chlorpyrifos orally for 21 days. (Bretmont Agro Ltd, Highbury lane, London, England Batch No. 20080625).
- Received 32.6 mg/kg bw of chlorpyrifos orally, daily for 21 days
- Were given 16.3 mg/kg bw of chlorpyrifos + Vitamin C 100 mg/kg bw daily for 21 days
- Oral 32.6 mg/kg bw of chlorpyrifos + Vitamin C 100 mg/kg bw daily for 21 days

Food and water was allowed ad libitum. 24 hours after the last dose of chlorpyrifos and vitamin C administration, the final body weights were recorded. Body weight change was calculated by determining the difference between the final and initial body weights. Under light chloroform anaesthesia, thoracostomy was done and blood was collected by direct cardiac tap which was transferred into dry plain bottles for the estimation of serum testosterone concentration.

The scrotal sac was dissected open and the testis, right epididymis and seminal vesicles were removed, cleared of fat and weighed with a Mettler P3 weighing machine. Organ body weight ratio was calculated as organ weight/body weight ×100 (%).

Histopathological examinations: The right testes, epididymis and seminal vesicle were then preserved in Boin’s fluid for histopathological examinations. Tissue slides were fixed and eventually stained with Haematoxylin and Eosin. Testicular and Epididymal lesions were graded using the method of (Sekoni et al., 1990), where 10 transverse sections of seminiferous and epididymal tubules were examined from slides prepared from each rat, the number of tubules affected were noted and percentage abnormal calculated from 50 tubules examined in each group.

Epididymal sperm concentration and motility: The left epididymis of each rat was used for the determination of epididymal sperm concentration using the Neubauer haemocytometer while % sperm motility was determined using the IV6 Analyser (Halmiton Thorn Research, Beverly, MA).

Serum testosterone determination: Serum obtained from the blood sample collected was used for the determination of serum testosterone at the Chemical Pathology Department, Ahmadu Bello University Teaching Hospital, Shika, Zaria, Nigeria using the Microwell EIA Kits (Diagnostic Automation Inc. Calabasas, California USA) as described by Tietz (1995).

Statistical analysis: Results were expressed as Mean±SEM and subjected to analysis of variance. Results were considered statistically significant with p<0.05.

Table 1: Showing body weight change and organ body weight ratio (%)

<table>
<thead>
<tr>
<th></th>
<th>Body weight change (g)</th>
<th>Testicular body weight ratio (%)</th>
<th>Epididymis body weight ratio (%)</th>
<th>Seminal vesicle body weight ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.80±7.73</td>
<td>0.065±0.01</td>
<td>0.039±0.02</td>
<td>0.046±0.013</td>
</tr>
<tr>
<td>CPF 16.3 mg/kg b.w</td>
<td>16.60±3.88**</td>
<td>0.056±0.01</td>
<td>0.006±0.00**</td>
<td>0.029±0.014</td>
</tr>
<tr>
<td>CPF32.6 mg/kg b.w</td>
<td>10.60±3.27**</td>
<td>0.018±0.01**</td>
<td>0.005±0.00**</td>
<td>0.01±0.002</td>
</tr>
<tr>
<td>CPF 16.3 mg/kg</td>
<td>23.40±5.19</td>
<td>0.065±0.02</td>
<td>0.016±0.01</td>
<td>0.038±0.015</td>
</tr>
<tr>
<td>+ Vit.C 10 0mg/kg b.w</td>
<td>22.80±6.46</td>
<td>0.058±0.01</td>
<td>0.019±0.01</td>
<td>0.044±0.001</td>
</tr>
<tr>
<td>CPF 32.6 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Vit.C 100 mg/kg b.w</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CPF: chlorpyrifos; **: p<0.05
RESULTS

CPF caused a significant dose dependent decrease in body weight (Table 1) in rats treated with 16.3 mg/kg (16.60±3.88 g) and 32.6 Smg/kg bw (10.60±3.27g) as compared with control (31.80±7.73). The weight loss was ameliorated on co-administration of vitamin C (p<0.05). Similarly, testicular, epididymal and seminal vesicle organ body weight ratio in the control rats (0.07±0.01, 0.04±0.02 and 0.05±0.01% respectively) were significantly higher (p<0.001) than those of rats treated with 32.6 mg/kg bw of CPF (0.02±0.01, 0.01±0.00 and 0.01±0.00). Co-administration of vitamin C significantly increased the organ weights (testicular and seminal vesicle and not epididymal) towards that of control (Table 1).

