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CHRONIC THIACLOPRID EXPOSURE IMPAIRS COGNITIVE FUNCTION AND TRIGGERS MITOCHONDRIAL APOPTOSIS PATHWAY IN RAT STRIATUM AND HIPPOCAMPUS: NEUROPREVENTIVE EFFECT OF BITTER APRICOT KERNELS EXTRACT *(PRUNUS ARMENIACA L.)*

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ABSTRACT

Thiacloprid (THI) is a nicotinic receptor agonist widely used as pesticide in Algeria, however it is susceptible to accumulate in various fruits and vegetables and pouring downstream into food plates and contributes to the development of neurodegenerative diseases. Conversely, several natural compounds are provided with cytoprotective potential and, therefore, are able to act against the harmful effects of toxicants such as pesticides. This study focused on striatum (str) and hippocampus (hipp) mitochondrial toxicity assessment, evaluation of behavioral function and intrinsic apoptosis pathway in rats exposed to THI at low-dose (0.020 mg/kg) for 3 months. In addition, this study examined neuroprotective potential of bitter apricot kernel extract when administered concomitantly with THI at the dose of 50 mg/kg. In current study, assessment of mitochondrial permeability transition pore (mPTP) and swelling, evaluation of mitochondrial redox status, cholinergic function (Ach E) and apoptosis markers (Caspase 9 and 3, Bax and Bcl2, Cytochrome c and cytosolic calcium) were performed in both brain areas, besides behavioral and histopathological examination. The results showed an increase of lipid peroxidation in both of str and hipp with a values of 1,14 ± 0,04 nmol/mg of proteins (pr) and 1,58 ± 0,09 nmol/mg pr. respectively and a significant decrease in GSH (0,09 ± 0,01 mmol/mg pr. in hipp and 0,08±0,01 mmol/mg pr. in str), the results also showed a change in the activity of antioxidants enzymes SOD (16,37±1,09 UI/mg pr. in hipp14,54±1,46 UI/mg pr. in str) , CAT (0,010±0,01 UI/mg pr. in hipp and 0,005±0,004 UI/mg pr. in str), GPx (0,01± 0,001 nmol/mg pr. in both hipp and str) and GST (23,73±1,68 UI/mg pr. in hipp and 17,56± 1,04 UI/mg pr. in str), as well as an abrupt increase in mPTP opening with a value of (0,057±0,005 in str and 0,054±0,005 in hipp) , which leaded to mitochondrial swelling where the level o mitochondrial swelling was (0,016±0,002 in str and 0,106±0,003 in hipp), the swelling was associated also with a high releasing of Cyt-c with a values of (4,48 ± 1,26 µg/ml in str and 5,32 ± 1,08 µg/ml in hipp) and Ca++(2,26±0,06 mmol/l in str and 2,32±0,07 mmol/l in hipp) into the cytosol, the results also showed a significant decreasing of Bcl2 (16,4 ± 1,86 ng/mg pr in str and 14,8± 0,82 ng/mg pr in hipp), in the other hand the rates of caspase-9 were (278±14 mAbs/mg pr.) in str and 212 ±24 mAbs/mg pr. in hipp), caspase3 (184± 16mAbs/mg pr.) in str and 250 ±14mAbs/mg pr. in hipp), and BAX (0,926 ng/mg pr in str and 1,189 ng/mg pr in hipp) were increased. The results of this study revealed also a decrease of memorization processes and learning abilities, at the same time a decrease in Ach E activity (14,02± 0,78 nmol/min/mg pr. in str and 22,35± 1,77 nmol/min/mg pr. in hipp) was recorded. Inversely, bitter apricot kernels extract showed higher cytoprotective potential against THI neurotoxicity, since mitochondrial redox homeostasis and membrane integrity were recovered, cognitive impairment and brain tissue damage were also prevented. In conclusion, THI induced mitochondrial disorders, triggered apoptosis signaling pathway and impaired cognitive functions which were prevented by bitter apricot kernels extract when associated with this pesticide.

Keywords: Thiacloprid, Extract, Striatum, Hippocampus, Cognitive function, Mitochondrial apoptosis

INTRODUCTION

It is recognized that the benefits of pesticides are inherent in crop protection and in increasing economic potential in agriculture and human health by increasing agri-food and crop production and controlling some disease-carrying insects **(Messiad et al. 2015)**. However; The main reason for their toxicity could be the presence of similar targets in both pests and non-target species, including humans. Neonicotinoids (NNs) are considered to be the most important synthetic pesticides used to control harmful insects in plant and animal health over the last few decades and were able to bind to nicotinic acetylcholine receptors (nAChRs), causing excitation, abnormal paralysis and death of harmful organisms, developed to replace organophosphorus and carbamate insecticides **(Schaafsma et al. 2015; Pang et al. 2020)**. However, if these pesticides have a potent agonist against nAChRs of target insects, there is multiple evidence that these pesticides have low affinity for the same receptors in vertebrates **(Costas-Ferreira and Faro 2021)**, raising the question of how neonicotinoids might act for animal non-target organisms can be neurotoxic. It has been reported that the toxicity of NN focuses on the cellular and molecular integrity of the organism, resulting in carcinogenic, endocrine disorders **(Sekeroglu et al. 2014)**, neurotoxic **(Cimino et al. 2017)**, hepatotoxic effects **(Alarcan et al . 2020)**. Among the most commonly used NNs in Algeria is Thiacloprid (THI), (Z)-N-[3-(6-Chloro-pyridin-3-ylmethyl)-thiazolidin-2-ylidene]cyanamide), an organochlorinated insecticide of the family the chloroprynydilic neonicotinoids **(Galdikova et al. 2019)**. This insecticide was registered in 2000 under the trade name Calypso 480SC (480 g.L-1) **(Schwarzbacherov et al. 2019).** The mode of action of this pesticide in target organisms is to interrupt the transmission process of nerve impulses after its binding to nAChRs in neurons **(Galdikova et al. 2019).** Literature data indicate that THI is toxic to mammals at both acute and chronic exposures at low or high doses, causes neurotoxicity and attenuates locomotor activity in rats treated with 3.1 mg/kg body weight THI **(Vivek et al .2020).** In addition, a previous study showed that this pesticide can cause central nervous system disorders (Alzheimer's disease, Parkinson's disease, schizophrenia, depression), which were thought to be associated with a change in the density of the nAChRs subtype in rats who have been exposed to high doses of THI **(Cimino et al. 2017).** It is worth noting that the

functional and molecular integrity of mitochondria allows for an adequate supply of ATP through the respiratory chain to support cellular function, signaling pathways and overall cellular activities, in addition to synthesizing key molecules and responding to redox state homeostasis imbalances **(AbdelDaim et al. 2015).** Additionally, mitochondrial membrane potential is an important parameter of mitochondrial health as it reflects mitochondrial membrane integrity, energy production, and calcium processing capacity **(Baranov et al. 2021).** There is strong evidence in the literature substantiating the possibility that mitochondrial integrity and its redox status play a primary role in pathogenesis, particularly in apoptosis and neural degeneration **(Qasim et al. 2021; Baranov et al. 2021).** Interestingly, these literature data indicate that mitochondria can be considered an excellent experimental model to study neurodegenerative diseases, especially in the case of environmental pollutant-induced neurotoxicity. On the other hand, it has been reported that acute or chronic exposure to members of the NN family can lead to cognitive disorders and various types of behavioral disorders at different life stages **(Costas-Ferreira and Faro, 2021)**. Related to such neuronal damage and dysfunction that can be induced by pesticides, there are several botanicals that have been identified as cytoprotective and therefore likely to prevent or treat various pathological aspects induced by pesticide residues, including phytochemicals extracted from bitter apricot kernels. such as the apricot (*Prunus armeniaca* L.) which belongs to the Rosaceae family **(Kovacova et al. 2019).** The first origin of this species is from Central Asia, western China, Iran and the Caucasus and has a hard core containing a single large seed or kernel **(Aamazadeh et al. 2019)**. Apricot kernels are divided into sweet apricot, bitter apricot and bitter apricot according to their taste. Since, in addition to amygdalin and carbohydrates, the bitter apricot kernel contains flavonoids and carotenoids, minerals (Zn, Mg, Fe, Ca+2 and K) and various vitamins (E, K and D) and lipids **(Moradi et al. 2017)** , therefore it can have antioxidant properties **(Kopcekova et al. 2017)**, and bitter apricot kernel has been reported to have a number of pharmacological properties such as anti-cancer, anti-asthmatic and anti-inflammatory properties **(Li et al. 2016).**

