Gestational Exposure to Pesticides Induces Oxidative Stress and Lipid Peroxidation in Offspring that Persist at Adult Age in an Animal Model

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Abstract

Pesticide exposure may induce biochemical alterations including oxidative stress and lipid peroxidation. However, in the context of developmental origin of health and disease, putative trans-generational effect of exposure to pesticides are insufficiently studied. We therefore aimed to evaluate the biochemical effect of gestational exposure to four pesticides on female Wistar rats and their offspring at adult age. We studied 30 female nulliparous Wistar rats divided into 5 equal groups. Group 1 served as the control group and received distilled water while group 2, 3, 4 and 5 received orally pesticide 1 (imidacloprid), pesticide 2 (chlorpyrifos), pesticide 3 (imidacloprid + lambda cyhalothrin) and pesticide 4 (oxamyl) respectively once daily throughout gestation at a dose equivalent to 1/10 lethal dose 50. The mothers were followed up until one month post gestation. The offspring were followed up from birth until adult age (12 weeks). In all animals at each time point we evaluated malondialdehyde (MDA), oxidative stress and liver function enzymes. There was similar variation of total body weight in all the groups during and after gestation. However, Female Wistar rats of the exposed groups had significant alterations in liver SOD (−30.8% to +64.1%), catalase (−38.8% to −85.7%) and GSH (−29.2% to −86.5%) and; kidney catalase (> 100%), GSH (> 100%). Moreover, MDA, alanine transaminase (ALT) and aspartate transaminase (AST) levels were significantly higher in pesticide exposed rats compared to the control group. Similar alterations in antioxidant enzymes, MDA and liver function enzymes were observed in offspring of treated rats evidenced at weaning and persisting until adult age. Exposure to

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Abbreviations: ALT, Alanine Transaminase; AST, Aspartate Transaminase; BSA, Bovine Serum Albumin; EC, Emulsifiable concentrate; EDTA, Ethylenediaminetetraacetic acid; GPx, Glutathione peroxidase; GR, Glutathione reductase; MDA, Malondialdehyde; NADP, Nicotinamide adenine dinucleotide phosphate; nAChR, Nicotinic acetylcholine receptors; OD, Optical Density; GSSG, Oxidized Glutathione; GSH, Reduced glutathione; SL, Soluble liquid; SEM, Standard error of mean; SOD, Superoxide dismutase.

pesticides causes oxidative stress and lipid peroxidation in exposed female Wistar rats and their offspring. The persistence in offspring at adult age suggests transgenerational adverse effects.

INTRODUCTION

Environmental contaminants especially pesticides are now responsible for the development of diseases both in wildlife and humans (1,2). The recent classes of pesticides are organophosphate, carbamates, pyrethroids and neo-nicotinoids. Common pesticides belonging to these classes which are currently used are oxamyl, lambda cyhalothrin, chlorpyrifos and imidacloprid (3). The principal mechanisms of action of these pesticides include phosphorylation and inhibition of the action of acetylcholinesterase by organophosphates and carbamates, blocking of the open gates sodium channels by pyrethroids while neonicotinoids act by binding to the nicotinic acetylcholine receptors (nAChRs) and acting as nAChR agonists (4,5). In addition to their principal mode of action, lambda cyhalothrin, chlorpyrifos and imidacloprid have been reported to cause oxidative stress and lipid peroxidation both *in vitro* and *in vivo* (6-12). The degree of oxidative stress was enhanced with the combination of two pesticides (13,14).

The effect of gestational exposure to maternal pesticides are understudied meanwhile there is increasing evidence on the role of various exposures during early life (pre-natal and post-natal) on adult metabolism and biochemical status. Moreover, investigators like Tukhtaev and collaborators reported that long-term exposure to lambda-cyhalothrin led to the development of oxidative stress in pregnant female rats and their offspring during breastfeeding (15). Whether the effect of this exposure could persist at adult age is unknown. We therefore undertook this study aiming at investigating whether biochemical alteration resulting from exposure to pesticides during pregnancy would persist at adult age. We studied alone and in combination, the most commonly used agricultural pesticides in Africa.

