ORIGINAL ARTICLE

Metabolic effects of exposure to pesticides during gestation in female Wistar rats and their offspring: a risk factor for diabetes?

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Abstract
Some pesticides increase the risk of type 2 diabetes, but whether fetal exposure carries transgenerational risk remains unknown. We evaluated the metabolic effects of gestational exposure to chlorpyrifos and imidacloprid in female Wistar rats and their offspring. We studied female nulliparous Wistar rats, including six exposed to imidacloprid (IMI) and six to chlorpyrifos (CPF) once daily throughout gestation at 1/10 lethal dose 50, while six (control group) received distilled water. These were explored 1 month after the birth of the offspring, while their offspring were explored at weaning (4 weeks) and adult age (12 weeks). Blood glucose, insulin and lipid profile were determined at each stage, while glucose transporter 4 (GLUT4) and nuclear factor kappa beta (NFkβ) protein expression was measured in skeletal muscle at the end of follow up. Exposure to pesticides was associated with significantly higher fasting glucose (+25.4 to 30.9%) and insulin (> 100%) levels, with > 100% increased insulin resistance (HOMA-IR), − 18.3 to − 21.1% reduced HDL-cholesterol and + 60.9 to + 102.6% increased LDL-cholesterol in mothers. GLUT4 expression was reduced by 28.9–42.3% while NFkβ expression increased by 32.8–35.4% in mothers. In offspring, similar abnormalities were observed at weaning (+ 18.4 to 67.4% fasting glucose, + 57.1 to 72.2% LDL-cholesterol, + 72.3 to 78.2% fasting insulin), persisting at adult age with decreased expression of GLUT4 (− 52.8 to 54.5%) and increased expression of NFkβ (+ 30.5 to 30.7%). Gestational exposure to imidacloprid and chlorpyrifos induces hyperglycemia, insulin resistance and dyslipidemia in female Wistar rats and their offspring. The effects on offspring persist until adult age, suggesting intergenerational adverse effects.

Keywords Pesticide · Hyperglycemia · Dyslipidemia · GLUT4 · NFkβ

Abbreviations
ACh	Acetylcholine
ANOVA	Analysis of variance
CPF	Chloropyrifos
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immuno-sorbent assay
EC	Emulsifiable concentrate
FBG	Fasting blood glucose

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GLUT4	Glucose transporter-4
HDL	High density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
HOMA-β	Homeostasis model assessment of beta cell function
ICGEB	International Center for Genetic Engineering and Biotechnology
IMI	Imidacloprid
LDL	Low density lipoprotein
NFkβ	Nuclear factor kappa beta
PBST	Phosphate buffered saline tween
SEM	Standard error of mean
TC	Total cholesterol