In this study, we will highlight the neurotoxic effects of THI, the most commonly used pesticide in Algeria, along with its possible mechanism of neurotoxicity in both rat str and hipp exposed to a low dose of these NNs of 0.020 mg/kg/day for 3 months. On the other hand, we will investigate a possible noxiousness to the brain health of animals, focusing on mitochondrial impairment and its impact on cognitive disorders and brain cell degeneration, alongside evaluating the neural protection of the hydroalcoholic extract of bitter apricot kernels on the cellular and molecular integrity of rat str and and hipp.

MATERIAL AND METHODS

Chemicals

The majority of chemical products used in this study were purchased from Sigma Aldrich compagny, Germany.

The pesticide selected in this study is thiacloprid (THI) (calypso 480g/l) at a dose of 0.020 mg/kg/day administered chronically by gastric gavages for 90 days. The choice of this dose was adopted based on the exploration of this insecticide in biological matrices which showed a value of 0.376 mg/kg of in fresh tomato (**Omirou et al. 2009),** to convert the concentration of this insecticide in tomato to a dose administered daily to rats, we used a conversion factor of 0.05 in the rat **(EFSA, 2019)**. In addition, the dose of hydroalcoholic extract used in preventive treatment of animals against the toxicity of THI was 50mg/kg/day **(Kovacova et al. 2019**).

Solvent extraction of plant materials

The plant material consists of bitter apricot kernels (*Parmeniaca* L.), the apricot is harvested in Ain-Elkhadra region (the state of M'sila, Algeria), carried out during the period from May 2019 to July 2019. After, the harvest of apricots, the pits were obtained after stoning and then crushed to obtain the kernel which is dried at room temperature away from light and then crushed to obtain a fine powder using an electric grinder. The powder is stored in hermetically sealed boxes.

The extraction is done by maceration according to the method reported by **Minaiyan et al. (2014),** in which a mass of 30g of the powder obtained is macerated in 90 ml aqueous ethanol (70:30) for 72 hours under continuous stirring, after cooling, the mixture is filtered on a gauze and then on wattman paper (3mm), the filtrate has been evaporated using a rotary evaporator which allows to remove the solvent under vacuum, the aliquots obtained are placed in kneading boxes and then dried using lyophilisation equipment. The residue is scraped and stored at 4°C in a hermetically sealed bottle.

Animal treatments

32 Female rats weighing about 250g were purchased from Pasteur Institute in Algiers, Algeria. Just upon arrival, rats were kept in the animal house of the Faculty of nature and life Sciences, University of Batna 2, Algeria and were adapted for 2 weeks before gavages in room temperature (23±2°C) with a humidity of 60%, with a natural photoperiodic cycle 12/12H. 8 rats were housed in cages oflargesize (70×40×30cm), with double bottles excluding any crowding effect and they had free access to rodent food and distilled water.

Distribution and treatment of rats

Treated animals were divided into four (04) groups with eight (08) rats per group. All rats were treated daily by gavage for 90 days.

Control group (CON): received 1 ml of distilled water (DW).

Extract group (EXT group): treated with hydroalcoholic extract of bitter apricot kernels receiving (50mg/kg).

Thiacloprid group (THI group): treated with thiacloprid receiving 0.020 mg/kg.

THI+EXT group: treated with extract (50mg/kg/day) + THI (0.020 mg/kg).

Behavioural and cognitive tests

Open field test (OF)

The open field test (OF) is widely used to examine locomotor activity and anxiety in animals **(Choleris et al. 2001**). This test consists of a square device (50 x 50 × 50 cm). The device floor is divided into 25 squares **(Tatem et al. 2014)**. In current study, initially the rats were acclimatized in the examination room at least 10 min before the carrying the test, then the rats were individually placed in the center of the devise, its movement and the different behavioral parameters were observed and filmed for 5 minutes**.** After each test, device is cleaned using water-alcohol (50%) before doing another test**.** During this test, ambulation (which is the number of squares crossed by the animal) and rearing (is how much times the animal rests on its hind legs) were evaluated (**Jurgenson et al. 2010**).

Elevated Plus Maze (EPM) test

EPM consists of two open arms (50X10cm) opposite each other, crossed by two closed arms (50X10X 40cm), the device is placed 50cm above the ground (Zhang et al. 2020).In this test, the animal is placed in the center of the maze facing a closed arm and left to explore for 5 min, its behavior is then observed to measure the time spent in the open and closed arms as well as the number of entries of each ratin each of these arms (Jurgenson et al. 2010).Rats that have remained confined in closed arms are described as being anxious (Frih et al. 2010).

Forced swimming test (FST)

The FST is a behavioral model consisting in inducing a state of despair in rats and mice (Frih et al. 2010) in which the animals are placed individually for 5 minutes in a cylinder in glass (54 cm high X 34 cm X 60 cm), two-thirds filled with water at 26 ± 1 ° C (Porsolt et al. 1978).The behavior of the animal in the device is filmed using a video camera; the time of immobility is measured.In this case, immobility is interpreted as a reflection of "behavioral hopelessness" where the duration of immobility is indicative of the degree of hopelessness in the animal (Taibi et al. 2020).

Evaluation of working memory and learning ability

Novel Object Recognition test (NOR)

NOR is a classic method for evaluating memory and spatial learning after alterations in the neurotransmission system, after lesions or brain diseases (Leger et al. 2013). This test is based on the ability of rats to recognize a new object in the environment; it consists of an illuminated square enclosure (the same for the open field). Briefly, this test consists of three phases: adaptation, familiarization and trial phase, in the adaptation phase, the rats freely explore the OF enclosure in the absence of objects for 5 min. 24 hours after adaptation, the rats are subjected to a familiarization phase in which two identical objects have been placed in two opposite positions inside the enclosure at the same distance from the nearest corner, so the rats explore them. identical objects for 10 minutes, a day later the animals are then placed back in the same enclosure, where one of the two familiar objects are replaced by a new one, to start a 5 minute test phase during which the behavior of the rats is recorded with a video camera in order to calculate the IR recognition index corresponding to the percentage of the exploration time of the new object compared to the total exploration time of the two objects (IR = exploration time of a new object (New object exploration time + Familiar object exploration time) × 100%) (Antunes and Biala 2011; Zhang et al. 2012).