MATERIALS AND METHODS

Chemicals and reagents. L-Drint 20 [(20% emulsifiable concentrate (EC) chlorpyrifos)], Confidor [(17.8% soluble liquid (SL) imidacloprid)], Parastar (2% imidacloprid + 2% lambda cyhalothrin) and Furaplant (10% oxamyl) were purchased from Bayer Company (New Delhi, India). Kits for ALT and AST were purchased from ThermoFisher Scientific (New Delhi, India), while antioxidant enzymes and MDA quantification was carried out with biochemical reagents of molecular grade.

Animals and handling. Nulliparous Wistar rats, aged 10-12 weeks and weighing on average 210 g, were used in this study. The animals were housed in the Animal House of Jamia Hamdard University in polypropylene cages under light controlled conditions with a 12/12-hr light-dark cycle and a temperature of $22 \pm 2^{\circ}C$ and had free access to water and food. The study was approved by the Animal Ethics Committee of Jamia Hamdard University and the animals used in this study were treated humanely with regards for the alleviation of suffering.

Experimental design.
• ***Female rat sub-study:*** This was a prospective study with a follow-up period of 16 weeks. After acclimatization for a period of 7 days, the rats were fasted overnight and body weight was measured. Animals were then divided into 5 groups of six rats each (Group 1 to Group 5) matched with weight. In each group, animals were mated at a ratio of two females to each male in a cage. At gestation (vaginal swap smear and staining with methyl blue stain and examine for presence of sperms in the smear), the male rats were removed and, pesticide administration was initiated in all female rats. Female rats of Group 2, Group 3, Group 4 and Group 5 received pesticide 1 (44 mg imidacloprid per kg body weight/day), pesticide 2 (13.5 mg chlorpyrifos per kg body weight/day), pesticide 3 (5.6 mg imidacloprid + 5.6 mg lambda cyhalothrin per kg body weight/day) and pesticide 4 (0.4 mg oxamyl per kg body weight/day) respectively with gastro-esophageal probe throughout gestation for 22 days while Group 1 received distilled water. These doses were chosen as shown to induce biochemical alterations without causing mortality and considered to be smaller than the exposed doses in humans during pesticide application, accidental poisoning (water or food containing pesticides). Each solution was freshly prepared by diluting in water and administered between 9 and 10 AM at a dose volume of 10 mL/kg rat per day. Pesticide administration was discontinued at birth while animals were maintained on normal diet and rats were followed for a month. At the end of follow up blood was collected by retro orbital puncture into ethylenediaminetetraacetic acid (EDTA) tubes, plasma was prepared and stored at $-20^{\circ}C$ for ALT and AST quantification. After collection of blood samples, the rats were sacrificed by decapitation after local anesthesia and dissected. Liver and kidney were removed, washed in ice cold normal saline solution and homogenates prepared and refrigerated at $-80^{\circ}C$ for quantification of antioxidant enzymes (SOD, catalase, GPx, GSH and GR) and MDA.
• ***Offspring sub-study:*** At birth, offspring were followed up by weekly recording of body weight. The off-

spring were weaned at 4 weeks of age and were followed up until adult age (12 weeks). At adult age, 8 offspring (4 male and 4 female) from each group were randomly selected and sacrificed at the end of follow up. Blood was collected and plasma prepared for ALT and AST quantification while liver and kidney samples were collected for antioxidant enzymes and MDA quantification.

Biochemical assays.

• **Super-oxide dismutase quantification:** Superoxide dismutase (SOD) was assayed using the method of Marklund and Marklund (16) with slight modifications. A single unit of enzyme was expressed as 50% inhibition of pyrogallol reduction/min/mg protein by superoxide is measured at 420 nm. Briefly, 0.1 mL of the sample solution (liver or kidney homogenate) was added to 2.8 mL of Tris-EDTA buffer solution in a cuvette, 0.1 mL of pyrogallol was added. The solution was mixed rapidly by inversion and the optical density was measured at 420 nm using the kinetic programme (30 sec interval for 3 min). A control tube was also prepared replacing the sample with 0.1 mL doubled distilled water. Enzyme activity was expressed as units/mg protein.

• **Catalase quantification:** Catalase was assayed using the method of Aebi (17). Briefly, samples were diluted in the ratio 1:50 with phosphate buffer. One mL of hydrogen peroxide (60 mM) was added into the diluted samples, mixed rapidly by inversion and the optical density read at 420 nm at 30 sec interval for 2 min.

