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Exposure to THI during gestation and lactation impairs the striatum, hippocampus and cognition in rat offspring (G1/G2): The role of apricot kernel extract

Dounia Djellal[1]*, Zehoura Lakroune[2], Salim Gasmi[3], Mohamed Kebieche[1]*, Smail Chafaa[1], Fateh Mimeche[4], Hamadi Fetoui[5] & Rachid Soulimani[6]

[1]Faculty of natural and life sciences, LPTPCMB laboratory, University of Batna 2, Constantine Road, 05078, Fesdis, Batna, Algeria

[2]University of Mohamed Seddik Ben Yahia, Jijel, 18000 Algeria

[3]University of Tebessa, Faculty of Exact Sciences and Natural and Life Sciences, Tebessa, Algeria

[4]Department of Agricultural Sciences, Faculty of Sciences, University of M'Sila, 28000 M'Sila, Algeria

[5]Toxicology-Microbiology and Environmental Health Unit (UR11ES70), University of Sfax, Tunisia

[6]LCOMS/ Neurotoxicology and Bioactivity, University of Lorraine, Metz, France

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Thiacloprid (THI) is a widely used neonicotinoid suspected to exert neurotoxic effects in mammals, while bitter apricot kernels are traditionally used in folk medicine for the treatment of various diseases. In the context of limited data on thiacloprid-induced multigenerational neurotoxicity, this study aimed to evaluate the neurotoxic effects of THI exposure in utero and during lactation in rat offspring, as well as to investigate the neuroprotective potential of bitter apricot kernel extract as a natural protective strategy. Female rats were gavaged with 0.02 mg/kg THI or/and 50 mg/kg bitter apricot kernel extract from day1 of gestation to day21 of lactation, neurobehavioral tests were performed to assess the cognitive function of the rats' offspring. In addition, bioanalytical methods were performed to determine swelling and mitochondrial permeability, AchE and cytochrome-c activities, and redox status in striatal and hippocampal mitochondria of rat generations (G1, G2) at 100 days of age. The results showed that thiacloprid exposure impaired neurological behavior and cognitive performance, including memory and learning, in both the G1 and G2 offspring. It also induced mitochondrial edema and disrupted the cellular redox status. Conversely, bitter apricot kernel extract exhibited a significant cytoprotective effect against this neurotoxicity. Finally, thiacloprid demonstrates multigenerational neurotoxicity, leading to neuronal impairments, which can be mitigated by apricot kernel extract.

Keywords: Thiacloprid, brain mitochondria, oxidative stress, cognition, multigenerational toxicity

Introduction

Neonicotinoids are a class of neurotoxic insecticide widely used around the world. They are less toxic to mammals than the organophosphate and carbamate insecticides previously used[1]. Over the past two decades, three families of neonicotinoids have been on the market: N-nitroimines (imidacloprid, thiamethoxam, clothianidin and dinotefuran); N-cyanoimines (acetamiprid and THI); and nitromethylenes (nitenpyram)[2]. Thiacloprid (THI), (3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidin-2-ylidenecyanamide) is a neonicotinoid insecticide that is widely used to protect vegetable and fruit crops, as well as many other plant species, against insects[3,4]. Several previous studies have demonstrated that THI produces immunotoxic, mutagenic and neurotoxic effects, as well as reproductive toxicity and endocrine disruption in experimental animals[5]. In addition, neonicotinoid insecticides are known to cause a range of cognitive disorders[6,7]. More recently, studies have shown that exposure to this class of insecticides during pregnancy and lactation induces behavioural abnormalities[8,9] and delays sexual maturation in mammals[10]. Another study revealed that the neonicotinoid insecticide clothianidin decreases the number of germ cells in male mice[11]. Furthermore, Oladosu and Flaws results showed that in utero and lactational exposure to imidacloprid increases alanine aminotransferase and glucose 6-phosphate dehydrogenase activity, while decreasing acetylcholine esterase activity, in the plasma and brains of generation 2 rats[12]. In addition, a recent study suggests that THI is a neurotoxic insecticide for vertebrate embryos and possibly humans. This insecticide increases markers of oxidative stress, such as protein carbonyl and

*Correspondence:
E-mail: m.kebieche@univ-batna2.dz
E-mail:douniadjellal1994@gmail.com

malondialdehyde (MDA) content, as well as DNA damage. It also increases the over expression of pro-inflammatory cytokines (IFN-γ; interferon gamma, tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), as well as stress-related and apoptotic genes (NF-κB and caspase-3) in the brain tissue of a chicken embryo[13]. It is worth noting that herbal medicines are widely used around the world due to their high content of natural compounds that are used as effective therapeutic agents to promote health, treat and cure various diseases[14]. The apricot (*Prunus armeniaca* L.) is a member of the Rosaceae family and is mainly cultivated in mediterranean regions[15]. Indeed, its kernels have antioxidant, antibacterial and anti-inflammatory properties[16,17]. Thanks to their richness in 40.23–53.19% total oil, phenolic compounds, vitamins, minerals, protein and fibre[18–20], as well as amygdalin, also known as vitamin B17[21]. However, the reviewed data on the brain toxicity of THI does not provide sufficient evidence regarding regional neurotoxicity or cognition, regardless of the cytoprotective power of apricot kernel extract. Therefore, a study evaluating the in utero and lactational neurotoxicity of THI in rat striatum and hippocampus, as well as the cytoprotective effect of bitter apricot kernels through two generations of male and female rats (G1, G2), could contribute to the scientific literature.

Materials and Methods

Plant material

Apricot fruits (*Prunus armeniaca* L.) were collected in M'sila (Algeria) in 2020 and were authenticated by Dr. Farid Bakdouche, professor of botany in the faculty of natural and life sciences, University of Batna 2. A voucher specimen (FL. N° 10/2020) has been deposited at the Herbarium of the Faculty, University of Batna 2. Then, the apricot kernels were separated from the shell and pulp, dried and then crushed using an electric grinder and stored in plastic bags until use.

Extraction

The hydroalcoholic extract was prepared according to Minaiyan's method[22], whereby 30 g of almond powder was mixed with 90 mL of aqueous ethanol (70:30) and stirred for 72 hours. The resulting suspension was filtered through Whatman No. 1 filter paper, concentrated using a rotary evaporator and placed in Petri boxes. The boxes were then dried in an oven at 40 °C until a dry extract was obtained. The dried extracts were stored at 4°C in hermetically sealed containers.

Most chemicals were supplied by Sigma-Aldrich, Germany. The selected insecticide is based mostly on THI; its trade name is Calypso (suspension concentrate with 480 g/L), soluble in water was purchased from Bayer AG (Germany) at a dose of 0.020 mg/kg, converted to a rat-equivalent daily dose using EFSA-recommended conversion factors[23]. This dose was justified by the fact that, the Acceptable Daily Intake (ADI) for thiacloprid is 0.01 mg/kg bw per day. The NOAEL for systemic toxicity in rats is 1.2 mg/kg bw per day[24]. Our selected dose (0.020 mg/kg) is twice the ADI but remains significantly below the NOAEL, representing a low-dose, environmentally relevant chronic exposure scenario mimicking dietary intake from contaminated food matrices (e.g., tomatoes), as reported by Alhamami *et al.*[25]. The hydroalcoholic extract of bitter apricot kernels was administered at a dose of 50 mg/kg, as previously described and validated for its protective effects against pesticide-induced toxicity[26].

Chemical analysis of apricot Kernels extract

Determination of total polyphenols

Total phenolic compounds were quantified using the Folin-Ciocalteu method according to Singleton and Rossi[27]. This method is based on the reduction of the phosphotungstic and phosphomolybdic mixture in the Folin-Ciocalteu reagent by the phenolic compounds' reducing groups in an alkaline medium. This leads to the formation of a blue-coloured reduction product. This exhibits an absorption maximum at 765 nm[28]. The determination of polyphenols was carried out according to the procedure of Nwozo *et al.*[29]. 200 µL of the extract was added to 1000 µL of Folin-Ciocalteu reagent (10%), and after four minutes, 800 µL of an aqueous sodium bicarbonate solution (10%) was added to the reaction medium. After two hours of incubation at room temperature (22±2°C) in the dark, the absorbance was measured at 765 nm. In parallel, a calibration range with gallic acid (0–100 µg/mL) was established; the results are expressed in µg gallic acid equivalent per mg extract (µg GAE/mg extract)[30].

Determination of total flavonoids

Total flavonoids were determined according to the aluminium trichloride method described by Bahorun[31]. It should be noted that flavonoids form yellow colour complexes with aluminium chloride, and the intensity of these complexes can be measured at 430 nm[32]. In brief, 1 mL of the extract is added to 1 mL of a 2% aluminium trichloride solution prepared in methanol.

After 10 minutes of incubation, the absorbance is measured at 430 nm. The flavonoid concentration is determined from a calibration range established using quercetin (2.5–40 µg/mL), and the results are expressed as µg of quercetin equivalent per mg of extract (µg EQ/mg extract).