Epididymal sperm concentration of 6.80±0.74 million/ml obtained in the control rats was significantly higher (p<0.05) than those of rats treated with 2 doses of CPF respectively (2.40±0.25, and 2.00±0.32). The reduction in sperm concentration was dose dependent. Co-administration with vitamin C significantly improved sperm concentration towards normal (Table 2 and Fig. 2). Sperm motility in the CPF treated group (26.6±1.60%, 12.6±2.30%) obtained in rats treated with 16.3 mg/kg and 32.6mg/kg bw of CPF, was significantly lower (p>0.05) than that of the control group (52.8±2.60% Co-administration did not improve the percentage motile sperm (Table 2).

The two doses of CPF significantly reduced serum testosterone concentration while concomitant administration of vitamin C significantly improved serum
testosterone levels (Fig. 1). Histopathological studies revealed decreased epididymal sperm reserve (Fig. 3), fatty infiltration and fibrosis of basement membrane of the seminal vesicles (Fig. 7 and 8). Fibrosis and atrophy of the seminiferous tubule with decreased spermatogenesis was also observed (Fig. 4, 5 and 10) as compared with normal sperm reserve and basement membrane in control rats (Fig. 2) as well as seminal vesicles with normal epithelial cells and secretions (Fig. 6). The histopathological features were ameliorated on co-administration with vitamin C (Fig. 3, 8 and 9).

**DISCUSSION**

The toxic effects of organophosphates are predominantly produced through the inhibition of
acetylcholinesterase, causing accumulation of acetylcholine at peripheral and central cholinergic receptors, resulting in overstimulation of the cholinergic system (Qiao et al., 2001a, b., 2002, 2003, 2004, 2005). The body weight of treated animals was significantly lower than that of control (Table 1). Other studies report similar findings. Kanga et al. (2004) reported a decrease in body weight on administration of 250mg/kg of chlorpyrifos to rats. Developing children (including infants and fetuses) are predicted to be more sensitive to chlorpyrifos to rats. Developing children (including infants and fetuses) are predicted to be more sensitive to chlorpyrifos (ATSDR, 1997).

The body weight of treated animals was significantly lower than that of control (Table 1). Other studies report similar findings. Kanga et al. (2004) reported a decrease in body weight on administration of 250mg/kg of chlorpyrifos to rats. Developing children (including infants and fetuses) are predicted to be more sensitive to chlorpyrifos (ATSDR, 1997).

Studies supporting this reveal a decrease in fetal weight in women exposed to chlorpyrifos (Whyatt et al., 2004). Result from the studies indicate that statistically significant decrease in fetal birth weights starts to be seen at 5.0 mg/kg/day. The weight loss due to chlorpyrifos administration has been found to be ameliorated by pre-treatment with vitamin E (Ambali et al., 2011). This implies that oxidative stress may be involved in chlorpyrifos induced body weight suppression and also a combination of toxic and cholinergic stress (Civen et al., 1977 and Corley et al., 1989). However, a study by Joshi et al. (2007), showed no significant change in body weight. Another study on chronic exposure to chlorpyrifos caused an increase in body weight which was found to be due to increase in adipose tissue (Meggs and Brewer, 2007).

There was an observed decrease in testicular weight in the group treated with chlorpyrifos without co-administration with vitamin C (Table 1). Epididymal weights were also lower in the treatment groups in a dose dependent fashion as compared to control with no difference in the weight of the seminal vesicle in the treatment group as compared to control (Table 1). Similar studies reveal the same finding (Joshi et al., 2007 and El-Kashoury and El-Din, 2010). The decrease in testicular weight is said to be due to reduced tubular size which were found to be due to degeneration and atrophy of seminiferous tubules. Another factor implicated in the reduced testicular weight is the decrease in thyroid hormone levels on administration of chlopyrifos which is essential to the development of the testes (El-Kashoury and El-Far, 2004).