Introduction

A pesticide is defined as any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feed stuffs [1]. They are classified as organochlorines, organophosphates, carbamates, pyrethroids and neonicotinoids according to their chemical composition. The effects of these pesticides on metabolic diseases such as type 2 diabetes have been reviewed [2]. Organochlorines were banned in the 1970s because of their resilience to environmental degradation through chemical, biological, and photolytic processes; their lipophilicity, environmental pollution, long-range transport, bioaccumulation in human and animal tissue, as well as bio-magnification in food chains, made them very toxic [3]. Currently, the most commonly used pesticides in agriculture and residential pest control throughout the world are chlorpyrifos and imidacloprid, belonging to the organophosphate and neonicotinoids classes respectively [4, 5]. Chlorpyrifos and imidacloprid directly or indirectly modulate the acetylcholine (ACh) pathway leading to excessive transmission of nerve impulse [6, 7]. The consequence is the involuntary twitching of muscles, paralysis and the death of the exposed organism. These two pesticides affect other biological pathways in ways that could be deleterious to non-target organisms, including humans. The main pathway involves alteration of the anti-oxidant system leading to the development of oxidative stress and lipid peroxidation. Chlorpyrifos and imidacloprid have been observed to induce oxidative stress and lipid peroxidation in the liver, kidney and other organs of rats [8–11]. These pesticides equally affect pathways associated with metabolic disorders such as diabetes and obesity. Acute oral exposure to chlorpyrifos causes hyperglycemia and hyperlipemia in rats [12]. Chronic oral administration of chlorpyrifos to male rats increased the blood glucose level while decreasing the serum insulin level [13]. Exposure of male rats to chlorpyrifos through subcutaneous injection caused hyperlipemia and hyper insulinaemia in adulthood [14]. In humans, fetal exposure to chlorpyrifos caused brain defects, as well as abnormalities of the eyes and other organ system in children [15–17], while acute exposure through poisoning induced drowsiness, dizziness, vomiting, disorientation, and fever [18, 19]. These signs and symptoms were recorded in a 69-year-old woman who ingested a formulated product containing 9.6% imidacloprid and who died 12 h after the exposure [20]. A 24-year-old man who accidentally inhaled a pesticide containing 17.8% imidacloprid while working on his farm was disoriented, agitated, incoherent, sweating and breathless following the exposure [21]. Likewise, direct exposure to imidacloprid may affect the insulin signaling cascade in cultured cell lines [22], and modulate adipocyte differentiation and lipogenesis [23]. Imidacloprid equally induces respiratory distress and neuropsychiatric features following accidental inhalational exposure in humans [24]. The potential for these pesticides to cause intergenerational alterations on oxidative stress and lipid peroxidation has been investigated. Chlorpyrifos, imidacloprid, lambda-cyhalothrin and oxamyl induced oxidative stress and lipid peroxidation in the offspring of exposed Wistar rats [11]. However, the effect on metabolic parameters such as glucose and lipid metabolism is unknown. In fact, the developmental origin of health and disease hypothesis stipulates that adverse fetal exposure may cause permanent fetal adaptations in structure, physiology and metabolism; these might be beneficial for short-term fetal survival, but may lead to fetal growth retardation, cardiovascular and metabolic diseases in adulthood [25]—as has been observed with several risk factors for type 2 diabetes. Possible programming factors investigated include diet and nutrition, environmental factors, maternal hormonal levels during pregnancy and the metabolic situation during pregnancy [26]. We thus investigated the metabolic effects of gestational exposure to the widely-used pesticides chlorpyrifos and imidacloprid in female Wistar rats and their offspring.

Materials and method

Chemicals and reagents

L-Drint 20 [(20% emulsifiable concentrate (EC) chlorpyrifos)] and Confidor [(17.8% soluble liquid (SL) imidacloprid)] were purchased from Bayer Company in New Delhi, India. Kits for insulin ELISA, lipid profile, plasma glucose quantification and western blotting analysis were purchased

from ThermoFisher Scientific in New Delhi, India. Both chemicals were stored at room temperature and diluted with distilled water to the required concentration before being administered to the rats.

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-h light–dark cycle and a temperature of 22 ± 2 °C. They 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 regard to 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 subjected to an overnight fast (approximately 16 h). Fasting blood glucose levels and body weight were measured. Animals were then divided into three groups of six rats each (group 1, group 2 and group 3), matched with weight and fasting glycemia. In each group, animals were mated at a ratio of two females to each male in a cage. At gestation, the male rats were removed and pesticide administration was initiated. Female rats in group 1 served as the control group, receiving water; female rats in groups 2 and 3 received imidacloprid (44 mg/kg body weight/day) and chlorpyrifos (13.5 mg/kg body weight/day), respectively, with a gastro-esophageal probe throughout gestation. This dose was chosen as previously shown to induce biochemical alterations without causing behavioral signs of toxicity or mortality. Behavioral signs of toxicity that were checked included changes in skin and fur, eyes and mucous membranes, respiratory and behaviour patterns, with particular attention paid to possible tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. 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 parents were followed for a month. At the end of follow up, blood was collected by cardiac puncture into dry tubes and ethylenediaminetetraacetic acid (EDTA) tubes. Serum and plasma were prepared and stored at -20 °C for glucose, lipid profile and insulin. After collection of the blood samples, the rats were killed by euthanasia after local anesthesia and dissected.