The different compounds in the hydroalcoholic extract were identified by HPLC-DAD

HPLC is a simple method for identifying and quantifying the different metabolites in an extract. In this study, the different compounds in the sample were analysed using an HPLC-DAD system (Shimadzu), model: CTO-20A Enable Oven with SIL-20ACXR Enable Autosampler; one Hypersil Gold reversed-phase Ultra C18 column (250 x 4.6 mm ID); and one SPD-M20A diode array detector. The flow rate remained constant at 1 mL/min throughout the analysis, and the injection volume was 10 µL. The operating conditions were as follows: solvent A (1% acetic acid/ultrapure water) and solvent B (acetonitrile), with the following gradient: 10% solvent B from 0.01 to 55 min, then 90% solvent A; 100% solvent B from 55 to 65 min (with 0% solvent A); 10% solvent B from 65 to 75 min; and 90% solvent A. The column was kept at 30 °C and UV-visible detection was recorded in the wavelength range of 190–800 nm. The extract compounds were identified by comparing the retention times of the sample spectrograms with the standard values used[33].

Animal treatments

Female and male rats (albino Wistar) were purchased from the Pasteur Institute (Algeria) and housed in the animal house of the University of Batna 2, with female rats (n=16) weighing approximately 180 g at 8 weeks of age and males (n=8) at 12 weeks of age. Rats were housed in cages of large size (70X40 X 30 cm), with double bottles excluding any crowding effect and they had free access to rodent food and distilled water at a controlled temperature (23±2 °C) and relative humidity (60%) with a 12 h day/night cycle. After15 days of adaptation period, every two females were mated with an untreated male per night. The following morning, a vaginal smear was observed under a microscope to confirm pregnancy. The parturition date for each female was designated as postnatal day 0 (PND 0). Pregnant rats were randomly distributed to four treatment groups (n=4/group) as follows:

1. Control (CON): receives distilled water during the gestation and lactation periods.
2. THI: treated with 0.020 mg/kg THI during gestation and lactation.
3. Extract (EXT): treated with 50 mg/kg of hydroalcoholic extract of bitter apricot kernels during gestation and lactation.
4. THI + extract (THI+EXT): treated with extract (50mg/kg/day) and THI (0.020 mg/kg/day) during gestation and lactation.

All experimental assays were carried out in conformity with international guidelines for the care and use of laboratory animals. In this study all animal experiments were approved by PIA according to the code of ethics: No. Batna-Univ 2.2020.231.

Generations G1 and G2

For generation 1, eight males (n = 8) and 16 females were kept for reproduction, and 24 males and 24 females were used for experiments. G1 females (at 8 weeks of age, n=16) were mated with G1 males at 12 weeks of age (n=8) to yield the G2 generation. G1 males and females were exposed to treatments *in utero* and during lactation; whereasG2 animals were not directly exposed. Therefore, G1 animals were considered intergenerational, and the effects observed in G2 were considered multigenerational[34]. On postnatal day 100, G1 and G2 males and females underwent behavioral tests and were subsequently sacrificed for biochemical analyses.

Behavioural and cognitive tests

Anxiety-Like Measurement

Open field test (OF)

The open-field test was used to assess locomotion and anxiety-like behaviors. This test consisted of an arena (50cm × cm 50× [50H] cm). The arena was divided into 25 squares, in this test, each rat was placed in the center of the arena, and its behavior was recorded for 5min with a camera mounted above the open field[35], total distance traveled (ambulation), and the number of rearing (vertical movement activities) were recorded for each rat[36].

Elevated Plus Maze (EPM)

The EPM test is used to assess anxiety behaviour[37].This test consisted of two arms closed by a 50 cm high wall and two open arms ledge 0.5 cm; the arms were placed at 50 cm height. In this test, the rats were placed in the center of the arena facing a closed arm, and the time spent in the test arms was recorded for 5min with a camera mounted above the test[38], The number of entries in the open or closed

arms (entries in the open or closed arms/total entries in the arms) and the percentage of time in the open or closed arms (time on the open or closed arms/total arms) were calculated[39].

Evaluation of working memory and learning ability

Novel Object Recognition test (NOR)

NOR is a test of memory and spatial learning. Briefly, the rats were accustomed to the test arena (the same arena as the open field test) in the absence of any object for 5 min; this is the adaptation phase. After 24 hours, the rats were placed in the arena for the familiarization phase with two identical objects for 10 min; a day later, each rat was placed in the arena for 5 minutes for the test phase with one of the two objects replaced by a novel object. The behaviour was observed and analyzed using a video camera[40], at the end we quantified the recognition index, which was defined as the ratio of time spent exploring a novel object to the total time spent exploring both objects, multiplied by 100[41].

Assessment of the muscle strength of treated animals

Konziela's Inverted Screen test (KIS)

Konziela's inverted screen test is used to assess muscle strength in animals. This test consists of placing the rat in the center of a porous metal screen with wooden borders; the test procedure consists of placing the rat in the center of the metal screen; then; the screen is rotated to the inverted position. The time the rat falls is counted. If the time is more than 60 s, the rat gets the highest score (4). If this time is between 1 and 10 s, the rat takes a score of 1; if it is between 10 and 26 s, it takes a score of 2; between 26 and 60, it takes a score of 3[42].

Biochemical analysis

Separation of the mitochondrial matrix from the striatum and hippocampus

Striatum or hippocampus was placed in TSE buffer (10mM tris, 250MM sucrose, and 1mM EDTA, pH= 7.2) at +4°C and homogenized to obtain a 10% tissue homogenate. Then, the homogenate was centrifuged at 600g for10 min at +4°C. The supernatant was centrifuged at 10,000g for 10 min, +4°C to obtain mitochondria in the pellet. In the next step, the pellet was washed twice and suspended in TS buffer (10mM tris, 250 mM sucrose, pH=7.2) at +4°C[43] for mitochondrial matrix. The mitochondrial suspension was then frozen and thawed 8 times with repeated potting, then, this suspension was centrifuged at 9600 rpm for 10 minutes, the obtained supernatant was used as a source of stress parameters such as (GSH, SOD, CAT, GST and MDA), all operations were performed on ice[44].

Evaluation of Mitochondria swelling and permeability

Mitochondrial swelling was performed according to the method of Nègre-Salvayre and Salvayre [45], the assessment of mitochondrial permeability based on the rate of Ca++ crossing the inner membrane of mitochondria, which leads to the increase in mitochondrial size detected at the wavelength of 540 nm for 3 minutes and every 30 seconds.

Evaluation of oxidative stress in the striatum and hippocampus mitochondria

Mitochondrial Protein concentration

Protein content was estimated by the method of Bradford[46] using bovine serum albumin as a standard.

Measurement of catalase activity

Catalase activity was performed according to the method of Clairbone[47]; the disappearance of hydrogen peroxide is recorded each 30 s for three minutes by a spectrophotometer at a wavelength of 240 nm.

Measurement of SOD activity

SOD activity was assessed using the method of Beauchamp and Fridovich[48], which is based on the ability of the SOD enzyme to inhibit the reaction between tetrazolium nitroblue (NBT) and the superoxide anion that is produced by the photoreaction of oxygen and riboflavin in the presence of an electron donor such as methionine. This reaction can be followed by spectrophotometry at 560 nm.

Assessment of the MDA levels

MDA was measured by the method of Trostchansky *et al.*[49] this method is based on the reaction of thiobarbituric acid with MDA, measured by a spectrophotometer at 532 nm.

Dosage of Glutathione (GSH)

Glutathione was determined according to the method of Ellman[50] and Moron *et al.*[51], which is based on measuring the absorbance of 2nitro-5-mercapturic at 412nm.

Measurement of glutathione -s-transferase activity (GST)

GST was determined according to the method of Habig *et al.*[52]. This enzyme binds and metabolizes glutathione with the help of the cofactor CDNB (1Chloro-2,4dinitrobenzene); the result of this

reaction is 1Sglutathionyl-2,4-dinitrobenzene, where with the help of this compound the activity of the GST is measured.

Preparation of the cytosolic fraction

Striatum and hippocampus were collected for homogenization in 3 ml of phosphate buffer containing 17% KCl; after homogenization, the samples were centrifuged at 3000 g for 10 min. The supernatant was then centrifuged at 10,000 g for 30 min. The final supernatant is called the "cytosolic fraction"[53].

Measurement of the enzymatic activity of acetylcholinesterase (Ach E)

The activity of AchE was determined by the method of Wang *et al.*[54]. This method was based on the increase in yellow color produced by thiocholine when it reacts with the dithiobisnitrobenzoate (DTNB) ion. Optical density was read at 412nm each 4min for 20min.

Ca++ cytosolic assay

Cytosolic calcium concentration was estimated with a commercial kit manufactured by SPINREACT, SPAIN was assessed using the method described by Akabari *et al.*[55], using a UV/Visible spectrophotometer, results are expressed in mmol/l[56].

Measurement of cytochrome c

After brain extraction of animals and conservation in the formalin solution[57], before the preparation of cytosol, the concentration of cytochrome c was determined using a commercial kit (CYTOCOX1); this colorimetric assay kit is based on the decrease of the absorbance of ferrocytochrome c, which is measured at 550 nm.

Histological Analysis

Brain tissue sections from G1 were prepared according to a standard histological protocol. Organs were fixed by immersion in a large volume of formalin. The tissues were then dehydrated through a graded ethanol series (70%, 95%, and 100%). Following dehydration, samples were cleared in two successive xylene baths, each lasting 45 minutes. Tissues were subsequently embedded in two successive baths of molten paraffin for one hour each at 56°C and then poured into plastic molds for solidification. Paraffin blocks were sectioned using a microtome at a thickness of 7µm. Sections were mounted on glass slides previously coated with an adhesive solution containing 2 g of albumin and 50 mL of glycerin dissolved in 1000 mL of distilled water. After deparaffinization, sections were stained with hematoxylin–eosin (H & amp; E) and cresyl violet for neuronal identification. This staining allowed the evaluation of cellular and tissue abnormalities. After drying, photomicrographs were taken using a microscope equipped with a camera (Zeiss)[58].