Enzymatic activity was expressed as µmoles of H_2O_2 decomposed/min/mg protein using molar extinction coefficient of H_2O_2 (43.6 $M^{-1}cm^{-1}$).

• **Glutathione peroxidase quantification:** This assay was carried out using the method of Wendel (18). Briefly, the reaction mixture (7.0 mg of sodium azide, 33.2 mg reduced glutathione (GSH), and 1.08 mg reduced NADPH in 100 mL of phosphate buffer) was prepared and 3 mL added into the cuvette followed by 50 µL of hydrogen peroxide (0.042%). Fifty µL of sample was then added, rapidly mixed by inversion and the optical density read at 340 nm at 30 sec for 3 min. The results were expressed as nmoles NADPH oxidized/min/mg protein, using molar extinction coefficient of NADPH (6.22×10^6 M^{-1} cm^{-1}).

• **Reduced glutathione quantification:** This assay was carried out using the method of Moron (19). Briefly 200 µL of samples were diluted to 1,000 µL with phosphate buffer and reduced Glutathione standards were prepared. A blank tube was equally prepared containing 1 mL of double distilled water. Into each of the tubes (blank standard and samples) was added 1 mL of Ellman's Reagent solution. Each of the tube was incubated and the optical density (OD) read between 2 and 10 min at 412 nm in a spectrophotometer. A graph of OD = f (concentration) of standard was used to determine the concentration of GSH in samples by extrapolation and expressed in nmole.

• **Glutathione reductase quantification:** This assay was carried out using the method of Mavis and Stellwagen (20). Briefly, 2.9 mL of the reaction mixture (0.65 mL distilled water, 1.5 mL phosphate buffer 100 mM pH 7.0, 0.1 mL GSSG 30 mM, 0.35 mL β-NADPH 0.8 mM and 0.3 mL 1% w/v BSA) was introduced into the cuvette followed by 0.1 mL of sample and mixed rapidly by inversion. The optical density was then read at 340 nm using a kinetic program (30 sec for 3 min). The activity of the enzyme was expressed as nmoles NADPH oxidized/min/mg protein, using molar extinction coefficient of NADPH (6.22×10^6 M^{-1} cm^{-1}).

• **Malondialdehyde quantification:** Malondialdehyde (MDA) was quantified as thiobarbituric acid reactive substrate using the method modified from that of Ohkawa (21). One hundred microliters of each sample was added into a Pyrex test tube followed by 400 µL of the thiobarbituric acid substrate reagent mixture and the tubes were closed tightly. The mixture was heated at 100°C for 15 min. After heating the cork was removed and the tubes were placed in an ice bath for cooling to occur. After the cooling process, the mixture was centrifuged at 1,500 g for 5 min. The optical density of the supernatant was read at 532 nm. The concentration of MDA was then calculated using the molar extinction coefficient of MDA ($\varepsilon = 1.56 \times 10^5$ M^{-1} cm^{-1}) as follows:

$$\text{Concentration of MDA } (\mu M) = \frac{\text{OD}}{\varepsilon} \times 1{,}000{,}000 \times \text{dilution factor}$$

• **Total protein quantification:** Total protein content in tissue samples was determined by the method of Lowry (22), using bovine serum albumin as the standard.

• **Alanine transaminase and aspartate transaminase quantification:** Alanine transaminase (ALT) and aspartate transaminase (AST) were quantified with ALT and AST reagent kits according to the manufacturer's protocol (ThermoFisher Scientific) in the plasma.

• **Statistical analysis:** The results were analyzed using GraphPad Software (GraphPad Software, CA, USA). Unpaired t-test followed by Welch's correction was used to compare data between control and exposed groups. The data obtained were expressed as mean ± standard error of mean (SEM). All analyses were carried out at 95% confidence interval and $p < 0.05$ was considered to be statistically significant.