Skeletal muscles were collected and refrigerated at -80 °C for western blood analysis of GLUT4 and NFKβ.

Offspring sub-study

At birth, offspring were followed up by weekly recording of body weight and weaned at 4 weeks of age. At weaning, eight offspring (four male and four female) were randomly selected from each group and fasted overnight. Blood samples were collected into EDTA and dry tubes. Serum and plasma were prepared and stored at -20 °C for glucose, lipid profile and insulin quantification. After collection of blood samples, the rats were killed by euthanasia after local anesthesia and dissected. The remaining offspring were followed up until 12 weeks and killed (four male and four female per group) after an overnight fast. Blood samples and skeletal muscles were collected for biochemical (blood glucose, insulin and lipid profile) measurements and western blotting analysis.

Biochemical assays

Plasma glucose concentration was measured with a glucose oxidase kit according to the TRINDER method [27]. Serum total cholesterol, High Density Lipoprotein (HDL) cholesterol and triglycerides were measured using standard enzymatic techniques [28–30]. Low Density Lipoprotein (LDL) cholesterol was calculated using Friedwald's formula [31]. Plasma insulin was measured with the ALPCO Rat Insulin ELISA kit as per the manufacturer's protocol (Biorbyt Ltd, UK).

Western blotting analysis

A muscle sample was chopped and homogenized on ice with a buffer solution four times its volume. It was kept on ice for 15 min, and 2.5 μL of Nonidet P-40 was added. The mixture was centrifuged at 20,000 g for 15 min at 4 °C and the supernatant collected as protein extract. A portion of the extract was used for protein quantification via the Lowry method, using BSA as standard [32]. Each sample was diluted to 5 mg/mL with the extraction buffer, mixed with the loading buffer in the ratio 4:1 and heated at 80 °C for 7 min. After cooling to r.t.p., 10 μL was loaded into the wells of a 10% separating and 5% stacking gel. The gel was run for 30 min at 90 volts and then 2 h at 120 volts. A nitrocellulose membrane and the gel were equilibrated in the transfer buffer for 2 min. The transfer assembly was set (negative electrode/ sponge/three Whatmann filter paper/gel/membrane/three Whatmann filter paper/sponge/positive electrode) while rolling to remove bubbles. The assembly was then fitted into the gel tank and filled with the transfer buffer. The current was switched on and the transfer was effected at 95 volts

for 2 h. The membrane was removed and blocked with 5% bovine serum albumin (BSA) overnight at 4 °C. The next day, BSA was discarded and the membrane was incubated in the primary antibody (GLUT4 or NFkβ) or beta actin as the control protein at r.t.p. for 2 h, washed trice with 1× phosphate buffered saline containing 0.5% tween-20 (PBST). The membrane was again incubated with the secondary antibody (Goat Anti-Mouse IgG) for 90 min at room temperature and washed thrice with PBST. The developing solution (luminol) was spread on the surface of the membrane and the bands visualized and captured. The band of interest was then quantified using the image J software.

Calculations

Insulin resistance was determined by the homeostasis model assessment (HOMA-IR) using the formula: $\text{HOMA-IR} = \frac{\text{Fasting Glucose(mg/dL)} \times \text{Fasting Insulin(mU/L)}}{405}$. Beta cell function was determined by HOMA-β using the formula: $\text{HOMA-}\beta = \frac{360 \times \text{Fasting Insulin(mU/L)}}{\text{Fasting Glucose(mg/dL)} - 63}$.

Statistical analysis

The results were analyzed using GraphPad Prism software. The one way ANOVA test and the Bonferroni Multiple Comparison Test were used to compare data between the different groups. The data obtained were expressed as mean ± SEM. All analysis were carried out at 95% confidence interval and $p < 0.05$ was statistically significant.