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). ANOVA and Tukey's test were used for statistical analysis using XLSTAT 2014.5.03 software. The differences were considered statistically significant at $P < 0.05$. The result of the comparison is as follows:

- (ns) $P > 0.05$ = difference is not significant ; - (*) $0.05 > P > 0.01$ = difference is significant ; - (**) $0.01 > P > 0.001$ = difference is highly significant, - (***) $P < 0.001$ = difference is very highly significant, groups compared to control group, #$P < 0.05$= difference is significant; ##$P < 0.01$= difference is highly significant; ###$P < 0.001$= difference is very highly significant), groups compared to THI group. Due to the complexity of the multifactorial design, the present study employed one-way ANOVA followed by Tukey's post hoc tests for statistical analyses in both female and male. The independence or interaction of factors such as gender and generations was not directly assessed in the current study.

Results

Chemical study of hydroethanolic Kernel extract

Determination of polyphenols and flavonoids

The content of total polyphenols in the extract was carried out with reference to the calibration range for gallic acid (y= 0,0109, R^2= 0,997). This content is expressed as the microgram equivalent of gallic acid per milligram of extract (µg GAE/ mg Extract). The total phenolic content in the examined extract was 48.8 µg GAE/mg extract.

The flavonoid assay was carried out by an aluminum trichloride (AlCl3) method using quercetin as a standard (y=0.0368, R^2=0.999), the flavonoid content of the extract is expressed as µg EQ/ mg Extract. The results of this assay show that the flavonoid content of the bitter apricot kernels extract is 8.07 µg EQ/ mg Ext.

Identification of the different compounds of the hydroalcoholic extract by HPLC-DAD

The identification of these substances contained in the extracts is done by comparing the retention time

of the peaks of the standards used to those obtained after the analysis of the extract. The chromatographic profiles of the EXT are represented in Fig. 1, while the results of qualitative analysis are given in Table 1. The results showed that the extract contains: caffeic acid, gallic acid, vanillic acid, tannic acid, catechin, quercitin, genistein, gossypin, naringin, rutin, sylimarin, amygdalin, dimethoxyflavone. It should be noted that this study reports qualitative identification only.

Assessment of behavioral and cognitive changes

Anxiety-Like Measurement

Assessment of rats' anxiety by OF test

The Open Field test was used to analyze the motor activity of adult offspring (reflected by total distance traveled); the results of this study showed a significant decrease in total distance traveled (ambulation) and rearing in males and females in the G1 and G2 groups treated with THI compared to the control group. A significant increase was observed in the distance traveled by males and females in the G1 group treated with the extract, but no significant difference was observed in ambulation or rearing in the same group in the G2 generation compared to the THI group (Figs. 2 and 3).

Evaluation of anxiety effects of the animals by EPM test

EPM test was used to assess anxiety; in this test, G1 females of THI treated group spent more time in closed arms than males compared to the control group, and G1 females of extract treated group spent more time in open arms than males compared to THI group. Moreover, a significant decrease in the number of entries into the open and closed arms in the THI-treated

Table 1 — HPLC-DAD peaks and their retention times for the extract of bitter kernels of *P. armenica* L. apricot nuts and the different standards

Peaks	Compounds	Retention time of the standards	Retention time of the extract's compounds
1	Tannic acid	2.82	2.89
2	Gallic acid	3.77	3.72
3	Caffeic acid	11.07	11.02
4	Vanillic acid	1.80	1.85
5	Catechin	9.19	9.23
6	Genistein	3.52	3.49
7	Gossypine	2.56	2.62
8	Naringin	15.25	15.27
9	Quercétine	21.80	21.84
10	Rutin	13.57	13.63
11	Sylimarin	18.60	18.66
12	Amygdalin	10.50	10.43
13	Dimethoxyflavone	14.29	14.32

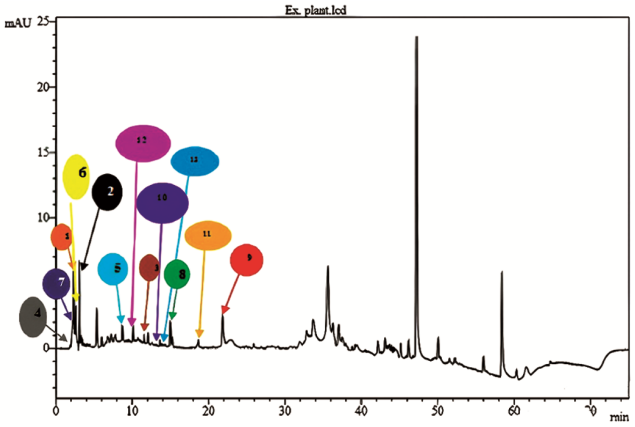

Fig. 1 — HPLC-DAD peaks of the hydroalcoholic extract of bitter apricot Kernels *P. armeniaca* L.

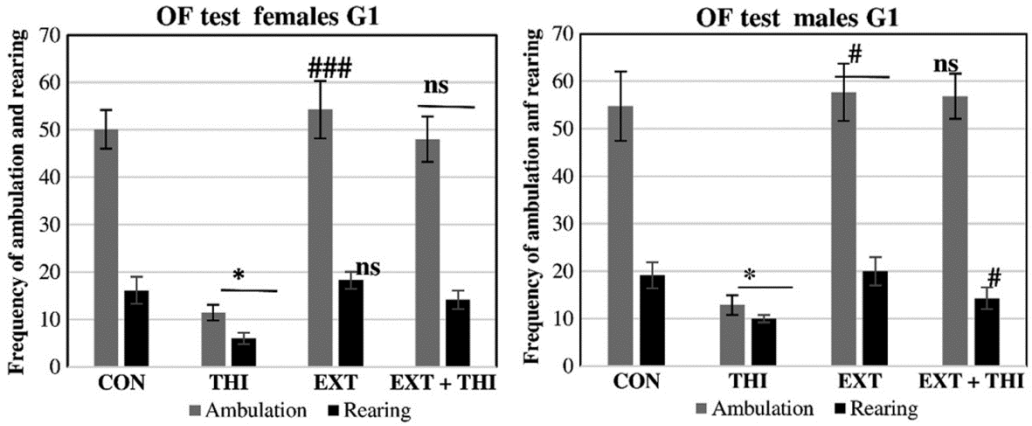

Fig. 2 — Evaluation of the level of anxiety in thefemales and males' rats of G1 exposed *in utero* and during lactation to THI and extract, THI + extract using the Open Field test (frequency of ambulation and rearing). Values are means ± SD, (n= 7), *P* value was detected compared to control group. ns $P > 0.05$: not significant, *$P<0.05$: the difference is significant, and the difference is significant (#) $0.05 > P > 0.01$, the difference was very highly significant (###) compared to THI group.

group of G1 rats compared to the control group. However, the males and females of G1 treated with extract made more entries into the open and closed arms compared to the THI group (Table 2), and the same results were observed in the G2 rats (Table 3)

Evaluation of working memory and learning ability Novel Object Recognition test (NOR)

The recognition index showed a significant decrease in both G1 and G2 males and females treated with THI compared to the control group; this recognition index was improved in all groups treated with hydroalcoholic extract compared to the THI group (Fig. 4).

Assessment of the muscle strength of treated animals Konziela's Inverted Screen test (KIS)

According to the results of the KIS test, the fixation time of rats in the inverted screen recorded a very highly significant increase ($P < 0.001$) in G1 rats treated with THI compared to the control group. On the other hand, a very highly significant decrease in the fixation time of

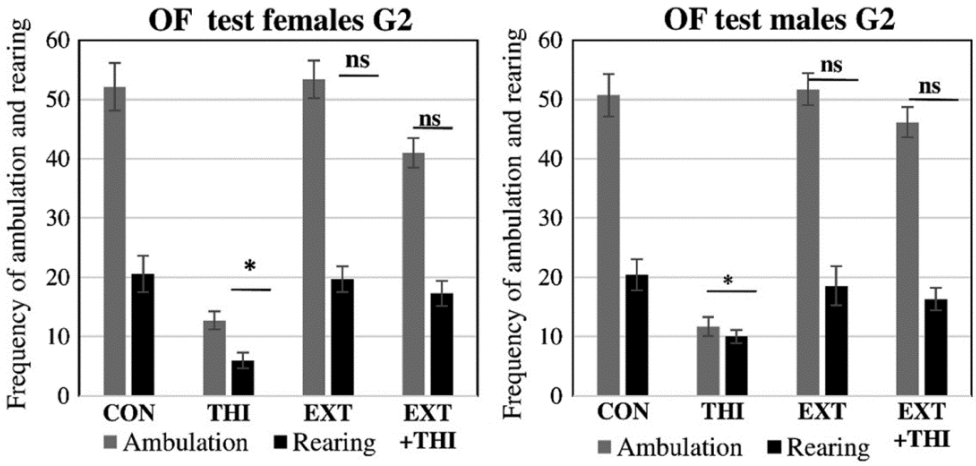

Fig. 3 — Evaluation of the level of anxiety in the females and males' rats of G2 exposed *in utero* and during lactation to THI and extract, THI + extract using the Open Field test (frequency of ambulation and rearing). Values are means ± SD, (n= 7), *P* value was detected compared to control group. ns $P > 0.05$: not significant, *$P<0.05$: the difference is significant, and ns $P > 0.05$: not significant compared to THI group.