Assessment of the muscle strength of treated animals

Konziela's Inverted Screen test (KIS)

This test used to assess neuromuscular diseases and coordination (Tabassum, 2017). The inverted screen is a square of wire mesh composed of squares of 1mm in diameter. It is bordered by a 4cm deep wooden bead. Before the test the rats are brought 5-20 min to the experimental room, the test procedure is to place the rat in the center of the metal screen and start the stopwatch, then rotate the screen to an inverted position. The moment the rat falls is noted. If this time is between 1 and 10 s the rat takes the score 1; if it is between 10 and 26 s it takes the score 2; between 26 and 60 it takes the score 3.Before the test the rats are brought 5-20 min in the experimental room, the test procedure consists of placing the rat in the center of the metal screen and start the stopwatch, then rotate the screen to an inverted position, and the moment when the rat falls is noted. If this time is between 1 and 10 sec the rat takes a score 1; if it is between 10 and 26 sec it takes a score 2; between 26 and 60 it takes a score 3 (**Deacon, 2013**)

Balance beam test (BBT)

BBT is a behavioral test to assess the balance and coordination of an animal crossing a beam (Luong et al. 2011). In this test the beams are consisted of long strips of wood (1m) with 4cm wide, they were placed horizontally 50 cm from the ground, with one end mounted on a narrow support and the other end attached to a black box (20cm) into which the rat can escape (Luong et al. 2011). This test is performed during 3 consecutive days, 2 days of training and another day for testing where the time required for the rat to cross the beam is measured, both the beam and the box are cleaned after each test (Hausser et al. 2018).

Separation of mitochondrial matrix from str and hipp

After decapitation of rats and brain extraction, both str and hipp brain regions were separated and immediately immersed in TSE buffer (10mM tris, 250MM sucrose and 1mM EDTA, pH= 7.2) at 4°C. After that, tissues were cut and homogenized in TSE to obtain a 10% tissue homogenate, which was centrifuged at 600g for 10 minutes to remove big cell debris, the resulting supernatant was also centrifuged at 10000g for 10 minutes, the pellet of this centrifugation was recovered and suspended in TS buffer (10mM tris, 250 mM sucrose, pH=7.2). This last suspension was centrifuged again at 10000 g for 10 minutes, the final pellet obtained constituted of the mitochondria, which was resuspended to obtain a mitochondrial suspension using TS buffer (**Sahu et al. 2014**). In order to recover the mitochondrial matrix, mitochondrial suspension was frozen and thawed several times, followed by intensified homogenization in an all-glass potter in order to burst the mitochondria, after which this potter homogenate was centrifuged at 9600 rpm/minute for 10 minutes at 4°C, the supernatant obtained was used as a source of stress parameters (GSH, SOD, CAT, GST, GPX, MDA), (**Lahouel et al. 2015**).

Evaluation of oxidative stress in the strand hipp mitochondria

Mitochondrial proteins concentration

Determination of protein was performed by the method of **Bradford (1976).** Briefly, to 5µl of the mitochondrial matrix 20µl of TS buffer is added, then the reaction is initiated by the addition of 1ml of Bradford's reagent, after 10 min of incubation, the optical density was measured at 595 nm, against the blank. The protein concentration is determined by comparison with a beef serum albumin (BSA) standard range (1 mg/ml) performed under the same conditions.

Measurement of catalase activity (CAT)

The activity of CAT is performed applying the method of **Clairbone (1985).** Briefly, 50 µl of the enzyme source (mitochondrial fraction) was added to 1ml of phosphate buffer (0.1M, pH7.4). The reaction is initiated after the addition of substrate H2O2 (0.019M). The optical densities are read at 240 nm every 30 s during 3 min. The results are expressed in IU/mg of proteins.

Measurement of superoxide dismutase activity (SOD)

SOD is evaluated according to the method of de **Beauchamp and Fridovich (1971).** Briefly, 2ml of a reaction medium containing : sodium cyanide 10^{-2}M , NBT $1.76x10^{-2}$M solution , EDTA 66 mM , riboflavin $2x10^{-3}$Mm , phosphate buffer at pH 7.8 , 50 µl of the enzyme source (mitochondrial fraction), and 0.1 ml of 10^{-2}M methionine solution, are subjected to radiation by a 15-W lamp for 15 minutes, after that absorbance is measured at 560 nm. A control tube is prepared with phosphate buffer (pH=7.8) under the same conditions as the samples.

Assessment of malondialdehyde levels (MDA)

MDA is commonly considered as a marker of polyunsaturated fatty acids peroxidation, which can be used a marker of oxidative stress. MDA rates were determined according to the method of **Shagirtha and Pari (2011).** Briefly,2ml of a reagent containing TBA 37%, TCA 15%, HCl 0.25N (1v, 1v, 1v) are mixed with 0.5 ml of the mitochondrial matrix, then the mixture was incubated in a water bath at 100°c for 15 min then it was cooled and centrifuged for 10 min, absorbance of the obtained supernatant was measured at 532 nm**.**

Evaluation of glutathione (GSH)

GSH assay measurement is performed according to the method of **Ellman (1959). Briefly,** 2ml of phosphate buffer at pH 8 was mixed with 50 µl of the mitochondrial fraction and 20µl of Ellman's reagent (DTNB), after 15minute of incubation at room temperature, the absorbance was measured at 412 nm against a blank prepared under the same conditions. The concentrations of reduced glutathione are expressed in mmol/ml. They are determined by comparison with a standard range of glutathione prepared under the same conditions as the assay.

Measurement of glutathione -s-transferase activity (GST)

The activity of GST was measured according to the method of **Habig et al (1974).** Briefly, incubation, in a water bath at 37°C for 10 min, of the reaction mixture consisting of 850µl phosphate buffer (0.1M and pH = 6.5) and 50 µl of CDNB reagent at 20 mM, then the reaction is initiated by the addition of 50µl of GSH (20Mm) solution and 50µl of mitochondrial fraction. The conjugation reaction between GSH and CDNB by the GST enzyme results a compound that absorbs at 340 nm. Thus, the absorbance is measured every 30 s for 5 minutes against a control prepared under the same conditions with phosphate buffer. The results are expressed in IU/mg of protein.

Evaluation of glutathione peroxidase activity (GPx)

Enzymatic activity of GPx was measured according to the method of **Flohé and Günzler (1984)**. Briefly, 0.2ml mitochondrial fraction is mixed with 0.4ml GSH 0.1mM and 0.2ml phosphate buffer 0.1M; pH 7.8, the mixture is incubated in a water bath at 25°C for 05min. The reaction is triggered by the addition of 0.2ml H_2O_2 1.3mM. After 10min, 1ml of TCA 1% (tri-chloroacetic acid) is added and the mixture is put on ice for 30min to stop the reaction, then the mixture is centrifuged for 10min at 3000t/min. 0.48 ml of obtained supernatant is placed in a cuvette to which 2.2ml of 0.32M Na_2HPO_4 is added with 0.32ml of 1mM DNTB. This mixture forms a colored compound and its optical density is measured at 412nm every 30sec for 05min.

Measurement of cytosolic acetylcholinesterase activity (AchE)

To obtain the cytosolic fraction, both brain str and hipp were homogenized with 3 volumes of 0.1M phosphate buffer containing 17% KCl, this potter homogenate was centrifuged at 3000 g for 10 minutes to remove cell debris, then the supernatant obtained was recovered and centrifuged at 10,000 g for 30 minutes, the final supernatant is considered as cytosolic fraction (**Iqbal et al.2003**).
Determination of AchE was performed according to the method described by **Ellman et al. (1961**). Briefly,100µl cytosolic fraction were mixed with 100µl DTNB (0.1M, pH 8) (obtained by dissolving 39.6mg DTNB, 15mg CO3Na in 10ml tris 0.1M, pH 7) and all mixed to 1ml tris buffer (0.1M, pH 7). After incubation for 5min, 100µl acetylthiocholine substrate (118mg Ach in 5ml distilled water) is added. The optical density is read at 412nm every 4min during 20min.

Evaluation of Mitochondria swelling and mPTP opening

Mitochondrial permeability is mediated by the opening of the permeability transition pore (PTP) located in the inner membrane. The evaluation of this phenomenon is based on the rate of Ca^{++} ions crossing mitochondrial membranes, the increased entry of Ca^{++} leads to an increase in mitochondrial size which is detected at a wavelength of 540 nm for 3 minutes and every 30sec (**Kristal et al. 1996**).