RESULTS

Reproductive outcome, weight variation and liver function enzymes level in parent rats. The weights of the dams were similar in all groups throughout the study (Fig. 1). The average gestation length was not signifi-
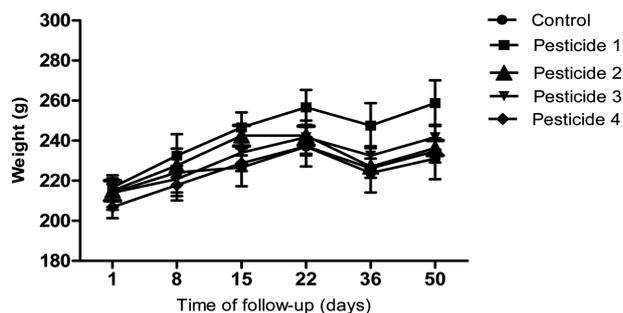
Fig. 1. Effect of pesticides exposure on body weight on female Wistar rats. Pesticide 1 = imidacloprid, pesticide 2 = chlorpyrifos, pesticide 3 = imidacloprid + lambda cyhalothrine, pesticide 4 = oxamyl. Data are reported as mean ± standard error of mean (SEM) for 6 samples.
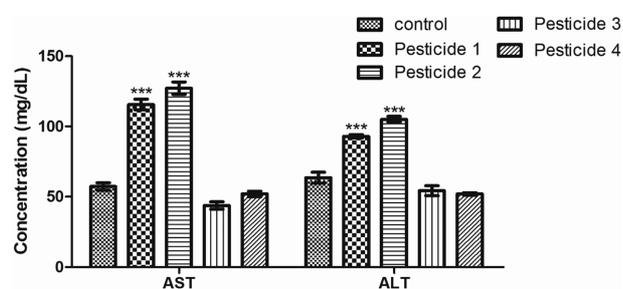

Fig. 2. Effect of pesticides on aspartate transaminase and alanine transaminase levels in female Wistar rats. Pesticide 1 = imidacloprid, pesticide 2 = chlorpyrifos, pesticide 3 = imidacloprid + lambda cyhalothrine, pesticide 4 = oxamyl, ***p < 0.001 compared with normal control. Data are reported as mean ± standard error of mean (SEM) for 8 samples.

cantly different between the control group (22 ± 1 day) and imidacloprid (23 ± 2 days), chlorpyrifos (22 ± 1 day), lambda cyhalothrin + imidacloprid (22 ± 2 days) and oxamyl (24 ± 3 days) exposed groups. However, parent rats exposed to imidacloprid and chlorpyrifos had a significant increase in aspartate transaminase (> 100%) and alanine transaminase (46.4% for pesticide 1 and 65.5% for pesticide 2) lev-

els compared to control (Fig. 2).

Antioxidant enzyme and malondialdehyde variation in liver and kidney of parent rats. Alterations in the level of antioxidant enzymes were recorded in the liver and kidney of parent rats exposed to pesticides when compared with the control group (Table 1). Liver SOD was significantly reduced in rats exposed to pesticide 1 (30.8%), pesticide 2 (38.5%) and 3 (51.3%) while a significantly higher value was recorded in rats exposed to pesticide 4 (64.1%) when compared with the control group. Exposure to all four pesticides led to a significant decrease in liver catalase and liver GSH of parent rats. Pesticide 1 and 2 significantly increase liver glutathione peroxidase (GPx) levels (50% and 64.9% respectively) while pesticide 3 and 4 significantly reduced liver GPx levels (81.1% and 83.9% respectively). Liver glutathione reductase (GR) was significantly reduced in rats exposed to pesticide 4 (64.5%) while liver MDA was significantly increased in rats exposed to pesticide 2 (> 100%). In the kidney, SOD was significantly increased in rats exposed to pesticide 1 (100%) while GPx was significantly increased in rats exposed to pesticide 4 (31.7%). Similarly, GSH was significantly increased in rats exposed to pesticide 1 (52.8%) and pesticide 2 (45.6%). Moreover, rats exposed to pesticide 1, 3 and 4 had a significantly higher MDA (> 100% in all cases) levels when compared to control. Kidney catalase levels were significantly higher in rats exposed to pesticide 3 and 4 (> 100% in both cases) while kidney GR levels were significantly lower in rats exposed to pesticide 3 (38.4%) and 4 (44.8%) compared to the control group.

Weight variation and liver function enzymes level in offspring. In offspring of pesticide exposed rats there was no significant different in body weight throughout the follow when compared with the control group (Fig. 3). However, offspring of rats exposed to all pesticides had a significant increase in ALT and AST levels when compared with the control group (Fig. 4).