Table 2 — Assessment of anxiety effects of females and males' rats of G1exposed *in utero* and during lactation to THI and extract, THI + extract using the EPM test

	Time spent in closed arms (%)		Time spent in open arms (%)		number of entries in open and closed arms	
	Females G1	Males G1	Females G1	Males G1	Females G1	Males G1
CON	6,71± 1,11	16,57±1,72	93,29±1,11	8,29±4,15**	14,71±2,69	1,57±0,53**
THI	92,00±4,40**	92,71±4,68*	82,43±1,27##	70,43±1,90#	14,57±2,70#	13,00±2,52 ns
EXT	17,57±1,27##	19,86±1,35#	83,43±1,72	5,00±4,68*	15,57±2,82	29±0,76**
THI+ EXT	29,57±1,90#	71±1,38#	80,14±1,35#	83,00±1,38#	15,29±2,36#	14,57±2,82#

Values are means ± SD, (n= 7), ns $P > 0.05$: not significant, *$P \leq 0.05$: significant; **$P \leq 0.01$: highly significant compared to control group, and the difference is significant (#) $0.05 > P > 0.01$, ##$P \leq 0.01$: highly significant compared to thiacloprid group.

Table 3 — Assessment of anxiety effectsof females and males' rats of G2 exposed in utero and during lactation to thiacloprid and extract, thiacloprid + extract using the EPM test

	Time spent in closed arms (%)		Time spent in open arms (%)		number of entries in open and closed arms	
	Females G2	Males G2	Females G2	Males G2	Females G2	Males G2
CON	12,14±4,78	88,00±1,73*	89,29±5,29	90,57±4,08	15,43±3,95	2,86± 1,57*
THI	9,43± 4,08	81,43±1,62**	12,00±1,73*	5,00± 1,62*	16,57±3,78	3,86±1,68*
EXT	8,29±3,20ns	10,29±4,89#	91,71±3,20ns	78,43±3,10ns	15,43±1,57ns	17,43±2,76ns
THI+ EXT	21,57±3,10ns	20,14± 2,67ns	89,71±4,89#	83,00±2,67ns	13,86±3,24ns	15,00±2,71ns

Values are means ± SD, (n= 7), ns $P > 0.05$: not significant, *$P \leq 0.05$: significant; **$P \leq 0.01$: highly significant compared to control group, and the difference is significant (#) $0.05 > P > 0.01$compared to thiacloprid group.

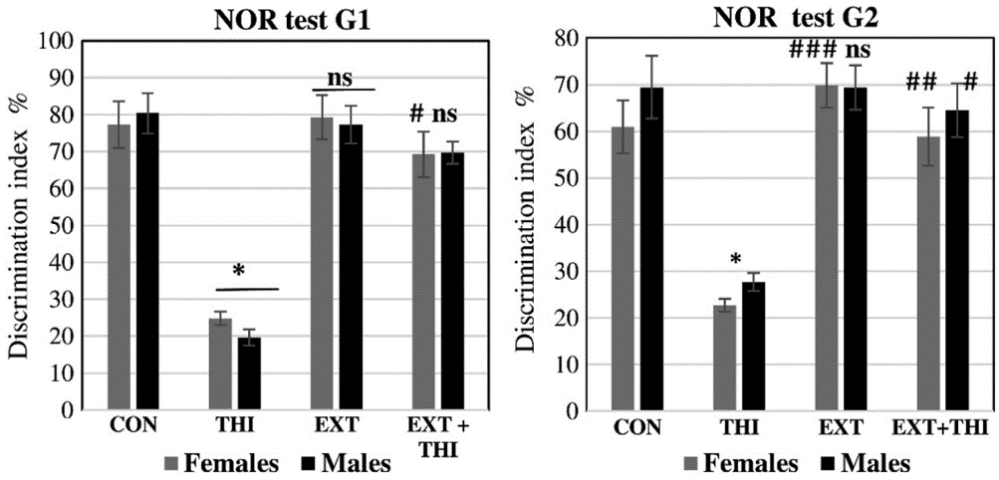

Fig. 4 — Evaluation of working memory and learning ability in the females and males' rats of G1 and G2 exposed *in utero* and during lactation to THI and extract, THI + extract using the NOR test. Values are means ± SD, (n= 7), *P* value was detected compared to control group. ns $P > 0.05$: not significant, *$P<0.05$: the difference is significant.and the difference is significant (#) $0.05 > P > 0.01$, the difference was highly significant (##) compared to THI group.

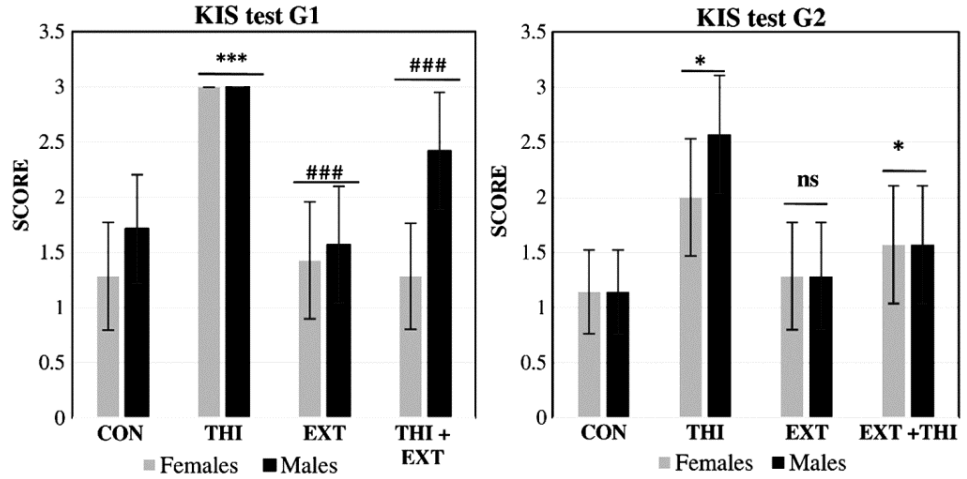

Fig. 5 — Evaluation of the muscle strength of females and males' rats of G1 and G2 treated *in utero* and during lactation with THI and extract and THI + extract using the KIS test. Values are means ± SD, (n= 7), p value was detected compared to control group. ns $P > 0.05$: not significant, *$P<0.05$: the difference is significant, ***$P \leq 0.001$: is very highly significant.and the difference was very highly significant (###) compared to THI group.

G1 rats in the inverted screen recorded in the extract treated group compared to the THI group, no significant difference ($P> 0.05$) recorded in the stabilization time of second generation G2 rats in the inverted screen compared to the control group (Fig. 5).

Biochemical exploration results

Evaluation of cholinergic function (AchE)

The results of the present study show a highly significant decrease and a significant decrease in the enzymatic activity of AchE in the hippocampus and striatum, respectively, in male and female rats of G1 and G2 treated with THI in comparison with the control group. This activity was significantly improved in the rats of G1 treated with the extract and the combination (EXT+THI) in both brain regions in comparison with the THI group (Figs. 6 and 7).

Cytosolic Ca^{++}

Cytosolic calcium concentration showed a highly significant increase in the striatum and hippocampus in G1 rats treated with THI compared to the control group. Also, G1 rats treated with extract and EXT+ THI recorded a highly significant decrease in this parameter in both brain regions compared to the THI group (Fig. 8); for G2 rats, the THI-treated rats

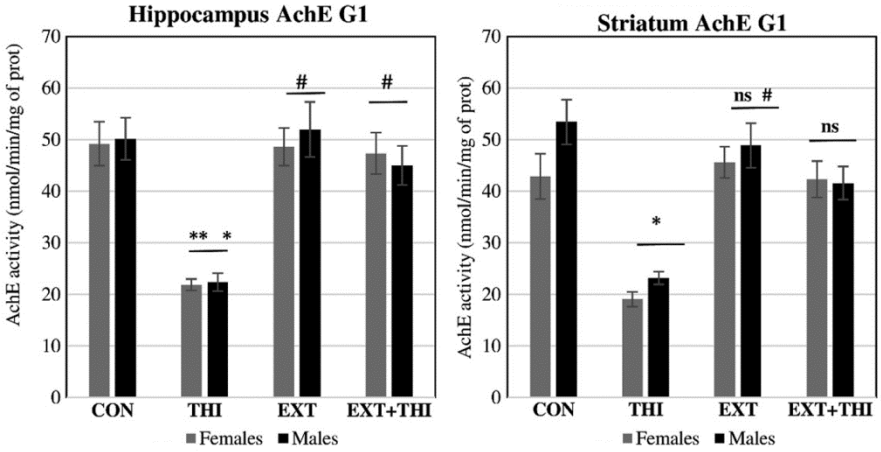

Fig. 6 — Variation of AchE activity in the hippocampus and striatum of females and males rats of G1exposed *in utero* and during lactation to THI and extract, THI + extract, Values are means ± SD, (n= 7);ns $P > 0.05$: not significant,*$P \leq 0.05$:the deference is significant; **$P \leq 0.01$: highly significant compared to control group, #$P \leq 0.05$: the deference is significant compared to THI group.