Evaluation of apoptotic indicators

Preparation of tissue lysates

Both striatum and hippocampus tissues were used to extract cell lysates applying the procedure described by **Ahmed et al. (2013).** Briefly, striatum or hippocampus tissues (0.5 g) were minced and then homogenized in 1 mLof lysis buffer [20 mM HEPES (pH 7.5), 150 mM NaCl, 1% NP-40, 0.1% SDS, 1 mM EDTA, and 1.0 mM DTT] with protease inhibitors (2 µg each of pepstatin A, leupeptin, aprotinin, and 0.5 mM phenylmethylsulfonyl fluoride, to prevent protease digestion of proteins), reaction medium was incubated on ice for 30 min, and then centrifuged at 10,000 ×g at 4°C for 20 min. Tissue lysates were extracted in order to assess indicated apoptosis intermediates.

Determination of Cytochrome- c, Caspase 3 and 9 activities, Bcl2 and Bax

Determination of the Cytochrome-c activity in tissue lysates was monitored by using Assay Kit (CYTOCOX1), measured at 550 nm, Sigma®, Germany. This colorimetric assay is based on the decrease of the ferrocytochrome-c rate in reaction medium by its cytochromic oxidation into ferricytochrome-c.
Determination of caspases-3 and 9 activities in both striatum and hippocampus tissue lysates was performed using commercial kits (Caspase-3 and caspase-9 Colorimetric Activity Assay Kits, respectively Part No. 90079 and Part No. 90083, Sigma®, Germany) which can provide a simple mean to measure caspase-3 and 9 activities. These enzymes recognize their respective substrates Ac-DEVD-pNA and Ac-LEHD-pNA and eliminate the chromophore p-nitroaniline (p-NA) which can be quantified by spectrophotometric detection at 405 nm. Both caspase activities are proportional to the quantity of p-NA. One unit of caspase-3 and 9 is the amount of enzyme required to cleave 1 pmol of its proper substrate per minute at 30°C. Levels were expressed as mAbsorbance/mg of proteins (mAbs/mg pr.)
Determination of Bcl2 (B-cell lymphoma 2) and Bax (Bcl2 associated X protein) levels in striatum and hippocampus tissue lysates was realized using ELISA kits (Uscn Life Science Inc.). Levels were expressed as ng/mg tissue proteins.
All procedures were performed according to instructions of manufacturer.
Determination of cytosolic Ca++ in cell lysates of both brain areas was carried out using commercial kits manufactured by SPINREACT, SPAIN. Results are expressed in mmol/l.
All assays were performed according to kit manufacturer's instructions.

Histological study

Histological sections of the brain were carried out as follows (**Houlot, 1984**): Fixation by immersion of the organs in a great volume of formalin, then the tissues were dehydrated in ethanol at different concentrations (70%, 95%; 100%). These steps were followed by clarification byputingtissues in 2 xylene baths, each bath lasts 45 minutes, the next step was the inclusion of the samples in two successive paraffin baths for one hour each at a temperature of 56 ° C, then poured into metal molds. After the paraffin inclusion, the blocks, containing the tissue fragments, were cut using a microtome to a thickness of 7 µm. Once the sections were made, the glass slides were covered with a containing solution (2g of albumin +50 of glycerin in 1000ml of distilled water), the next step is deparaffinization fining for the purpose of rehydrating the section for staining. The colored slides were dried and then photographed using a camera linked to a magnifying glass (23625, 147,5px).

Statistical Analysis

The results obtained in this study were expressed as the mean of six replicates (n=6) (mean ± standard deviation), for obtained graphs and histograms the office Excel 2016 was used. The statistical analysis was performed using software XLSTAT 2014.5.03using the ONE-WAY ANOVA analysis. The significance of the difference between the control and treated groups, and with the THI and treated lots is verified using the Tukey test and the comparison result as follows: $p > 0.05$ = the difference is not significant; (*) $0.05 > p > 0.01$ = the difference is significant; (**) $0.01 > p > 0.001$ = the difference is highly significant; (***) $p < 0.001$ = the difference is very highly significant when treated groups compared to the control group. #$p < 0.05$= the difference is significant; ##$p < 0.01$= the difference is highly significant; ###$p < 0.001$= the difference is very highly significant), different groups compared to the THI-group.

RESULTS

Assessment of behavioral and cognitive changes in rats exposed chronically to THI and hydroethanolic extract

Anxiety-Like Measurement

Assessment of rats' anxiety by OF test

Statistical analysis of the results of this test revealed an alteration in locomotors activity, since a significant decrease was recorded ($p<0.05$) respectively in horizontal activity (ambulation: the number of squares crossed by the animal) and in locomotor vertical activity (rearing: the position where the animal rests on both hind limbs only) in THI- treated rats compared to control. Also, the same results showed an increase in horizontal activity in EXT-treated rats and THI+EXT-group compared to THI- treated group (fig 1).

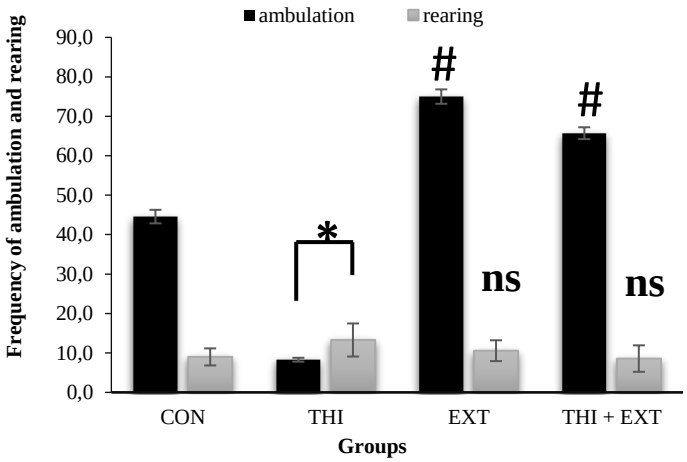


Figure 1 Evaluation ofthe level of anxiety in the control and the treated groups after 90 days of treatment using the Open Field test (frequency of ambulation and rearing). Values are means ± SD, (n= 6), p value was detected compared to control group. ns $p > 0.05$: not significant, *$p<0.05$: the difference is significant, and ns $p > 0.05$: not significant, the difference is significant (#) $0.05 > p > 0.01$compared to thiacloprid group

Evaluation of anxiety effects of the animals by EPM test

Obtained results showed a significant increase in time spent in closed arm and a significant decrease in time spent in open arm in the THI-treated rats compared to control group, while a nonsignificant decrease in time spent in closed arm was observed in both extract-treated group and THI+EXT-treated rats compared to THI-group, at the same time, this test recorded a significant variation ($p< 0.05$) in the number of entries in open and closed arms in THI-group when compared with control rats (Tab 1).

Table 1 Assessment of anxiety effects in the rats exposed chronically to THI and EXT, THI + EXT using the EPM test

Groups of rats	Time spent in closed arms (%)	Time spent in open arms (%)	number of entries in open and closed arms
CON	5,57 ± 0,53	94,43 ± 0,54	9,00±2,16
THI	97,00 ±1,41 *****	3,00 ± 1,41 *****	2,00±0,82*****
EXT	30,29 ±3,50 **ns**	69,71 ± 3,50 **ns**	8,43±0,98 **ns**
THI + EXT	34,00 ±1,15 **ns**	66,00 ± 1,15 **ns**	6,29±0,95 **ns**

Values are means ± SD, n= 6, p value was detected compared CON and THI group. **$p \leq 0.01$ is highly significant; ***$p \leq 0.001$: is very highly significant.