Table 1. Effect of pesticides on antioxidant enzymes and malondialdehyde levels in liver and kidney of female Wistar rats

Sample	Liver					Kidney				
	Control	Pesticide 1	Pesticide 2	Pesticide 3	Pesticide 4	Control	Pesticide 1	Pesticide 2	Pesticide 3	Pesticide 4
SOD	3.9 ± 0.4	2.7 ± 0.3*	2.4 ± 0.2*	1.9 ± 0.4**	6.4 ± 0.4**	1.4 ± 0.3	2.8 ± 0.4*	2.0 ± 0.3	1.3 ± 0.2	1.2 ± 0.2
CAT	24.5 ± 1.9	14.7 ± 1.4**	15.0 ± 1.7**	3.5 ± 0.8***	12.7 ± 1.9**	14.9 ± 6.4	22.4 ± 0.9	31.7 ± 4.6	35.9 ± 1.9*	32.9 ± 2.5*
GPx	14.8 ± 1.2	22.2 ± 1.9*	24.4 ± 2.8*	2.8 ± 0.5***	2.4 ± 0.1***	20.8 ± 2.2	20.6 ± 1.4	21.1 ± 1.1	15.54 ± 2.6	14.2 ± 1.4*
GSH	26.7 ± 2.4	18.9 ± 1.9*	17.3 ± 2.4*	3.8 ± 0.2***	3.6 ± 0.3***	19.3 ± 1.4	29.5 ± 1.7**	28.1 ± 2.9*	15.0 ± 1.8	16.8 ± 1.6
GR	3.1 ± 0.5	1.7 ± 0.6	1.9 ± 0.5	2.2 ± 0.4	1.1 ± 0.3*	48.4 ± 3.4	50.1 ± 2.7	48.5 ± 3.0	29.8 ± 4.9*	26.7 ± 3.1**
MDA	0.7 ± 0.06	5.3 ± 0.3	5.9 ± 0.6***	0.6 ± 0.1	0.6 ± 0.1	1.0 ± 0.1	2.4 ± 0.3*	1.6 ± 0.3	3.5 ± 0.6***	5.0 ± 0.3***

Pesticide 1 = imidacloprid, pesticide 2 = chlorpyrifos, pesticide 3 = imidacloprid + lambda cyhalothrine, pesticide 4 = oxamyl, SOD = super oxide dismutase, CAT = Catalase, GPx = Glutathione peroxidase, GSH = Reduced Glutathione, GR = Glutathione Reductase, *p < 0.05, **p < 0.01, ***p < 0.001 compared with normal control. Data are reported as mean ± standard error of mean (SEM) for 6 samples.
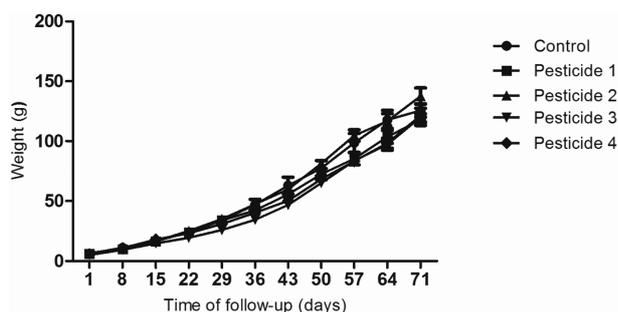
Fig. 3. Effect of pesticides on body weight in offspring. Pesticide 1 = imidacloprid, pesticide 2 = chlorpyrifos, pesticide 3 = imidacloprid + lambda cyhalothrine, pesticide 4 = oxamyl. Data are reported as mean ± standard error of mean (SEM) for 8 samples.

Fig. 4. Effect of pesticides on aspartate transaminase and alanine transaminase levels in offspring of female Wistar rats. Pesticide 1 = imidacloprid, pesticide 2 = chlorpyrifos, pesticide 3 = imidacloprid + lambda cyhalothrine, pesticide 4 = oxamyl, *** = $p < 0.0001$ compared with normal control. Data are reported as mean ± standard error of mean for 8 samples.

Antioxidant enzyme and malondialdehyde variation in liver and kidney of offspring.