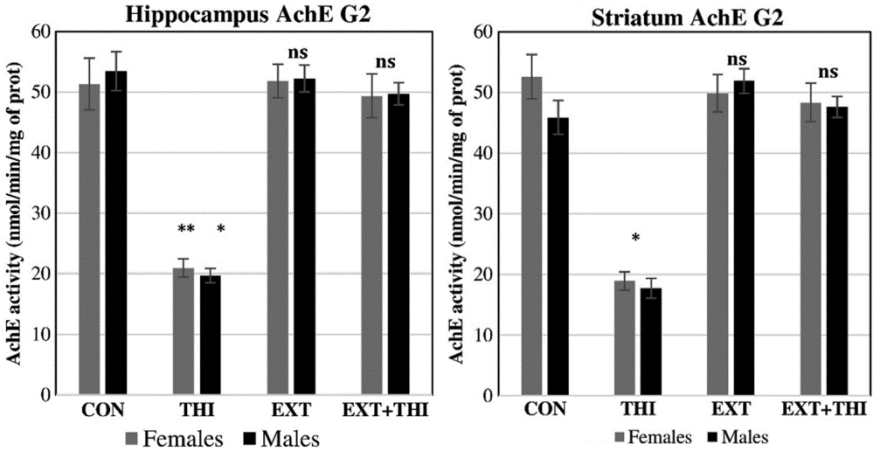

Fig. 7 — Variation of AchE activity in the hippocampus and striatum of females and males' rats of G2exposed in utero and during lactation to THI and extract, THI + extract. Values are means ± SD, (n= 7); ns $P > 0.05$: not significant, *$P \leq 0.05$: the deference is significant; **$P \leq 0.01$: highly significant compared to control group.

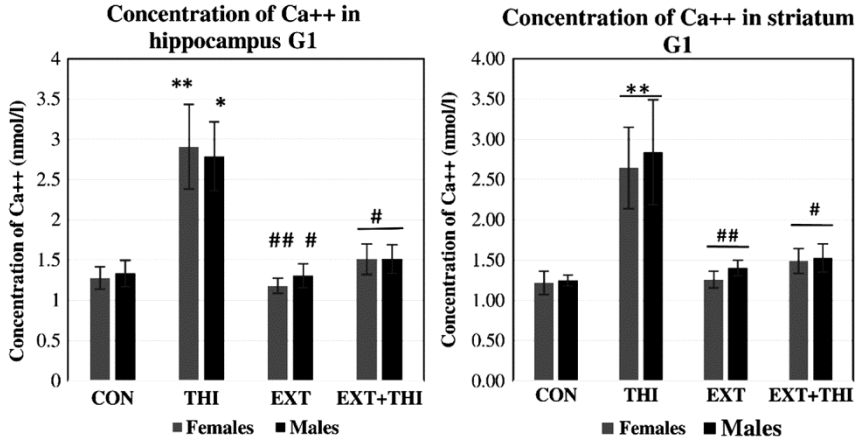

Fig. 8 — Change in brain cytosolic calcium in the hippocampus and striatum of females and males' rats of G1exposed *in utero* and during lactation to THI and extract, THI + extract. Values are means ± SD, (n= 7); *$P \leq 0.05$: significant, **$P \leq 0.01$: highly significant compared to control group. #$P \leq 0.05$: the deference is significant; ##$P \leq 0.01$: highly significant compared to THI group.

recorded a very highly significant and highly significant increase in females and males respectively in the striatum compared to the control group. The same group shows a significant increase of calcium concentration in the hippocampus of males and females rats compared to the control group. On the other hand, the females of the groups (EXT and EXT+THI) showed a very highly significant decrease of the calcium concentration in the striatum, and a significant decrease in the hippocampus compared to the THI group (Fig. 9).

Cytochrome c release into the cytosol

For G1 rats, the concentration of cytochrome c shows a significant increase in the striatum of male and female rats treated with THI compared to the control group. This concentration was decreased in male rats of EXT and EXT+THI groups compared to the THI group; this parameter shows a significant and very highly significant increase in the hippocampus of female and male rats respectively in the THI treated group compared to the control group. Females in the EXT+THI group and males in the EXT and EXT+THI groups show a highly significant and very highly significant decrease in the concentration of cytochrome c respectively compared to the THI group (Fig. 10). The females and males of G2 treated with THI showed a significant and highly significant increase of this parameter, respectively, in the striatum compared to the control group. In the

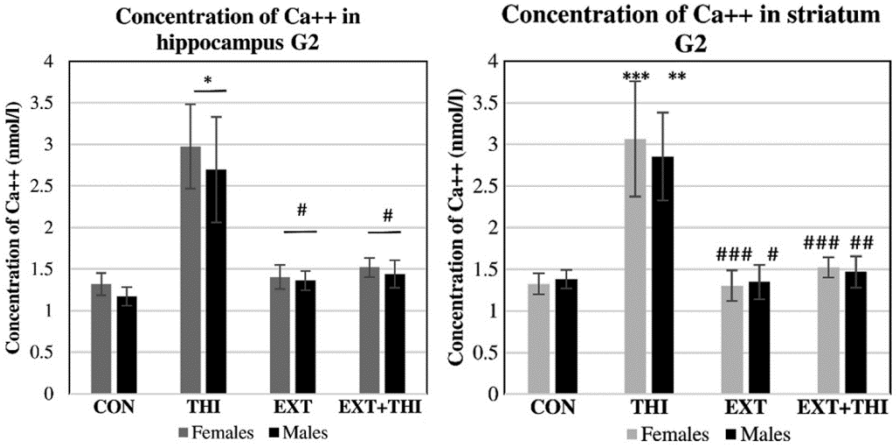

Fig. 9 — Change in brain cytosolic calcium in the hippocampus and striatum of females and males' rats of G2exposed *in utero* and during lactation to THI and extract, THI + extract. Values are means ± SD, (n= 7), **P* ≤ 0.05: significant; ***P* ≤ 0.01: highly significant; ***P ≤ 0.001: very highly significant compared to control group, and the difference is significant (#) 0.05 > *P* > 0.01, ##*P* ≤ 0.01: highly significant, the difference was very highly significant (###) compared to THI group.

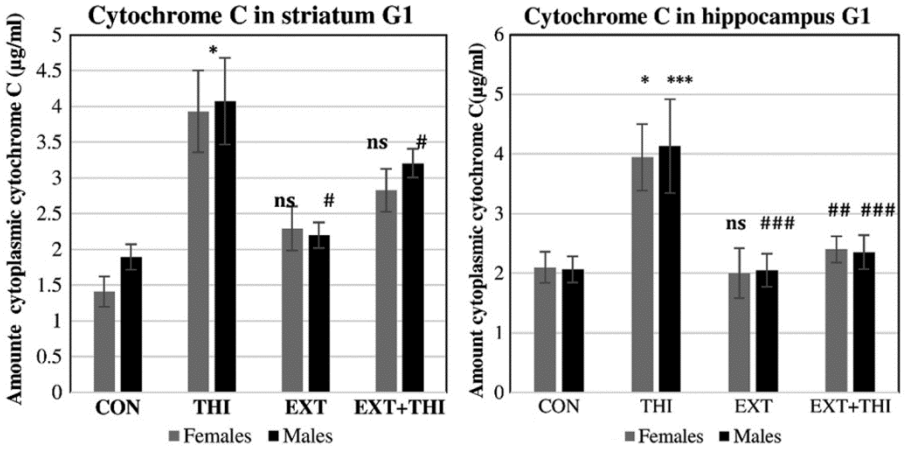

Fig. 10 — Change in brain cytosolic Cytochrome c in the hippocampus and striatum of females and males' rats of G1exposed *in utero* and during lactation to THI and extract, THI + extract. in the control group and the treated groups, Values are means ± SD, (n= 7),ns *P* > 0.05: not significant, **P* ≤ 0.05: significant; ****P* ≤ 0.001: very highly significant compared to control group, and the difference is significant (#) 0.05 > *P* > 0.01,##*P* ≤ 0.01: highly significant, the difference was very highly significant (###)compared to THI group.

hippocampus, the female and male rats of THI group show a very highly significant and significant increase respectively of the concentration of cytochrome C in comparison with the control group, our extract significantly decreases the concentration of this parameter in the striatum and hippocampus in comparison with the THI group (Fig. 11).

Evaluation of Mitochondria swelling and permeability

In this study, we recorded an increase in the mitochondrial swelling of the striatum and hippocampus of G1 females in comparison with the control group. On the other hand, the group treated with hydroalcoholic extract shows a highly significant protection of these mitochondria in comparison with the THI group. For the G1 males, our results show a highly substantial swelling of hippocampal mitochondria and a significant swelling of striatum mitochondria compared to the control group, a significant protection in the striatum and hippocampus recorded in the extract treated groups compared to the THI group (Fig. 12). For both males and females of the G2 group treated with THI, the results showed a significant swelling of hippocampal mitochondria and a very highly significant swelling of striatum mitochondria in comparison with the control group. The extract treated groups show a highly significant protection of striatum mitochondria and hippocampus for both generations in comparison with the THI group (Fig. 13).