Results

## Reproductive outcome of dams and pups

The weights of the dams in the pesticides-exposed groups were similar to those in the control group throughout the study. The average length of gestation was 25 days, 23 days and 27 days in the control, imidacloprid-exposed and chlorpyrifos-exposed groups, respectively. Pregnancy was recorded in four rats of the control group, five rats of the imidacloprid-exposed group and two rats of the chlorpyrifos-exposed group. The total number of pups was 31 (15 males), 32 (17 males) and 17 (11 males) for the control, imidacloprid-exposed and chlorpyrifos-exposed groups, respectively. There was no significant difference in the weight at birth of the pups of imidacloprid (5.9 ± 0.4) and chlorpyrifos (6.7 ± 0.4) exposed rats when compared to the control group (6.2 ± 0.2). No sign of toxicity was recorded in dams and their pups upon observation throughout the study.

Metabolic parameters in female Wistar rats

The metabolic parameters investigated were blood glucose, insulin, and lipid profile. Insulin sensitivity and insulin secretion were assessed as HOMA-IR and HOMA-β respectively, calculated from fasting glucose and fasting insulin values. In parent rats, the baseline fasting blood glucose (FBG) was similar in the three groups (79.6–82.0 mg/dL). At the end of the follow up, FBG and insulin were significantly higher in rats exposed to imidacloprid (25.4% and 123.9% for FBG and insulin, respectively) and chlorpyrifos (30.9% and 97.8% for FBG and insulin, respectively) compared to the control group. HOMA-IR values were significantly higher in rats exposed to imidacloprid and chlorpyrifos (Fig. 1), while no difference in HOMA-β values were recorded (103.9 ± 23.9–141.8 ± 63.1). Similarly, alterations in lipid profile parameters were recorded in pesticide-exposed rats (Fig. 2). Rats exposed to imidacloprid had a significant higher total cholesterol (51.9%) and LDL-cholesterol (> 100%) levels, while rats exposed to chlorpyrifos had a significant higher LDL-cholesterol (60.9%) level. Equally, HDL-cholesterol was significantly lower in rats exposed to both pesticides (18.3% IMI and 21.1% CPF) than in rats in the control group (Tables 1, 2).

Fig. 1 Effect of pesticide exposure on fasting blood glucose, insulin, HOMA-IR and HOMA-β in female Wistar rats. G1 = normal control, G2 = imidacloprid, G3 = chlorpyrifos, HOMA-IR = homeostasis model assessment of insulin resistance, *$p < 0.05$, Values are given as mean ± SEM (n = 6)
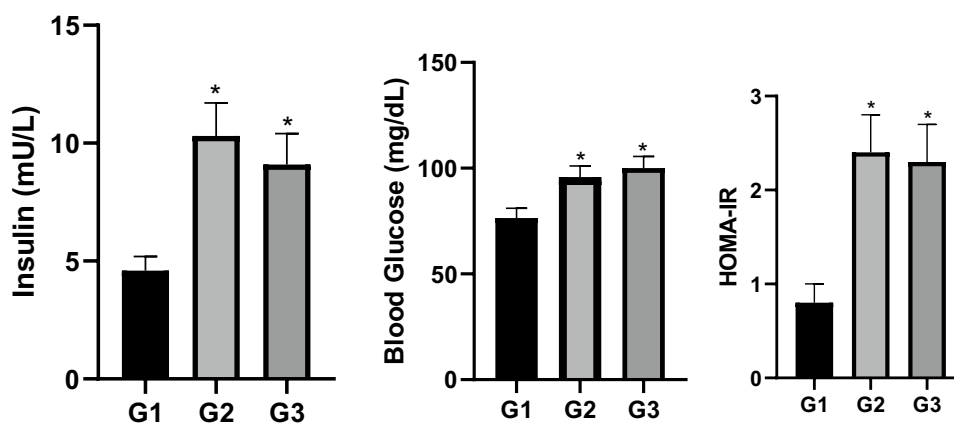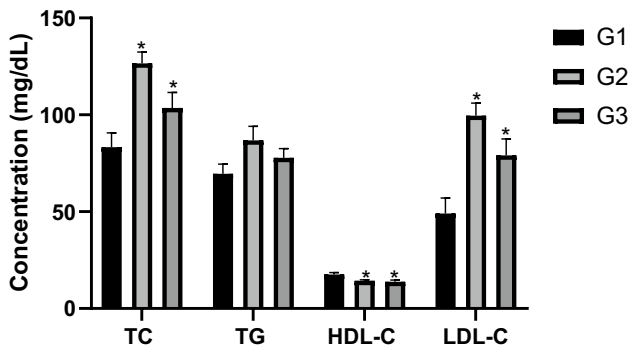
Fig. 2 Effect of pesticide exposure on lipid profile in female Wistar rats. G1 = normal control, G2 = imidacloprid, G3 = chlorpyrifos, TC = total cholesterol, TG = triglycerides, HDL–C = high density lipoprotein cholesterol, LDL–C = low density lipoprotein cholesterol, *$p < 0.05$, Values are given as mean ± SEM (n = 6)