Similar alterations in liver and kidney antioxidants and MDA levels were recorded in offspring of pesticide exposed rats when compared to the control group at weaning (Table 2) and persisted until adulthood (Table 3). Liver SOD was significantly lower in offspring of rats exposed to pesticide 1 (87.6%) and pesticide 2 (84.9%). Liver catalase was significantly higher i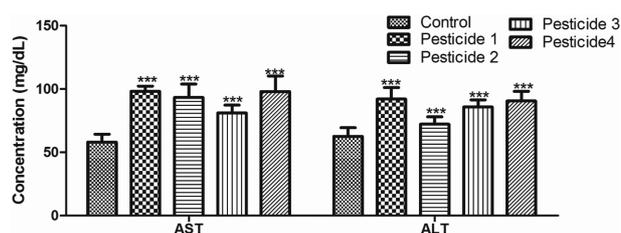n offspring of rats exposed to pesticide 1 (30.3%) and pesticide 2 (36.9%) while a significantly lower catalase level (39.4%) was recorded in offspring of rats exposed to pesticide 4. GPx level was significantly reduced in offspring of rats exposed pesticide 1 (77.9%), pesticide 2 (78.1%) and pesticide 4 (30.3%) when compared to the control group. Similarly, liver GSH was significantly lower in offspring of rats exposed to pesticide 1 (30.4%), pesticide 3 (91.2%) and 4 (90.9%). Likewise, a significantly lower liver GR was recorded in offspring of parent rats exposed to pesticide 3 (41.4%) and 4 (34.5%). A one-fold increase in liver

Table 2. Effect of pesticides on antioxidant enzymes and malondialdehyde levels in liver and kidney of offspring Wistar rats sacrificed at aweaning

Sample	Liver					Kidney				
	Control	Pesticide 1	Pesticide 2	Pesticide 3	Pesticide 4	Control	Pesticide 1	Pesticide 2	Pesticide 3	Pesticide 4
SOD	1.9 ± 0.2	1.7 ± 0.2	1.9 ± 0.2	1.3 ± 0.2*	1.3 ± 0.2*	2.8 ± 0.6	5.0 ± 0.4*	5.7 ± 0.6**	4.6 ± 1.1	2.9 ± 0.5
CAT	9.2 ± 0.9	14.1 ± 1.8*	8.6 ± 0.7	11.9 ± 2.5	13.4 ± 5.8	10.7 ± 5.6	24.6 ± 13.7	48.9 ± 29.4	8.0 ± 1.0	8.3 ± 0.8
GPx	28.2 ± 2.3	29.8 ± 4.9	26.7 ± 3.1	27.2 ± 2.3	33.5 ± 1.4	4.2 ± 0.5	13.4 ± 5.8	11.9 ± 2.5*	7.0 ± 2.2	8.3 ± 0.9**
GSH	21.4 ± 3.3	15.0 ± 1.8	16.8 ± 1.6	15.5 ± 2.6	14.2 ± 1.4	33.5 ± 1.4	69.1 ± 6.5**	75.7 ± 4.9***	52.0 ± 5.9*	53.2 ± 7.0*
GR	7.1 ± 3.7	1.8 ± 0.3	1.4 ± 0.4	1.3 ± 0.2	1.2 ± 0.2	3.3 ± 0.4	11.1 ± 2.4*	6.9 ± 1.4	6.1 ± 1.2	6.8 ± 1.2*
MDA	3.5 ± 0.4	3.5 ± 0.6	5.0 ± 0.3*	4.4 ± 1.1	3.3 ± 0.4	0.3 ± 0.02	0.9 ± 0.2*	1.6 ± 0.1***	0.9 ± 0.1*	0.8 ± 0.1**

Pesticide 1 = imidacloprid, pesticide 2 = chlorpyrifos, pesticide 3 = imidacloprid + lambda cyhalothrine, pesticide 4 = oxamyl, SOD = super oxide dismutase, CAT = Catalase, GPx = Glutathione peroxidase, GSH = Reduced Glutathione, GR = Glutathione Reductase, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with normal control. Data are reported as mean ± standard error of mean (SEM) for 8 samples.