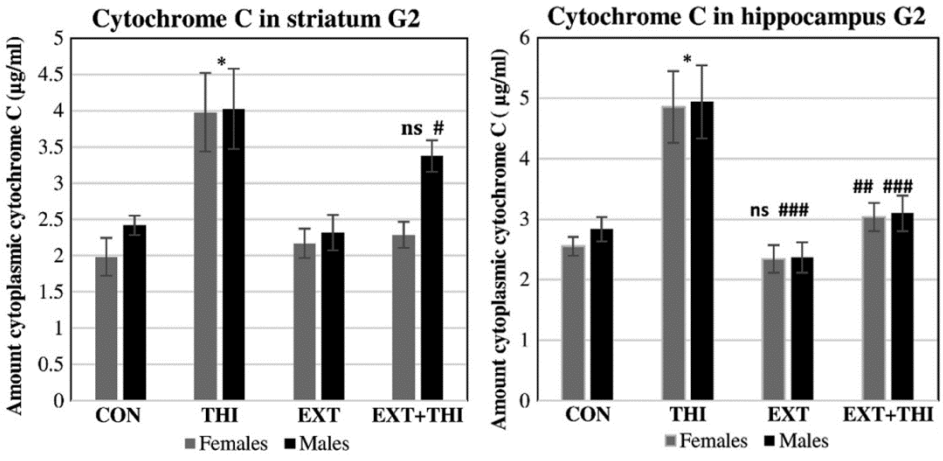

Fig. 11 — Change in brain cytosolic Cytochrome c in the hippocampus and striatum of females and males' rats of G2exposed *in utero* and during lactation to THI and extract, THI + extract. in the control group and the treated groups, Values are means ± SD, (n= 7), ns $P > 0.05$: not significant, *$P \leq 0.05$: significant; **$P \leq 0.01$: highly significant; ***$P \leq 0.001$: very highly significant compared to control group, and the difference is significant (#) $0.05 > P > 0.01$, ##$P \leq 0.01$: highly significant compared to THI group.

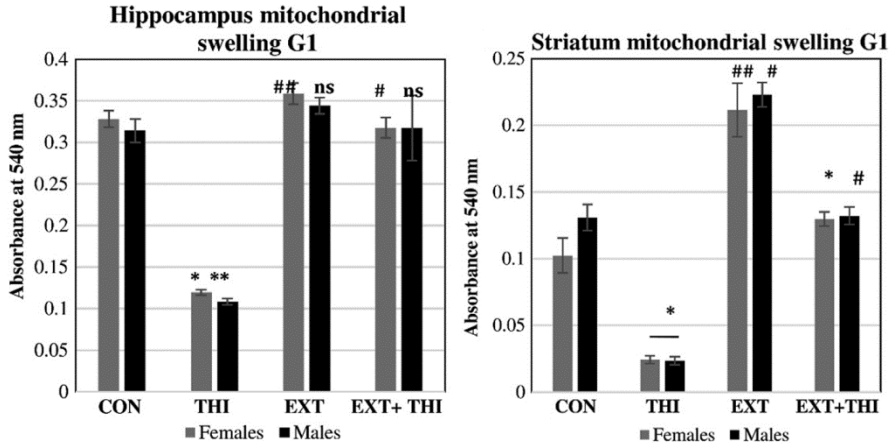

Fig 12 — Changes in mitochondrial swelling in the hippocampus and striatum of females and males rats of G1exposed *in utero* and during lactation to THI and extract, THI + extract, Values are means ± SD, (n= 7); ns $P > 0.05$: not significant, *$P \leq 0.05$: significant; **$P \leq 0.01$: highly significant compared to control group, and the difference is significant (#) $0.05 > P > 0.01$, ##$P \leq 0.01$: highly significant compared to THI group.

About permeability, our results show a highly significant increase in mitochondrial permeability of the striatum and hippocampus in male and female rats of G1 and G2 compared to the control group. The extract and combination (EXT+THI) treated groups of G1 and G2 showed a highly significant decrease in mitochondrial permeability in striatum and hippocampus compared to the THI group (Table 4).

Oxidative stress assessment (Tables 5 and 6)

For G1 rats treated with THI, we recorded a highly significant increase in the enzymatic activity of CAT and SOD in striatum (str) and hippocampus (hipp); both brain regions (str and hipp) show a high rate of lipid peroxidation marked by the highly significant increase of MDA in both male and female G1 offspring. The non-enzymatic antioxidant GSH shows a significant decrease in the striatum and hippocampus; and the enzymatic activity of GST shows a significant decrease in the striatum and hippocampus compared to the control group.

Our results show that the EXT-treated groups and the combination (EXT+THI) recorded an improvement in all stress parameters, where CAT activity recorded a highly significantly decreased in the striatum and hippocampus. SOD activity and MDA concentration were significantly decreased in the striatum, and highly significant in the hippocampus; on the other hand, GST activity and GSH concentration were significantly improved in both regions compared to the THI group.

For G2 rats treated with THI, we recorded a significant decrease of CAT activity in the striatum and hippocampus in female offspring, and significantly and highly significantly in the striatum

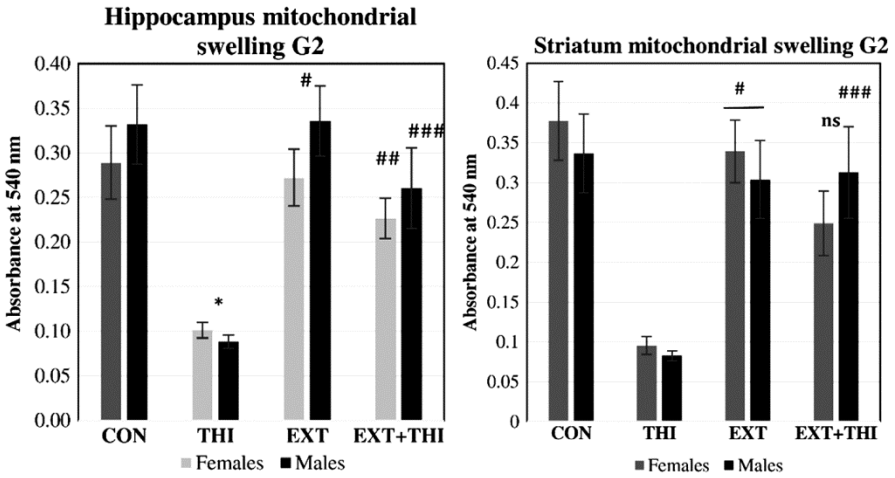

Fig. 13 — Changes in mitochondrial swelling in the hippocampus and striatum of females and males rats of G2 exposed in utero and during lactation to THI and extract, THI + extract, Values are means ± SD, (n= 7); ns $P > 0.05$: not significant, *$P \leq 0.05$: significant; **$P \leq 0.01$: highly significant; ***$P \leq 0.001$: very highly significant compared to control group, and the difference is significant (#) $0.05 > P > 0.01$,##$P \leq 0.01$: highly significant, the difference was very highly significant (###)compared to THI group.

Table 4 — Change of mitochondrial permeability in the brain of females and males' rats of G1 and G2 exposed in utero and during lactation to thiacloprid and extract, thiacloprid + extract

	Mitochondria permeability as (ΔDO/Δt) G1		Mitochondrial permeability as (ΔDO/Δt) G2	
	females	Males	females	Males
CON	Str 0,0006 ± 0,0002	Str0,0005 ± 0,0003	Str0,0004 ± 0,0001	Str0,0004 ± 0,0001
	Hipp 0,0005 ± 0,0001	Hipp 0,0005 ± 0,0001	Hipp 0,0005 ± 0,0001	Hipp 0,0004 ± 0,0001
THI	Str 0,004 ± 0,001 **	Str 0,005± 0,001*	Str 0,004± 0,001**	Str 0,005± 0,001**
	Hipp 0,003 ± 0,001**	Hipp 0,005± 0,002**	Hipp 0,004± 0,002*	Hipp 0,005± 0,002**
EXT	Str 0,0006 ± 0,0002 ##	Str 0,0005± 0,0001#	Str 0,0004± 0,0001##	Str 0,0004± 0,0001##
	Hipp 0,0004 ± 0,0001 ##	Hipp0,0005 ±0,0001##	Hipp 0,0006 ± 0,0002#	Hipp 0,0006 ± 0,0002##
THI + EXT	Str 0,0008 ± 0,0003##	Str 0,0009 ± 0,0003 ns	Str 0,0004 ± 0,0001 #	Str 0,0003 ± 0,0001#
	Hipp 0,0005 ± 0,0001##	Hipp0,0006 ±0,0002##	Hipp 0,0009 ± 0,0003#	Hipp 0,0006 ± 0,0002##

Values are means ± SD, (n= 7), ns $P > 0.05$: not significant, *$P \leq 0.05$: significant; **$P \leq 0.01$: highly significant compared to control group, and the difference is significant (#) $0.05 > P > 0.01$, ##$P \leq 0.01$: highly significant compared to THI group. Hipp: hippocampus, Str: striatum.

Table 5 — Variation of glutathione (mitGSH), malondialdehyde (mitMDA), the enzymatic activity of glutathione peroxidase (mitGPx), glutathione Stransferase (mitGST), superoxide dismutase (mitSOD), and catalase (mitCAT) in hippocampus and striatum mitochondria of offemales and males' rats of G1exposed in utero and during lactation to thiacloprid and extract, thiacloprid + extract.