Table 1 Effect of pesticide exposure on fasting blood glucose, insulin, HOMA-IR and HOMA-β

Variables	Parents		
	Control group	Imidacloprid group	Chlorpyrifos group
FBG 1 (mg/dL)	80.4 ± 5.4[a]	79.6 ± 6.6[a]	82.0 ± 4.2[a]
FBG 2 (mg/dL)	76.4 ± 4.7[b]	95.8 ± 5.3[a]	100.0 ± 5.6[a]
Insulin (mU/L)	4.6 ± 0.6[b]	10.3 ± 1.4[a]	9.1 ± 1.3[a]
HOMA-IR	0.8 ± 0.2[b]	2.4 ± 0.4[a]	2.3 ± 0.4[a]
HOMA-β	141.8 ± 63.1[a]	133.4 ± 32.5[a]	103.9 ± 23.9[a]

Group 1= normal control, Group 2= imidacloprid, Group 3 = chlorpyrifos, FBG 1 = fasting blood glucose before treatment, FBG 2 = fasting blood glucose after treatment, HOMA-IR = homeostasis model assessment of insulin resistance, HOMA-β = homeostasis model assessment of beta cell function. Values are given as mean± SEM (n = 6). Means with common lower case letter in column do not differ significantly at 5 % ($p < 0.05$) level of significance

Table 2 Effect of pesticide exposure on lipid profile

Variables	Parents		
	Control group	Imidacloprid group	Chlorpyrifos group
TC (mg/dL)	83.3 ± 7.4[b]	126.5 ± 5.9[a]	103.5 ± 8.1[a,b]
TG (mg/dL)	69.50 ± 5.0[a]	86.8 ± 7.2[a]	77.8 ± 4.7[a]
HDL-C (mg/dL)	17.5 ± 1.0[a]	14.3 ± 0.5[b]	13.8 ± 0.9[b]
LDL-C (mg/dL)	49.1 ± 7.9[b]	99.5 ± 6.6[a]	79.0 ± 8.5[a]

Group 1= normal control, Group 2= imidacloprid, Group 3 = chlorpyrifos, TC = total cholesterol, TG = triglycerides, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol. Values are given as mean± SEM (n =6). Means with common lower case letter in column do not differ significantly at 5 % ($p < 0.05$) level of significance