Evaluation of depression by FST test

Results analysis showed a highly significant ($p<0.01$) immobility time (TIM) in THI-treated rats compared to control rats, however, EXT-treated rats showed a significantly longer mobility time than THI-treated rats. Our results show a significant decrease in immobility time in THI+EXT-treated rats compared to THI-group (fig 2).

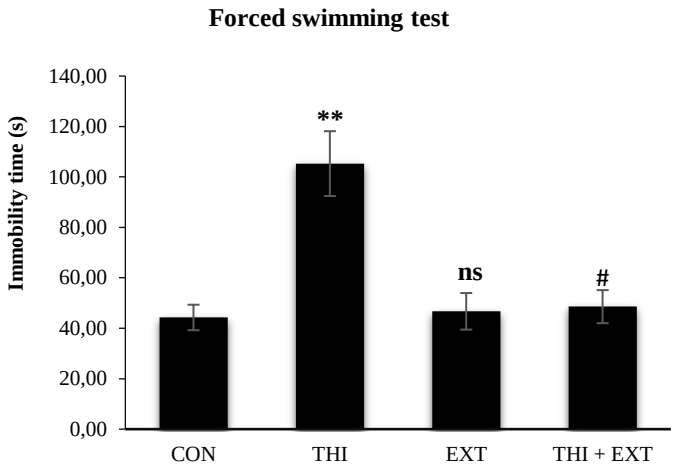


Figure 2 Evaluation of the level of depression in the rats exposed chronically to low dose of THI and THI+EXT using the forced swimming test (immobility time). Values are means ± SD, (n= 6), p value was detected compared to control group. ns $p > 0.05$: not significant, **$p \leq 0.01$: is highly significant. and ns $p > 0.05$: not significant, the difference is significant (#) $0.05 > p > 0.01$compared to thiacloprid group

Evaluation of working memory and learning ability

Novel Object Recognition test (NOR)

According to the results obtained in this test, the recognition index (RI)which presents the time to explore the new object, showed a highly significant decrease ($p<0.01$) in THI-treated rats compared to control. But RI increased significantly ($p<0.05$) in EXT-treated group and THI+EXT-treated rats, (fig 3).

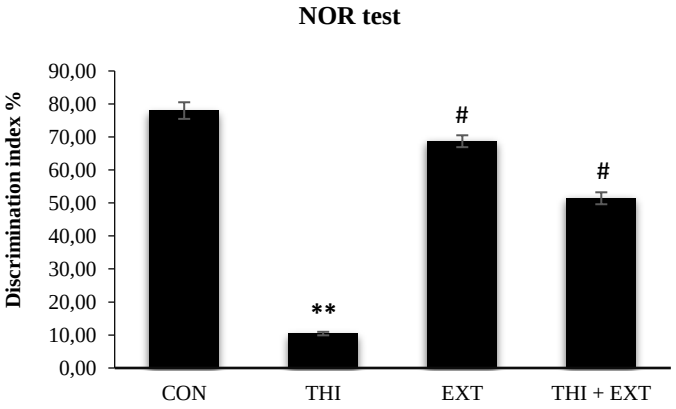


Figure 3 Evaluation of working memory and learning ability in the rats exposed chronically to THI and THI+EXT extract using the NOR test. Values are means ± SD, (n= 6), p value was detected compared to control group. **$p \leq 0.01$: is highly significant, and the difference is significant (#) $0.05 > p > 0.01$compared to thiacloprid group

Assessment of the muscle strength of treated animals

*(KIS)*test*:* The results obtained from the KIS test, represented a very highly significant ($P < 0.001$) increase in time of rat fixation in the inverted screen, in THI-treated group, in addition, a significant increase of this time recorded in EXT-treated group and THI+EXT-treated rats compared to control. Moreover, the same results showed a highly significant decrease in the time of fixation of the rats in the inverted screen in EXT-treated group and THI+EXT-treated group compared to THI-group (fig 4).

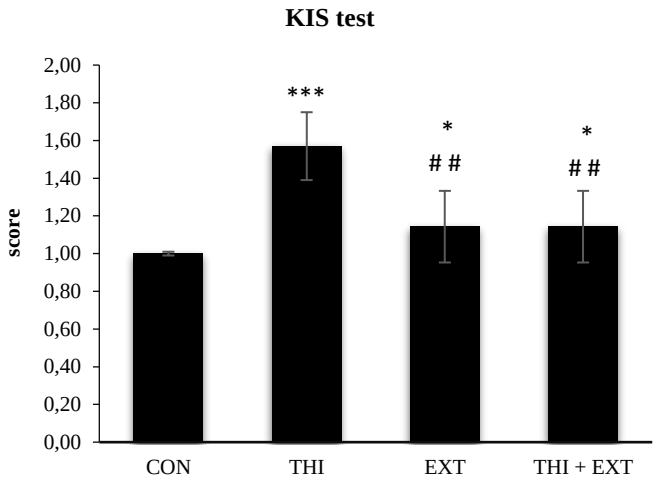


Figure 4 Evaluation of muscle strength of rats treated with THI and THI-EXT using the KIS test. Values are means ± SD, (n= 6), p value was detected compared to control group. *$p<0.05$: the difference is significant, ***$p \leq 0.001$: is very highly significant. and the difference was highly significant (##) compared to thiacloprid group.

Balance evaluation by BBT test: The results of this study revealed significantly ($p<0.05$) deficits in coordination and equilibrium in the rats exposed to THI and THI+EXT compared to control, expressed by walking time on the beam. In addition, these results showed a non-significant decrease in the time of walking on the beam, in the groups treated with EXT and THI+ EXT, in comparison with the THI-treated group (fig 5).

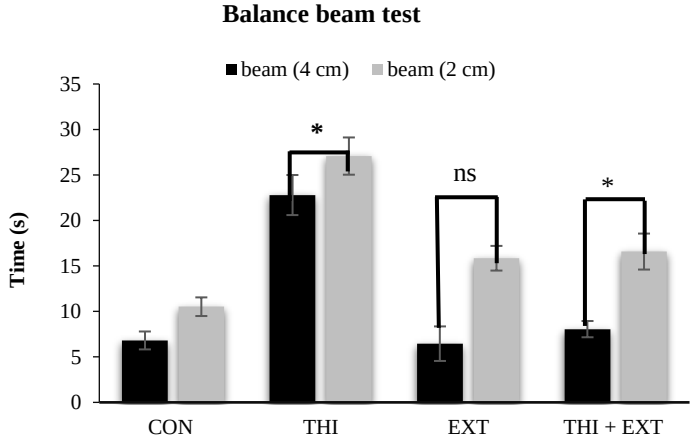


Figure 5 Evaluation of coordination and balance of rats treated with THI and THI+EXT using the KIS test. Values are means ± SD, (n= 6), p value was detected compared to control group. ns $p > 0.05$: not significant, *$p<0.05$: the difference is significant, and the difference is not significant ns $p > 0.05$compared to thiacloprid group

Evaluation of cytosolic AchE activity

The results of this essay showed a highly significant ($p< 0.01$) and significant ($p< 0.05$) decrease in AchE activity respectively in str and hipp; in THI-treated group compared to control group. However, the same results noticed a significant increase in AchE activity in rats treated with the EXT and THI+EXT, (fig 6).