		CON G1		THI G1		EXT G1		THI+EXT	
		Females	Males	Females	Males	Females	Males	Females	Males
CAT (UI/mg of proteins)	str	0,01± 0,002	0,01± 0,001	0,16± 0,02**	0,17± 0,02 **	0,01± 0,001##	0,01± 0,001##	0,01± 0,005#	0,01± 0,001##
	hipp	0,01± 0,001	0,01± 0,001	0,10± 0,02**	0,12± 0,02**	0,01± 0,002##	0,01± 0,001##	0,02± 0,001*	0,01± 0,001##
SOD (UI/mg of proteins)	str	22,42 ± 0,72	30,06± 0,62	66,75± 2,15 *	62,24± 3,60 *	23,27± 0,93#	28,50± 0,85#	27,89± 1,15ns	31,13± 0,81#
	hipp	24,54± 1,63	33,13± 0,53	69,22± 6,69*	72,98± 3,64 **	22,09± 0,58##	31,06± 0,83##	34,33± 0,61*##	34,66± 0,43##
MDA (nmol/mg of proteins	str	0,65± 0,03	0,58± 0,04	1,36± 0,18**	1,35± 0,22**	0,51± 0,05#	0,57± 0,04#	0,68± 0,04#	0,61± 0,04##
	hipp	0,64± 0,05	0,62± 0,05	1,58± 0,27**	1,58± 0,27**	0,68± 0,04##	0,56± 0,03##	0,69± 0,06#	0,71± 0,03##
GSH (mmol/mg of proteins	str	0,28± 0,03	0,27± 0,04	0,05± 0,01*	0,05± 0,01*	0,29± 0,02#	0,29± 0,03#	0,27± 0,04##	0,25± 0,03#
	hipp	0,47± 0,06	0,51± 0,04	0,09± 0,01*	0,10± 0,01*	0,43± 0,05###	0,48± 0,05#	0,33± 0,05#	0,39± 0,04#
GST (UI/mg of proteins)	str	24,14± 1,59	26,11± 1,74	7,79± 0,46*	8,14± 0,50*	22,79± 1,10ns	25,22± 1,51#	22,26± 1,28ns	25,55± 1,51ns
	hipp	29,38± 2,50	27,38± 2,41	8,94± 0,54*	8,04± 0,52*	25,94± 2,65##	24,46± 1,59#	24,11± 2,15#	26,99± 2,08#

Values are means ± SD, (n= 7); ns $P > 0.05$: not significant, *$P \leq 0.05$: significant; **$P \leq 0.01$: highly significant compared to control group, and the difference is significant (#) $0.05 > P > 0.01$, ##$P \leq 0.01$: highly significant, the difference was very highly significant (###) compared to thiacloprid group. Hipp: hippocampus, Str: striatum

Table 6 — Variation of glutathione (mitGSH), malondialdehyde (mitMDA), the enzymatic activity of glutathione peroxidase (mitGPx), glutathione S transferase (mitGST), superoxide dismutase (mitSOD), and catalase (mitCAT) in hippocampus and striatum mitochondria of of females and males' rats of G2 exposed in utero and during lactation to different treatments.

		CON G2		THI G2		EXT G2		EXT+THI G2	
		Females	Males	Females	Males	Females	Males	Females	Males
CAT (UI/mg of proteins)	str	0,09± 0,01	0,10± 0,02	0,01± 0,001**	0,004± 0,001*	0,08± 0,02##	0,09± 0,03###	0,06± 0,04#	0,07± 0,02###
	hipp	0,11± 0,02	0,09± 0,03	0,01± 0,001**	0,01± 0,001***	0,11± 0,03###	0,10± 0,04##	0,07± 0,03#	0,08± 0,001##
SOD (UI/mg of proteins)	str	31,78± 1,88	25,23± 1,33	77,51± 4,31*	76,68± 4,31*	24,34± 1,19###	24,30± 1,61#	32,20± 1,51##	26,28± 1,58#
	hipp	23,76± 1,76	24,66± 1,07	70,65± 5,53*	72,95± 5,02*	25,13± 1,52#	24,71± 1,57ns	25,17± 1,77#	26,26± 2,29ns
MDA (nmol/mg of proteins	str	0,48± 0,05	0,56± 0,04	1,78± 0,20*	1,56± 0,27**	0,50± 0,06#	0,48± 0,05##	0,52± 0,08ns	0,51± 0,07##
	hipp	0,46± 0,07	0,57± 0,06	1,59± 0,24*	1,72± 0,25**	0,52± 0,09#	0,45± 0,05###	0,52± 0,08#	0,56± 0,05##
GSH (mmol/mg of proteins)	str	0,33± 0,06	0,34± 0,06	0,02± 0,01**	0,03± 0,01***	0,33± 0,06##	0,36± 0,07#	0,23± 0,04##	0,32± 0,06###
	hipp	0,53± 0,06	0,56± 0,08	0,06± 0,01**	0,04± 0,01**	0,53± 0,06##	0,56± 0,08#	0,49± 0,05#	0,51± 0,07##
GST (UI/mg of proteins)	str	8,52± 0,50	8,96± 0,50	25,24± 1,54*	24,27± 1,65*	8,74± 0,59#	8,48± 0,69ns	8,25± 0,72#	7,88± 0,54ns
	hipp	7,79± 0,46	8,85± 0,47	23,08± 1,31*	23,79± 1,26*	8,64± 0,73ns	8,25± 0,56#	8,19± 0,58ns	8,47± 0,62#

Values are means ± SD, (n= 7); ns $P > 0.05$: not significant, *$P \leq 0.05$: significant; **$P \leq 0.01$: highly significant; ***$P \leq 0.001$: very highly significant compared to control group, and the difference is significant (#) $0.05 > P > 0.01$, ##$P \leq 0.01$: highly significant, the difference was very highly significant (###) compared to thiacloprid group. Hipp: hippocampus, Str: striatum.

and hippocampus, respectively. In male rats, the enzymatic activity of SOD is significantly increased in both brain regions; MDA shows a significant and highly significant increase in female and male rats respectively in the striatum and hippocampus. Concerning the tripeptide GSH, the results show a highly significant decrease of this parameter in the striatum and hippocampus, GST shows a significant increase in comparison with the control group.

Conversely, the extract enhanced the antioxidant defence system. Groups treated with the extract alone or in combination (EXT+THI) exhibited highly significant increases in catalase activity in the striatum and hippocampus, respectively.

Superoxide dismutase (SOD) activity decreased very significantly in the striatum of female rats and significantly in the striatum of male rats and the hippocampus of female rats. Lipid peroxidation decreased significantly and highly significantly in the striatum and hippocampus of female and male rats, respectively.

Our extract significantly increased glutathione (GSH) levels in both brain regions of female and male rats. However, glutathione S-transferase (GST) activity decreased significantly in the striatum of female rats and in the hippocampus of male rats compared to the THI-treated group.

Histopathological examination of G1 Rat Brain

Fig. 14 shows representative photomicrographs of brain sections from the different experimental groups. Brain sections from the G1CON and G1EXT groups

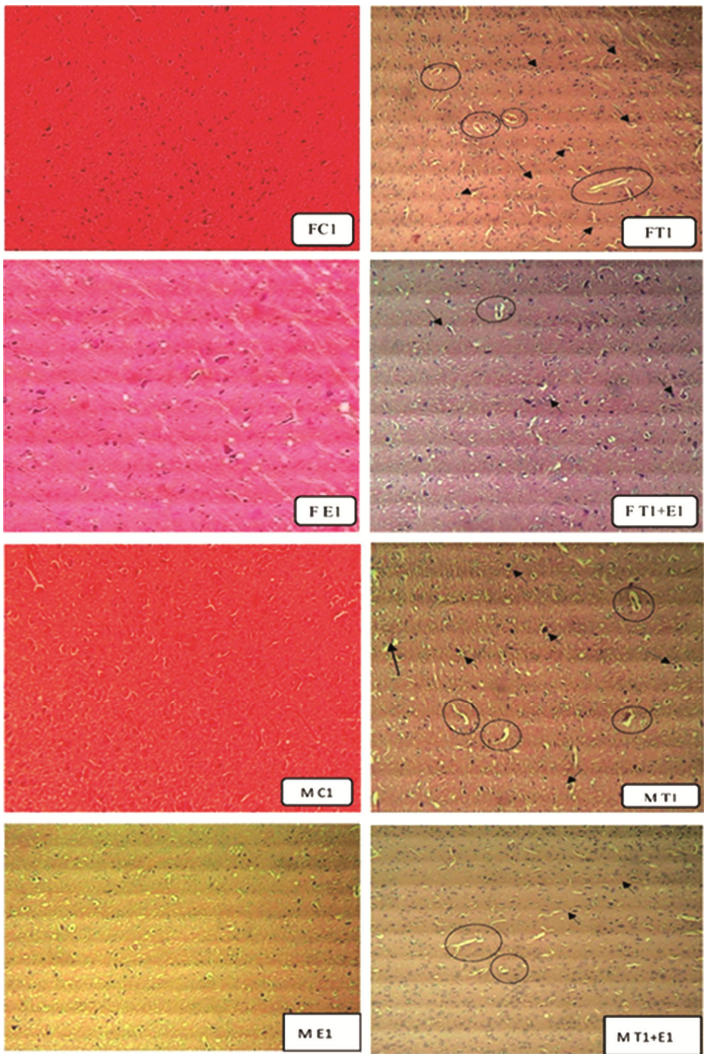

Fig. 14 — Histological sections of rat brain from males (M) and females (F) of G1(1) exposed during gestation and lactation to different treatments (H&E, X400). (C) brain section of G1-CON rats; (E) brain section of G1-EXT rats; (T) brain section of G1-THI rats; (T+E) brain section of G1-EXT+THI rats.

exhibited normal histological architecture, characterised by intact neuronal organisation and well-preserved granular layers. By contrast, sections from the G1THI group showed perivascular haemorrhage (circled) and neuronal degeneration associated with erythrocyte extravasation (indicated by the arrow), as well as moderate lesions and vascular congestion. Furthermore, brain sections from the G1THI+EXT group displayed largely preserved architecture comparable to the control group (G1CON), however moderate neuronal degeneration was evident.