Metabolic parameters in offspring of female Wistar rats

The results of the evaluation of metabolic parameters in offspring after weaning (4 weeks of age) and at adult age (12 weeks) are presented in Fig. 3. Fasting blood glucose, insulin and HOMA-IR values were significantly higher in the 4 weeks-old offspring of rats exposed to imidacloprid (67.4% FBG, 78.2% insulin and > 100% HOMA-IR), while rats exposed to chlorpyrifos had a significant increase in insulin (72.3%) and HOMA-IR (> 100%) levels when compared to rats in the control group. This increase was observed to persist until adult age, despite a reduction in magnitude in rats exposed to imidacloprid. However, HOMA-β values were not significantly different between the offspring of exposed and control groups sacrificed at weaning (152.3 ± 23.4–315.5 ± 65.4), as well as those sacrificed at adult age (128.1 ± 27.4–150.4 ± 87.9). Alteration in lipid profile parameters was equally recorded in the offspring of exposed rats sacrificed at weaning (22.1% and 16.9% increase in TC for IMI and CPF, 72.2% and 57.1% increase in LDL-cholesterol for IMI and CPF, respectively) and those sacrificed at adult age, compared to the control group (Fig. 4).

Glucose transporter 4 and nuclear factor kappa beta protein expression

In dams, the expression of GLUT4 was significantly lower in the imidacloprid (28.9%) and chlorpyrifos (42.3%) exposed groups compared to the control group (Fig. 5A), while NFKβ expression was significantly higher in the imidacloprid (35.4%) and chlorpyrifos (32.8%) groups compared to the control group (Fig. 5B). Similarly, in pups sacrificed at adult age, the expression of GLUT4 was significantly lower in imidacloprid exposed (52.8%) and chlorpyrifos exposed (54.5%) groups compared to the control group (Fig. 6A), while NFKβ expression was significantly higher in imidacloprid exposed (30.5%) and chlorpyrifos exposed (30.7%) groups compared to the control group (Fig. 6B).

Discussion

Pesticide poisoning is presently considered a public health problem in Africa. This is largely due to misuse of pesticides resulting from, among other factors, a lack of training, the discharge of remains into nearby rivers, and the absence of personal protection equipment during pesticide applications [33]. Chlorpyrifos, imidacloprid and other insecticides and fungicides are extensively used, with inhalation and ingestion being the main exposure routes [33–35]. This study is highly relevant as it investigates
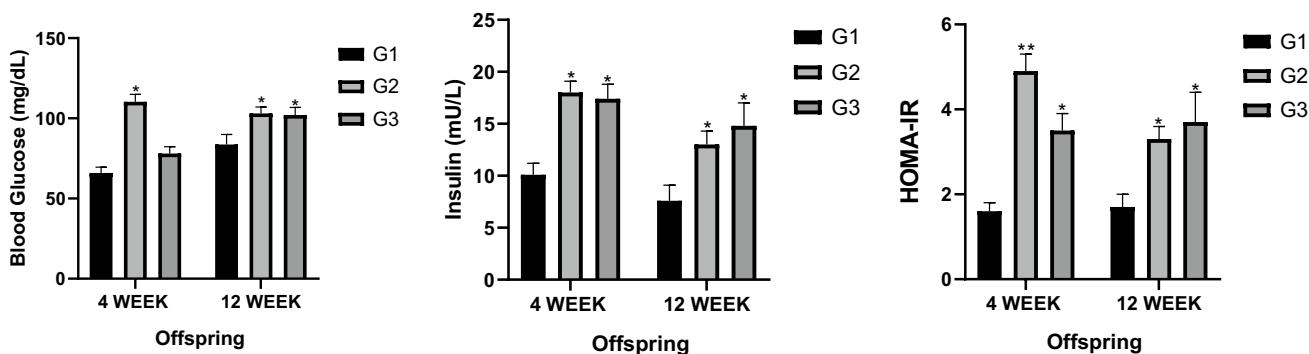
Fig. 3 Effect of pesticide exposure on fasting blood glucose, insulin, HOMA-IR and HOMA-β in offspring. G1 = normal control, G2 = imidacloprid, G3 = chlorpyrifos, HOMA-IR = homeostasis model assessment of insulin resistance, $*p < 0.05$, $**p < 0.01$, Values are given as mean ± SEM (n = 8)