The sperm concentration of the Chlorpyrifos treatment groups (without co-administration with vitamin C), showed a dose dependent decrease in sperm concentration. While the group with vitamin C co-administration showed no significant difference in sperm concentration as compared to the control group (Table 2). Faraga et al. (2010) in their study also observed an associated reduction in morphologically normal spermatozoa along with the decrease in sperm count in groups treated with chlorpyrifos. It resulted in a decreased number of live fetuses and an increased number of dead fetuses. Chlorpyrifos administration also produced a marked reduction in epididymal and testicular sperm counts in exposed males (Joshi et al., 2007). The oxon metabolites of organophosphorus insecticides like chlorpyrifos were found to participate in organophosphorus sperm genotoxicity (often with a higher toxicity) alongside the parent compound (Salazar-Arrendo et al., 2008).

Sperm motility for the control group was significantly higher than that of the treated groups (Table 2). In accordance with the finding of this study, El-Kashoury and El-Din (2010) also reported reduced sperm motility. This was suggested to be due to accumulation of proteins in the testes and epididymis secondary to androgen deprivation resulting in increased abnormal spermatozoa (Rao and Chinoy, 1983). Similar decreased motility was noticed in the study by Faraga et al. (2010) principally caused by defective spermatozoa. Sperm DNA damage occurred secondary to oxidative stress exacerbated by a decline in local anti-oxidant protection especially during epididymal maturation resulting in a variety of adverse clinical outcomes (Aitken and Iuliis, 2004). It produced defective spermiogenesis with cells characterized by retention of excess residual cytoplasm, persistent nuclear histones, poor zona binding, disrupted chaperone content and referred to as dysmature cells. Elevated levels of Malondialdehyde levels (induction of a lipid peroxidation process) and decrease in superoxide dismutase reaction corroborated their findings.

A significant decrease in serum testosterone concentration among the treatment groups as compared to control was observed (Fig. 1). Similar findings were observed by Jeong et al. (2006), Joshi et al. (2007) and Kanga et al. (2004). Chlorpyrifos displayed an anti-androgen activity which manifested as inhibition of testosterone stimulated increase in the weight of accessory sex organs (Kanga et al., 2004). The decrease in serum testosterone was ameliorated by administration of vitamin C in our study.

Histopathological investigation of the epididymis (Fig. 2), seminiferous tubules and seminal vesicles (Fig. 6) of the control group showed normal sperm
reserve, spermatogenesis and secretion respectively. Histological findings of the epididymis for the treated groups with co-administration of vitamin C (Fig. 3) revealed reduced sperm reserve. Reduced sperm reserve in the epididymis is due to oxidative stress causing inhibition of spermatogenesis as evidenced by the changes in the seminiferous tubules of the treatment groups which showed extensive fibrosis (Fig. 4, 10 and 11) and reduction in sperm count (Table 1) in the study groups. Joshi et al. (2007) observed similar findings. Examination of the testes in his study showed mild to severe degenerative changes in seminiferous tubules at various dose levels with a significant reduction in the sialic acid content of testes and testicular glycogen whereas the protein and cholesterol content was raised at significant levels. This was further potentiated by degenerating changes on other accessory sex organs like the seminal vesicles (Fig. 7 and 8). The decreased sperm reserve and features of fibrosis due to oxidative stress is found to be associated with an increased number of abnormal forms of spermatozoa due to DNA damage (Aitken and Iuliis, 2004). Oxidative stress in these structures is ameliorated by administration of vitamin C as evidenced by improved parameters (Fig. 5 and 9) similar to the findings of Ambali et al. (2010) on haematological parameters.

CONCLUSION

CPF significantly reduced body weight, testicular and epididymal weights, sperm concentration, sperm motility and serum testosterone of Albino rats with no effects on the weight of seminal vesicles. Vitamin C significantly improved above parameters except % sperm motility. CPF caused significant tissue degeneration in the male reproductive organs of Albino rats. Oxidative stress is a possible mechanism of action of CPF induced toxicity in the male reproductive organs.

RECOMMENDATIONS

We recommend studies on markers of oxidative stress (malonaldehyde, superoxide dismutase and glutathione peroxidase) on administration of chlorpyrifos especially with co-administration of vitamin E. We also recommend studies to determine the efficacy of exogenous paraoxonase in ameliorating the effects of chlorpyrifos and the possibility of its use on long term basis.

ACKNOWLEDGMENT

We wish to acknowledge Dr. M. O. A. Samaila of the Department of Pathology, Ahmadu Bello University Teaching Hospital, Shika, Zaria for reviewing the histological slides and Mr. M. A. Ekanem of the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Shika, Zaria for his technical support during the course of the study.

REFERENCES