Discussion

Exposure to environmental pollutants (such as pesticides) during pregnancy and lactation can be associated with teratogenic effects and various adult diseases[59]. Neonicotinoids are a new generation of insecticides that structurally resemble nicotinic acetylcholine receptors (nAChRs) and act as agonists, like nicotine itself [3]. THI is one of the most widely used neonicotinoids worldwide and has been reported to exert neurotoxic[60,61], hepatotoxic[62], and genotoxic[63] effects. In this study, we evaluated the cognitive potential, oxidative stress, mitochondrial integrity, cholinergic function of offspring rats (G1 and G2) following gestational and lactational exposure to THI, either alone or in combination with EXT. According to literature data, the phytochemical analysis of apricot kernel extract revealed moderate phenolic compounds content including caffeic acid, gallic acid, chlorogenic acid, p-coumaric acid, syringic acid, sinapic acid, quercetin, and ferulic acid[64,65], which have been reported to exert neuroprotective and antioxidant effects, particularly in the context of neurodegenerative disorders such as Alzheimer's disease[66,67]. Although the extract alone showed moderate biochemical activity, its co-administration with THI resulted in a marked cytoprotective effect, as evidenced by the normalization of the evaluated parameters. Apricot kernel extracts are also known to contain amygdalin, the main cyanogenic glycoside, as well as higher levels of natural antioxidants compared to the fleshy part of the fruit[68,69]. HPLC-DAD analysis confirmed the presence of several bioactive phenolic compounds, including amygdalin, which has been reported to exhibit antioxidant and cytoprotective properties. Previous studies have suggested that *Prunus armeniaca* L. extracts may contribute to the improvement of learning deficits and synaptic alterations in experimental models of neurodegeneration, with bitter apricot kernel constituents potentially supporting neuronal function[70]. The extract used in this study was characterized by a moderate richness in phenolic compounds such as quercetin, catechins, naringin, gallic acid, rutin and silymarin, which have been widely reported for their antioxidant and neuroprotective potential. When co-administered with THI, the extract was associated with an attenuation of THI-induced alterations across several behavioral and biochemical parameters. These observations suggest a possible protective association, although no direct mechanistic conclusions can be drawn. Behavioral assessment using the open field test revealed a significant reduction in locomotor activity in male and female offspring from both G1 and G2 generations following developmental exposure to THI. This finding is consistent with previous reports describing decreased motor activity after exposure to neonicotinoids (THI, clothianidin) during gestation and lactation[10,71]. In parallel, EPM test results indicated increased anxiety-like behavior in THI-exposed offspring, as reflected by reduced exploration of the open arms. Similar behavioral profiles have been documented in rodent models exposed to other neonicotinoids (imidacloprid)[9] or nicotine during early developmental periods[72,73]. Cognitive performance assessed by the novel object recognition test showed impaired recognition memory in both generations of THI-exposed offspring. Given the known involvement of the hippocampus in learning and memory processes[74], these deficits may reflect alterations in hippocampal function; Comparable impairments in learning and memory have been reported following pre- and postnatal exposure to neonicotinoids in experimental models[75,76]. The KIS test, which is commonly used to evaluate neuromuscular strength and coordination, showed a significant increase in both male and female offspring from G1 and G2 following developmental exposure to THI. Such changes may reflect alterations at the level of the central nervous system, peripheral nervous system, or muscle function[77]; however, the present data do not allow distinction between these possibilities. Nicotinic acetylcholine receptors are known to play an important role in neurodevelopment and motor regulation. Prolonged activation of these receptors by nicotine or neonicotinoids during critical developmental windows has been associated with functional disturbances in motor behavior. In the present study, gestational and lactational exposure to THI was associated with a significant decrease in acetylcholinesterase (AChE) activity in the striatum and hippocampus of G1 and G2 offspring. Although neonicotinoids are not classified as direct AChE

inhibitors, reduced AChE activity has been reported in association with neonicotinoid exposure and may reflect secondary neurochemical alterations rather than direct enzymatic inhibition[78]. In agreement with these observations, Oladosu JI and Flaws[12] reported decreased brain AChE activity in female rats following exposure to imidacloprid. An increase in extramitochondrial calcium concentration was observed in the striatum and hippocampus of G1 and G2 rats exposed to THI. This observation is consistent with reports from the European Food Safety Authority (EFSA)[79], which described enhanced calcium influx following exposure to neonicotinoids such as acetamiprid and imidacloprid, as well as nicotine. Similar calcium-related alterations have also been reported by Ramirez Cando *et al.*[80], who; observed Ca^{2+} release in association with mitochondrial permeability transition. In current study, THI exposure was associated with increased cytosolic cytochrome c levels and alterations in mitochondrial permeability and swelling in both generations. The scientific literature reported that the opening of the MPTP contributes to mitochondrial swelling, which can lead to necrotic or apoptotic cell death in some conditions such as ischemia or degenerative diseases[81,82]. Although increased cytosolic cytochrome c is frequently mentioned in discussions of apoptotic signaling, we did not carry out direct molecular validation of specific apoptotic markers, such as caspase-3 activity or the Bax/Bcl-2 ratio. Therefore, the observed mitochondrial changes should be interpreted as indicative rather than definitive evidence of apoptosis. **T**hese changes are commonly discussed in the literature in relation to mitochondrial dysfunction under conditions of calcium overload and oxidative stress[83]. The mitochondrial permeability transition pore (MPTP), located in the inner mitochondrial membrane, is known to respond to elevated intracellular Ca^{2+} and reactive oxygen species; however, direct evidence of MPTP opening or pore regulation was not assessed in this study. Therefore, the observed mitochondrial alterations should be interpreted as associative rather than mechanistically established. However, co-administration of the apricot kernel extract was associated with a normalization of mitochondrial permeability and swelling parameters compared to THI exposure alone. This modulatory effect may be related to the antioxidant properties of the extract[59], THI exposure was also associated with a disruption of oxidative balance, as reflected by increased MDA levels, indicating enhanced lipid peroxidation[13]. Concurrently, GSH levels were observed, which may reflect increased utilization or altered redox homeostasis[84], consistent with previous findings following nicotine exposure[85]. SOD activity was elevated in THI-treated rats, which may represent a compensatory response to increased reactive oxygen species production[13]. Catalase (CAT) activity showed a differential pattern, with increased activity in G1 offspring and decreased activity in G2 offspring; similar inhibitory effects on CAT activity under conditions of excessive hydrogen peroxide production have been reported in developmental exposure models[86-87]. GST activity decreased in G1 rats but increased in G2 rats, suggesting possible generational differences in oxidative stress adaptation rather than a uniform enzymatic response[88].

Exposure to THI during gestation and lactation induced marked histopathological alterations in the offspring brain, including neuronal and glial cell degeneration, parenchymal vacuolization, and vascular congestion. These findings are consistent with those reported by Oladosu JI and Flaws[12], who demonstrated perivascular hemorrhage, neuronal degeneration, and nuclear migration in G1 rats passively exposed to imidacloprid at doses of 10 and 20 mg/kg/day. Conversely, treatment with EXT preserved brain architecture and attenuated the observed alterations, suggesting a cytoprotective and neuroprotective effect, as previously described by Vauzour, (2008)[89].

Conclusion

This study demonstrates that gestational and lactational exposure to thiacloprid (THI) is associated with significant neurobehavioral deficits, mitochondrial dysfunction, cholinergic imbalance, and oxidative stress in both first (G1) and second (G2) generation offspring. These alterations were accompanied by histological evidence of neuronal distress in the striatum and hippocampus. Notably, co-administration of apricot kernel extract, rich in phenolic compounds, was associated with a marked attenuation of THI-induced behavioral and biochemical disruptions, suggesting a potential protective effect. However, given the correlational nature of the data and the absence of direct molecular validation of apoptotic pathways or nicotinic receptor subtypes, the precise mechanisms underlying these effects remain to be fully elucidated. These findings highlight the potential multigenerational neurotoxicity of THI and support further investigation into genetic and epigenetic

modification in order to invest towards transgenerationality of THI-neurotoxicity and natural compounds as protective strategies against pesticide-induced developmental toxicity.

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Competing Interests

The authors declare that they have no competing interests.

Ethical statement

In this study, all animal experiments were approved by PIA according to the code of ethics: No. Batna-Univ 2. 2020.231 and all the experimental protocols adhered strictly to ethical guidelines and received approval from the committee of the ''Algerian Association of Sciences in Animal Experimentation'' under law No.88-08/1988, related to veterinary medical activities and animal health protection (N° JORA: 004/1988).

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