Fig. 4 Effect of pesticide exposure on lipid profile in offspring. G1 = normal control, G2 = imidacloprid, G3 = chlorpyrifos, TC = total cholesterol, LDL–C = low density lipoprotein cholesterol, $*p < 0.05$, $**p < 0.01$, values are given as mean ± SEM (n = 8)
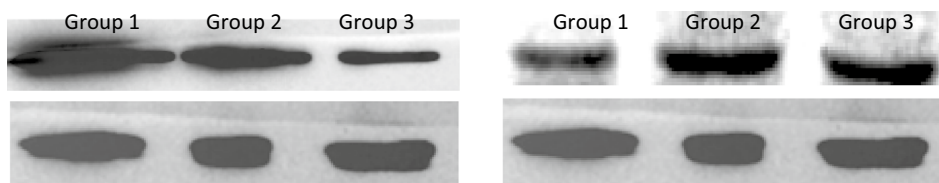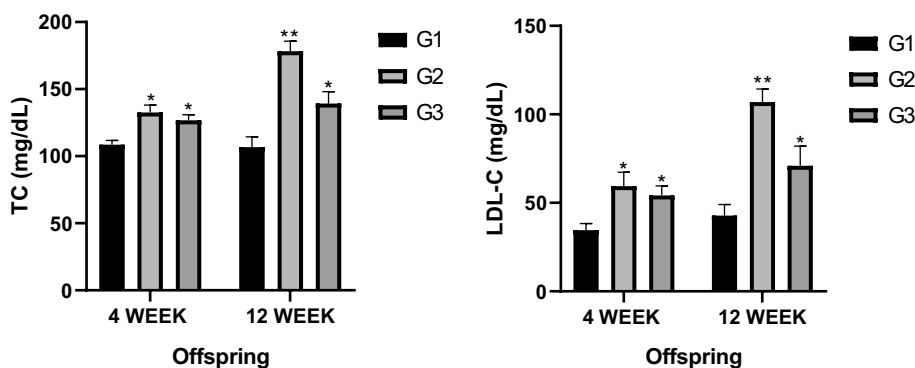

Fig. 5 Effect of imidacloprid on GLUT4 (left) and NFkβ (right) expression in exposed rats (n = 6). The blots below are beta actin control. Group 1 = normal control, Group 2 = imidacloprid, Group 3 = chloropyrifos. Data are reported as mean ± standard error of mean (SEM)
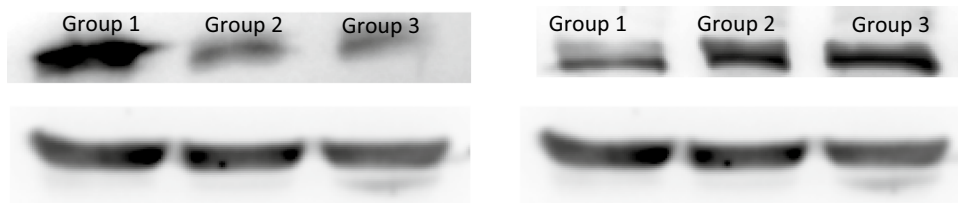

Fig. 6 Effect of imidacloprid on GLUT4 (left) and NFkβ (right) expression in offspring at adult age (n = 8). The blots below are beta actin control. Group 1 = normal control, Group 2 = imidacloprid, Group 3 = chloropyrifos. Data are reported as mean ± standard error of mean (SEM)

pesticides that are widely used for agricultural purposes as well as in public health disease control. We showed in previous studies that these pesticides were associated with the development of oxidative stress and lipid peroxidation [11], considered to be responsible for defects in glucose and lipid metabolism. Rats were exposed to a dose assumed to be the same as or inferior to a human exposure dose in an agricultural setting, with a similar mode of exposure. Given that chronic long-term exposure to pesticides has been reported to affect metabolic pathways, protein expression analysis was effected to confirm the possible mechanistic pathway. Investigations were also carried out on offspring exposed throughout gestation, to explore the intergenerational effects. The present study shows that exposure to imidacloprid and chlorpyrifos throughout gestation caused hyperglycemia, hyper insulinaemia and dyslipidemia in female Wistar rats. Our findings are in conformity with other studies which have reported hyperglycemic and hyperlipemic effects for chlorpyrifos and imidacloprid exposure [12–14].