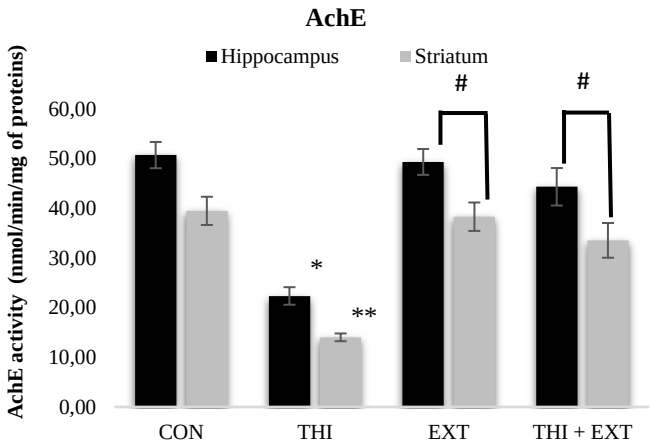


Figure 6 Variation of AchE activity in rat hippocampus and striatum after chronic different treatments with THI and EXT during 90 day, Values are means ± SD, (n= 6); *p ≤ 0.05: the deference is significant; **p ≤ 0.01: highly significant compared to control group, #p ≤ 0.05: the deference is significant compared to Thiacloprid group

***Evaluation of Mitochondria swelling and* (mPTP)**

Results of this evaluation showed a very highly significant (p<0,001) and a significant (p<0, 05) increase respectively in hipp and str mitochondrial swelling, in THI-treated rats compared to control. These same results showed also very highly significant (p<0,001) and a highly significant (p<0, 05) decrease in mitochondria swelling in EXT-treated group compared to control. However, results revealed that the association of THI with EXT decreased significantly (p<0, 01) mitochondrial edema in hipp, compared to THI-treated group (Tab 2).

Table 2 Mitochondrial swelling and permeability in the hippocampus and striatum of CON rats and treated after 3 months of treatment

Groups of rats	Brain areas	Mitochondria swelling as (optical density)	Mitochondrial permeabilityas (ΔOD/Δt)
CON	Str	0,064 ±0,001	0,014 ± 0,001
	hipp	0,209 ±0,001	0,004 ±0,0001
THI	Str	0,016 ±0,002 *****	0,057±0,005*****
	hipp	0,106 ± 0,003*******	0,054±0,0005******
EXT	Str	0,220 ±0,003 ******	0,013±0,001**#**
	hipp	0,381 ± 0,003 *******	0,005 ±0,0001$^{\#\#}$
THI + EXT	Str	0,196 ± 0,002 *****	0,020 ±0,002 **ns**
	hipp	0,244 ± 0,001$^{\#\#}$	0,005 ±0,0002$^{\#\#}$

Values are expressed as mean ± standard deviation; we use ANOVA test, treated group compared with THI and EXT, and the THI + EXT compared to CON group, and THI group, Values are means ± SD, (n= 6); *p ≤ 0.05: significant; **p ≤ 0.01: highly significant; ***p ≤ 0.001: very highly significant; ns p > 0.05: not significant groups compared to CON group, #p ≤ 0.05: significant; ##p ≤ 0.01: highly significant; ###p ≤ 0.001: very highly significant; ns p > 0.05: not significant groups compared to THI group. hipp: hippocampus, Str: striatum.

Regarding membrane permeability, the results showed a highly significant (p<0, 01) and significant (p<0, 05) increase, respectively in hipp and str mitochondrial permeability in THI-treated group compared to control. Conversely, the groups treated with the combination of THI and EXT showed a highly significant (p<0,01) and significant (p<0,05) decrease, respectively in hipp and str mitochondrial permeability, compared to THI-group (Tab 2).

Evaluation of hipp and str mitochondrial redox status

Study results showed a highly significant (p<0,01) and significant (p<0,05) decrease in SOD activity respectively in str and hipp mitochondria in rats exposed to THI and recorded no significant variation in the other groups compared to the control. At the same time, the EXT and THI+EXT groups showed a significant (0.05) enhance in SOD activity in hipp and str mitochondria compared to the THI-treated group (Tab3).

Assessment of CAT activity revealeda highly significant (p<0, 01) decrease in str and hipp mitochondria, in THI-treated group compared to control. The same assessment also showed a significant (p<0.05) improvement in CAT activity in rats treated with the EXT compared to THI-treated group (Tab 3).

Evaluation MDA demonstrated a highly significant (p<0.01) and significant (p<0.05) increase in its str and hipp mitochondrial rate, in THI-treated compared to control. However, same results, noticed a decrease in hipp mitochondrial MDA level in THI+EXT-treated group compared to THI-treated group (Tab 3).

Determination of GSH revealed a significant depletion of mitochondrial GSH in str and hipp in rats treated with THI compared to control. At the same time, EXT and THI+EXT groups presented a highly significant increase of GSH in hippcompared to the THI-treated group (Tab3).

GST evaluation showed a significant increase in mitochondrial GST activity in both str and hipp in THI-treated group compared to control. The results of this evaluation also noticed a significant (p<0.05) decrease in GST activity in hipp mitochondria matrix, in rats exposed to the EXT and combination of THI and EXT compared to THI-treated group(Table3).

Assessment of matrix GPX activity revealeda highly significant (p< 0.01) and significant (p<0.05) decrease respectively in both str and hipp mitochondrial GPX rates, compared to control group. The same results showed, however, a highly significant (p< 0.01) enhance in GPX activity in the EXT and THI+EXT groups compared to THI-treated group(Table3).

Table 3 Variation of GSH and MDA rates and GPx, GST, SOD, and CAT activities, in both rat hippocampus and striatum mitochondria following long-term chronic exposure (90 days) to THI, EXT and THI+EXT

Groups	areas	CON	THI	EXT	THI +EXT
CAT (UI/mg of proteins)	str	0,05±0,004	0,005±0,001*	0,047±0,002#	0,033±0,002 **ns**
	hipp	0,08±0,01	0,010±0,002**	0,058±0,01##	0,065±0,01**ns**
SOD(UI/mg of proteins)	str	77,23±8,0	14,54±1,46**	72,89±9,00 ##	48,28±5,08 #
	hipp	83,39±2,92	16,37±1,09*	52,40±2,22**ns**	55,00±3,16 #
MDA(nmol/mg of proteins)	str	0,89±0,11	1,14±0,04**	0,70±0,06*	0,66±0,05*
	hipp	0,74±0,03	1,58±0,09*	0,59±0,03#	0,085±0,04**ns**
GSH(mmol/mg of proteins)	str	0,20±0,02	0,08±0,01*	0,27±0,03**ns**	0,14±0,02**ns**
	hipp	0,28±0,02	0,09±0,01*	0,27±0,02 ###	0,21±0,02###
GPX (nmol/mg of proteins)	str	0,04±0,005	0,01±0,001*****	0,044±0,003 ##	0,03±0,005**ns**
	hipp	0,06±0,01	0,01±0,001*****	0,06±0,01#	0,040±0,010##
GST (UI/mg of proteins)	str	7,68±0,20	17,56±1,04*****	8,17±0,47**ns**	7,60±0,47**ns**
	hipp	12,33±0,60	23,73±1,68*****	10,22±0,53#	9,03±0,42#

Each value is expressed as mean ± standard deviation; we used ANOVA test. Treated groups with THI and EXT, and the THI + EXT compared to CON group, and THI group.Values are means ± SD, (n= 6); *p ≤ 0.05: significant; **p ≤ 0.01: highly significant; ***p ≤ 0.001: very highly significant; ns p > 0.05: not significant groups compared to CON group, #p ≤ 0.05: significant; ##p ≤ 0.01: highly significant; ###p ≤ 0.001: very highly significant; ns p > 0.05: not significant groups compared to THI group. Hipp: hippocampus, Str: striatum

Evaluation of Apoptosis markers in strand hipplysates

Evaluation of the different apoptotic markers are summarized in the table 4. The results showed significant enhancement of Cyt-c, Bax, caspase-3 and 9 and Cu^{++} levels in both brain regions in THI-treated group, respectively with different degrees of statistical signification ($p< 0.05$, $p< 0.001$, $p< 0.01$, $p< 0.01$, $p< 0.01$), compared to CON-group, while Bcl2 content in tissue lysates of both areas decreased significantly ($p<0.05$). On the other hand, such apoptotic markers changes have not been observed in both EXT and THI+EXT groups when compared to control. Otherwise, administration of the bitter apricot kernels extract associated with THI prevented significantly the change in the rates of these apoptosis markers.