Table 3. 0Effect of pesticides on antioxidant enzymes and malondialdehyde levels in liver and kidney of offspring Wistar rats at adulthood

Sample	Liver					Kidney				
	Control	Pesticide 1	Pesticide 2	Pesticide 3	Pesticide 4	Control	Pesticide 1	Pesticide 2	Pesticide 3	Pesticide 4
SOD	23.3 ± 3.8	2.9 ± 0.3**	3.5 ± 0.4**	19.3 ± 1.4	16.6 ± 1.0	3.8 ± 0.3	3.1 ± 0.2	3.4 ± 0.3	5.0 ± 0.4*	5.7 ± 0.6*
CAT	16.5 ± 1.0	21.5 ± 1.2**	22.6 ± 0.7***	17.9 ± 2.5	10.0 ± 2.2*	17.4 ± 2.5	21.2 ± 1.5	26.9 ± 2.9*	24.6 ± 13.7	48.9 ± 29.4
GPx	70.9 ± 4.4	15.7 ± 1.9***	15.5 ± 1.3***	55.7 ± 8.7	49.4 ± 4.5**	7.5 ± 0.6	10.4 ± 0.5**	12.5 ± 0.8***	13.4 ± 5.8	11.9 ± 2.5
GSH	30.9 ± 3.3	21.5 ± 1.9*	24.3 ± 1.7	2.7 ± 0.2***	2.8 ± 0.2***	26.4 ± 3.3	21.5 ± 1.6	14.6 ± 3.3*	17.1 ± 4.7	20.7 ± 4.1
GR	2.9 ± 0.4	2.5 ± 0.6	1.9 ± 0.3	1.7 ± 0.3*	1.9 ± 0.2*	31.8 ± 1.2	34.3 ± 1.9	37.1 ± 1.4*	13.2 ± 1.6***	12.6 ± 1.4***
MDA	5.8 ± 1.4	10.0 ± 1.2*	10.3 ± 0.9*	5.6 ± 0.1	6.1 ± 0.2	2.2 ± 0.2	3.2 ± 0.3*	4.8 ± 0.8*	2.4 ± 0.5	2.6 ± 0.5

Pesticide 1 = imidacloprid, pesticide 2 = chlorpyrifos, pesticide 3 = imidacloprid + lambda cyhalothrine, pesticide 4 = oxamyl, SOD = super oxide dismutase, GPx = Glutathione peroxidase, GST = Glutathione S transferase, GSH = Reduced Glutathione, GR = Glutathione Reductase, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with normal control. Data are reported as mean ± standard error of mean (SEM) for 8 samples.

MDA was recorded in rats exposed to pesticide 1 (72.4%) and 2 (77.6%).

In kidney, SOD was significantly increased in offspring of rats exposed to pesticide 3 (31.6%) and pesticide 4 (50%). Kidney catalase was significantly higher (54.6%) while kidney GSH was significantly lower (44.7%) in offspring of parent rats exposed to pesticide 2. Moreover, kidney GPx and MDA levels were significantly higher in offspring of parent rats exposed to pesticide 1 (38.7% and 45.5% for GPx and MDA respectively) and pesticide 2 (66.7% and 118% for GPx and MDA respectively) when compared to the control group. GR was significantly higher in offspring of rats exposed to pesticide 2 (16.7%) and significantly reduced in offspring of rats exposed to pesticide 3 (58.5%) and pesticide 4 (60.4%).

DISCUSSION

Pesticide poisoning constitute a public health problem as 3,000,000 cases of poisoning and 220,000 deaths were recorded annually across the globe, the majority of which are reported from developing countries (23). Pesticide usage/hectare (ha) is less in Africa (1.23 kg a.i./ha) compared to Latin America and Asia (23). However, the concern in Africa is alarming due to poor status of legislation, insufficient education and training in pesticide usage amongst others. In Cameroon, pesticide exposure mainly occur in agricultural sector, practiced by local farmers or industrialized companies such as Cameroon Development Corporation (CDC), a major development partner in Cameroon which cultivates rubber, oil palm and banana (24). Lack of respect of pesticide application measures, high run off of pesticides into nearby water sources as a result of aircraft spraying and dumping of pesticide waste amongst others result to pesticide poisoning in Cameroon (25). This has as consequence, killing of aquatic organisms, intoxication of the applicators and local population which can result to dead.