Hyperglycemia and oxidative stress are well-known complications/outcomes of pesticide exposure [11, 36]. Moreover, the oxidative stress caused by pesticide exposure may exacerbate hyperglycemia and vice versa. Both conditions (oxidative stress and hyperglycemia) contribute to an oxidative environment that may alter insulin sensitivity, either by increasing insulin resistance or impairing glucose tolerance. A significantly high HOMA-IR value characteristic of insulin resistance in pesticide-exposed rats confirms the insulin resistant effect of imidacloprid and chlorpyrifos through the oxidative stress and/or hyperglycemic pathway. The possible link between oxidative stress and insulin resistance include ROS impaired insulin signaling caused by inducing IRS serine/threonine phosphorylation, disturbing cellular redistribution of insulin signaling components, decreasing GLUT4 gene transcription, or altering mitochondrial activity [37, 38]. Our study reported low expression levels of GLUT4 proteins in pesticide exposed rats, which should result from a decrease in gene transcription. Chronic oxidative stress has also been reported to induce a number of stress-sensitive signaling pathways, such as NF-κB JNK/SAPK, and p38 MAPK. Our study confirms such findings, as oxidative stress resulting from pesticide exposure significantly increased NF-κB protein levels.

Metabolic alterations in exposed parents were equally observed in their offspring, persisting until adult age despite no postnatal exposure. Our study therefore provides new findings as it is the first to report metabolic alterations such as hyperglycemia, dyslipidemia, insulin resistance as well as alterations in GLUT4 and NFkβ protein expression in offspring with in utero exposure. These were in evidence at weaning and persisted with similar magnitude at adult age without additional exposure.

This suggests that chronic exposure to pesticides induces permanent changes affecting the metabolic pathway directly or through the cholinergic system. Given that the pesticides were neither quantified in the biological fluids of parents nor offspring, we cannot affirm that the adverse effects observed in offspring were mainly due to fetal exposure, as alterations in the parents could be transmitted to offspring through fetal programming. Another limitation is the fact that we did not study the second generation necessary to establish putative trans-generational effect, or conduct methylation analysis to confirm the effect on the epigenome as well as the expression of other proteins involved in the insulin signaling pathway such as pPKB, p-mTOR, and p-IRS-1.

The 7th edition of the International Diabetes Federation Atlas shows that Africa will be faced with the highest increment in the prevalence of diabetes by 2040 [39]. It is also a continent that relies heavily on pesticide applications for the control of agricultural and household pests. With the possible metabolic alterations observed in this study, especially in offspring that persist until adult age, it seems likely that pesticide is a major contributor to the global burden of diabetes. The entire human population should be therefore be sensitized on the adverse effects of these chemicals, while a search is conducted for a biological replacement, since pesticides are essential to agriculture. Additionally, there is a need for further study of the epigenome to investigate the molecular mechanisms, with emphases on the insulin signaling pathway and on the second and third generations, to ascertain the trans-generational effects. Studies in humans are equally needed to create awareness and enable policy makers to regulate the use of these pesticides.

Imidacloprid and chlorpyrifos induce hyperglycemia, insulin resistance and dyslipidemia in female nulliparous Wistar rats and their offspring with in utero exposure throughout gestation. The biochemical alterations in offspring remain until adult age, suggesting that the chemicals responsible persist in the offspring even after the cessation of exposure, or modify the epigenome through epigenetic mechanisms. Further research on the epigenome is therefore required to investigate these molecular mechanisms.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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