Table 4 Changes of apoptosis markers in both striatum and hippocampus in different treated rats exposed during 90 days to a low-dose of thiacloprid

Rat Groups	Brain areas	CON	THI	EXT	THI +EXT
Cyt-c(µg/ml)	str	1.42 ± 0.22	4.48 ±1.26*	1.34 ± 0.88 ##	2.42 ± 0.42 #
	hipp	1.12 ± 0.18	5.32 ±1.08*	0.98 ± 0.16 #	1.98 ± 0.22 #
Bax(ng/mg pr.)	str	07.5 ± 0.42	15.2 ± 0.82***	06.6 ± 0.62 ###	14.64 ± 1.22 ns
	hipp	08.6 ± 0.94	17.6 ± 1.24***	07.2 ± 1.44##	12.52 ± 2.64#
Bcl2(ng/mg pr.)	str	24.6 ± 2.24	16.4 ± 1.86*	25.2 ± 2.60 #	18.46 ± 1.22 ns
	hipp	18.2 ± 0.55	14.8 ± 0.82*	19.4 ± 1.80 #	18.54 ± 0.86 #
Bax/Bcl2 ratio(mean value)	str	*0.304*	*0.926*	*0.261*	*0.793*
	hipp	*0.307*	*1.189*	*0.371*	*0.675*
Caspase-3(mAbs/mg pr.)	str	090 ± 14	184 ± 16**	080 ± 12 #	160 ± 12 ns
	hipp	110 ± 18	250 ± 42 *	070 ± 16 #	096 ± 14 #
Caspase-9(mAbs/mg pr.)	str	120 ± 20	278 ± 14**	130 ± 14 ##	238 ± 21ns
	hipp	098 ± 08	212 ± 24**	102 ± 12#	152 ± 18 #
Cytosolic Ca++ (mmol/l)	Str	1.65 ± 0.10	2.26 ± 0.06*	1.64 ± 0.12#	1.65 ± 0.08 ns
	hipp	1.29 + 0.02	2.32 ± 0.07 *	1.37 ± 0.03 ns	1.54 ± 0.06 ns

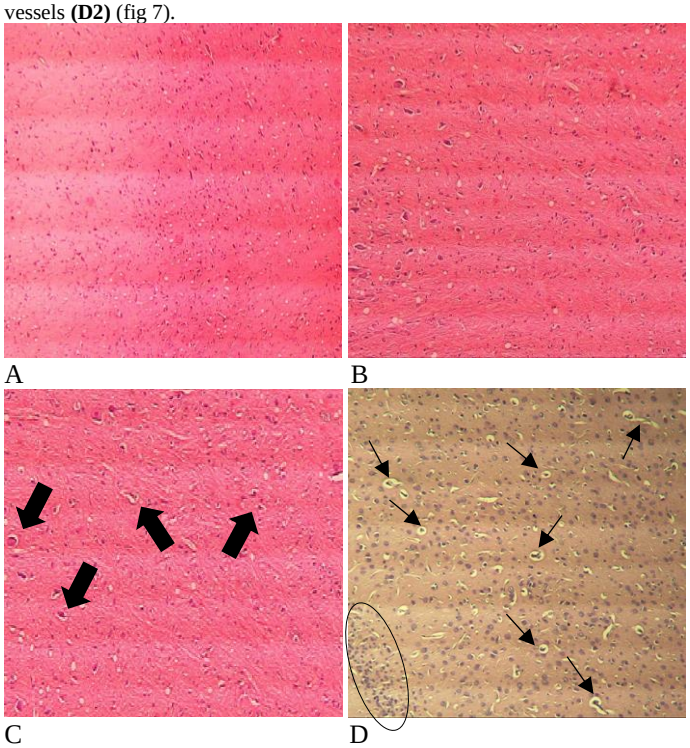
Values are means ± SD, (n= 6); *p ≤ 0.05: significant; **p ≤ 0.01: highly significant; ***p ≤ 0.001: very highly significant; ns p > 0.05: not significant groups compared to CON group, #p ≤ 0.05: significant; ##p ≤ 0.01: highly significant; ###p ≤ 0.001: very highly significant; ns p > 0.05: not significant groups compared to THI group. hipp: hippocampus, Str: striatum

Histopathological observations

Microscopic observation of whole brain sections in control group revealed normal architecture and distribution of neurons. In addition, both neuronal and glial cells are intact in neuropil of total brain **(A).**

Rats treated with EXT **(B)** and the combination (THI+EXT) have normal architecture and similar structure to that of control, however a moderate neuronal degeneration appeared in this last group **(C).**

Observation of histological sections of whole rat brain treated with THI showed conversely however, a morphologic change compared to control brain rats, presenting degeneration of neurons haemorrhages, accompanied with severe degeneration of microglial cells and focal aggregation of microglial cells was also recorded, in addition to mild degeneration of cells of the purkinje cell layer **(D1).** The same sections noticed spongiosis and vacuolation of neuropil with multiple areas of extravasted erythrocytes with moderate lesions and congestion of blood vessels **(D2)** (fig 7).



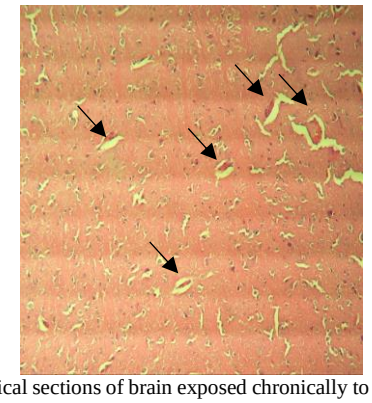


Figure 7 Histological sections of brain exposed chronically to different treatments :**(A)** intact neurons and glial cells in neuropil of total brain; (B) Rats treated with EXT; (C) moderate neuronal degeneration in the group of the combination (THI + EXT) have normal architecture and a structure similar to that of control; (D)group of rats treated with THI showed severe degeneration of microglial cells and focal aggregation of microglial cells; (D1) the same group of rats treated with THI showed multiple areas of extravasted erythrocytes

DISCUSSION

The aim of this study was to evaluate *in vivo* neurotoxic effects associated with the exposure of rats to realistic dose of THI during 3 months. Of not, NNs pesticides cause various toxic effects, such as neurotoxicity, immunotoxicity, hepatotoxicity, nephrotoxicity and recently reproductive effects have been also recognized in animals and humans **(Wang et al. 2018; Taibi et al. 2020).** In current study, biochemical examinations and behavioral tests were approached to assess cellular and mitochondrial integrity in two brain regions, namely str and hipp. In this context, another study is required to investigate also the repercussions of these organic abnormalities on cognitive and neurobehavioral functions. In this sense, prior to sacrifice, animals were submitted to some approved tests, evaluating the effect of THI on cognitive and behavioural potential such as locomotion, memory, learning, anxiety and depression, muscle strength, and coordination/ balance. Indeed, the OF test revealed an altered locomotor activity in THI-group compared to the control. This locomotion decrease may be due to the direct influence of this NNs on cholinergic function, especially since the results of this study showed a reduction in Ach Eactivity. The same result was reported by numerous studies having used both THI and imidacloprid at higher doses (**Taibi et al. 2020).** On the other side, when **a**nxiolytic behavior was evaluated using EPM test, the results obtained showed that THI was anxiogenic compared to the control group. Similarly, the study of **Hirano et al. (2018)** reported that administration of clothianidin (5-250/kg/ day), for 4 weeks induced anxiety behavior in mice. Forced swimming test showed inability to swim in THI-treated group which proves the depressant effect of THI Of note, the administration of imidacloprid at different doses in rats has resulted in SNC depression as a consequence of disturbances in the serotonergic system and neuronal nicotinic acetylcholine receptors (nAChRs) (**Taibi et al. 2020).**