Organophosphate, carbamates and neonicotinoids insecticides as well as fungicides are extensively used in agricultural settings in Cameroon with inhalation and ingestion being the main exposure routes (25-27). This study is therefore relevant as it investigated pesticides which are widely used especially for agricultural purposes as well as in public health disease control. Rats were exposed to a dose assumed to be same or inferior to human exposed dose in agricultural setting with similar mode of exposure in humans. Investigation of parameters in offspring was essential so as to depict possible transgenerational effect. The present study shows that all the pesticides tested induced oxidative stress in female Wistar rats, characterised by alterations in circulating levels of at least one antioxidant enzyme in liver and kidney tissues. The observed increase in lipid peroxidation as well as high plasma ALT

and AST levels in rats exposed to imidacloprid and chlorpyrifos were confirmatory of tissue damage. Similar alterations observed in parents were equally recorded in offspring at adult age. Oxidative stress in liver and kidney were observed in offspring of rats exposed to pesticides when compared to the control group characterized by alterations in antioxidant levels. Moreover imidacloprid and chlorpyrifos were shown to induce lipid peroxidation in offspring of rats exposed to these two chemicals. In a similar manner, *in utero* exposure to all 4 pesticides induced liver injury as observed by a 3 fold increase in plasma ALT and AST when compared to the control group.

Our findings in directly exposed animals are in accordance with most previous studies. Lambda-cyhalothrin was observed to induced oxidative stress both *in vivo* and *in vitro* (6-9). It significantly increased liver and kidney malondialdehyde (MDA) of male adult Wistar rats (9,10). Similarly dysregulation in oxidative stress and lipid peroxidation biomarkers in rats exposed to imidacloprid was reported (11,28-30). Furthermore, chlorpyrifos was observed to induce oxidative stress as well as increase levels of lipid peroxides in the (12,31-35). Notwithstanding, ALT and/or AST levels were significantly increased in plasma of exposed rats and their offspring (36,37). However, the pesticide mixture (5.6 g/kg imidacloprid + 5.6 g/kg lambda cyhalothrine) did not affect the liver function enzymes which can be explained by the low dose.

The mechanism of generation of oxidative stress is related to pesticide biotransformation as in the case of chlorpyrifos. Biotransformation of chlorpyrifos by cytochrome P450 generate its metabolites chlorpyrifos oxon and 3,5,6-trichloro-2-pyridinol. These metabolites being more toxic than the parent compound directly or indirectly generate reactive oxygen species such as highly reactive superoxide radical and hydrogen peroxide (38). At the onset, there is an increase in the level of antioxidants to counteract the effect of these ROS. However, prolonged exposure leads to depletion of the antioxidant system leading to cellular damage (39) and oxidative stress. The end stage of oxidative stress is impairment of cellular functions and potential tissue damage which is characterized by lipid peroxidation (increased MDA levels) and leakage of cytosolic enzymes from hepatocytes (ALT and AST).

What is new in our findings is the effect on offspring with *in utero* exposure that can be evidenced at weaning (Table 3) persist with similar magnitude at adult age without additional exposure. Tukhtaev *et al.* (15) observed similar alterations in oxidative stress enzymes in offspring of rats with inutero exposure to pesticides. However, they reported that the alterations decreased both in exposed rats and their offspring with cessation of breast milk. The difference could be because of the dose used since that in our study was 10 times higher. Therefore, pesticide exposure at

certain doses could induce permanent alteration in exposed organisms. Even though pesticides were not quantified in body fluids of offspring, we can affirm that alterations in offspring resulted from foetal exposure since these chemicals have been shown to cross the placenta. Therefore, further studies looking at the epigenome to investigate the molecular mechanism as well as the second and third generation to characterize the trans-generational effect are warranted.

Commonly used pesticides in Africa (imidacloprid, chlorpyrifos, lambda cyhalothrin and oxamyl) induced oxidative stress, lipid peroxidation and caused liver injury in female Wistar rats and their offspring. These biochemical alterations on offspring remain until adult age suggesting that these chemicals persist in the offspring even after cessation of exposure or modify the epigenome through epigenetic mechanisms. Therefore, further studies looking at the epigenome to investigate the molecular mechanism are warranted.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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