The results of NOR test showed clearly a functional alteration of the different brain regions involved in declarative memory. Similar data reported that, after acute exposure of rats to acetamiprid (100 mg/kg), animals showed blocked memory formation at 30% (M**ondal et al. 2014)**. According to **Xiao et al. (2011),** the repetitive opening of nAChR causes desensitization of the receptor and ion channel and thus prevents the flow of Ca^{++} through the membrane explaining this memory blockage. Similarly, administration of a higher dose (8 mg/kg during 3 months) of THI showed a decrease in learning activity in adult rats (**Kara et al. 2015).**The groups treated with EXT and EXT+THI exposed an increase in the recognition index, which indicates that the extract of bitter apricot kernels shows a preventive effect against memory disorders induced by THI. These results are in agreement with numerous works revealing that bitter apricot kernel aqueous extract demonstrated treatment efficacy against memory loss and neuroprotective potential versus neuronal death **(Gasmi 2018, Vahedi-Mazdabadi et al. 2019).** In this study**,** KIS test revealeda very highly significant increase in muscle strength in THI-group compared to the control. These results can be explained by continuous activation of the acetylcholine receptor, leading to signs of neurotoxicity and continuous membrane depolarization under THI effect, which is defined to be a non-hydrolysable acetylcholine (Ach) agonist, knowing also that AchE is unable to degrade it **(Anadón et al. 2020),** also the binding of THI to nAChR results in a surplus of Ach, which is normally degraded by AchE. However, according to our results, THI leads to a deficit in the activity of this enzyme, thus this inhibition produces a prolongation of the duration of Ach action **(Cavalcante et al. 2020).** Administration of the extract showed a significant increase in muscle strength compared to the control, these results can be explained by the difference in body weight between treated and control rats whose weight gain reduces muscle strength **(Shoji et al. 2016),** where apricot kernels extract improved muscle strength most likely due to its richness in flavonoids, knowing that these compounds are neuroprotective agents **(Moradi et al. 2017;Vahedi-Mazdabadi, 2019)**. Worthy of note is that mitochondrial pathway of cell death begins with permeabilization of mitochondrial membrane which results in swelling and rupturing outer membrane and externalization of proapoptotic proteins such as Cyt-c **(Gasmi et al. 2019).** Indeed, data of this study showed an increase in mitochondrial permeability and swelling in THI-treated group, but when the pesticide was associated with extract a significant protection of the mitochondrial membrane permeability and oedema were prevented compared to control group. The prevention of mitochondrial integrity was reported similarly by numerous authors when quercetin (flavonoid) was administered to animals in combination with NNs (**Beghoul et Sal. 2017; Gasmi et al. 2017**). Of note, among the manifestations resulting from chronic exposure to neurotoxic pesticides, oxidative stress is widely indicated **(Gasmi et al. 2019),** let's also remember thatbrain is the most sensitive organ to the imbalance of redox status able to induce most neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases **(Mayada et al. 2019; Mesole et al. 2020).** Evaluation of mitochondrial redox status in the both brain regions showed a depletion of mitochondrial GSH and a significant decrease in mitochondrial SOD, CAT and GPx activities in THI-treated rats. This weakening of the antioxidant systems may be explained by excessive generation of free radicals under the prooxidant effect of THI. Similarly, previous studies demonstrated the depletion of antioxidant enzymes activities due to their involvement in detoxification processes **(Moeen et al. 2018; Kanane et al. 2019; Katić et al.2020).** The results of this study showed an increase in MDA production in THI-treated group. These data indicate the presence of lipid peroxidation caused by ROS on polyunsaturated fatty acids of cell membranes, which leads to the loss of the cell membrane structure and its function **(Beghoul et al. 2017).** This interpretation could be supported by another previous study which recorded that the resulting oxidative damage is the main cause of neuronal degeneration after chronic treatment of rats with 1 mg/kg of imidacloprid (**Abd-Elhakim et al. 2018; Moeen et al. 2018)**. Also, these results are in agreement with the outcomes of our previous studies where it was noticed that all antioxidant systems collapsed with intensive production of MDA (**Gasmi et al. 2016**; **Beghoul et al. 2017)** in rats exposed chronically NNs. Of note, it is well known that polyphenols are used against various diseases caused by oxidative stress which can increase the capacity of antioxidant defences and they can also reduce toxicity and loss of neurons **(Qin et al. 2019)**. In this context, results of previous study (**Moeen et al. 2019)** showed that apricot kernels extract is able to reduce the toxic effects of pesticides which are shown by a reduction in ROS and a significant increase in antioxidant enzymes such as CAT and SOD levels. Incurrent study, administration of the bitter apricot kernels extract associated with THI, prevented significantly variation of oxidative stress indicators, normalized cholinergic function and prevented cognitive disorders. Indeed, the work of **Li et al. (2016) and Geng et al. (2016)** showed the richness of bitter apricot kernels by different antioxidant components such as iron, potassium, amygdalin, vitamin B17, flavonoids, which can have different biological activities such as antioxidant and anti-apoptotic activities.In the present study, significant decrease in AchE activity in the strand hipp was recorded in THI-group compared to the control. This effect has been recorded also by **Katić et al. (2020)** where theyshowed inhibition of whole brain AchE activity after treatment of rats with 0.06 and 0.8 mg/kg of THI during 28 days; furthermore, the same decrease in AchE activity recorded by **Topal et al. (2017)** and **Moeen et al. (2018)** after long duration exposure to 10 and 20 mg/kg/of imidacloprid in rats. Ca^{++} is considered a key regulator not only of cell survival, but also of cell death in response to various apoptotic stimuli and neuronal degeneration **(Gunter et al. 2004; Kumar et al. 2015).** The results of this study showed an increase in extra-mitochondrial calcium rate in str and hipp in THI-treated rats compared to the control, which could trigger the lysis of many proteins and cell death, but apricot kernels extract prevented the enhancement of cytosolic Ca^{++}. Indeed, previous study showed that high concentrations of extra-mitochondrial calcium cause the release of Cyt-c and activate calcium dependant proteases, thus initiating apoptosis processes **(Gasmi et al. 2019; Chouit et al. 2020).** For this reason, the used extract is considered provided with a neurocytoprotector activity, able to neutralize the toxicity of the THI.

According to literature data, the observed mitochondrial permeability impairments can lead the release of cyt-c and Ca++ into cytosol during the early step of cell apoptosis, which participate in the maturation of caspase-9, this process is required for apoptosis triggering (Gasmi et al. 2017; Chouit et al. 2021). Indeed, the results of this study showed an enhancement in cytosolic cyt-c, caspase-3 and 9 activities, and in cytosolic Ca++ and Bax rates, in THI-treated group. All these markers indicated that the used pesticide is able to trigger intrinsic apoptosis signaling pathway **(Toualbia et al. 2017; Saranya et al. 2020**). This apoptotic THI effect was supported by a significant down-regulation of Bcl2 which is considered a potential anti-apoptotic member. So, the results of this study explored primary mitochondrial membrane alterations in the form of edema and membrane permeability disorders allowing that release of apoptotic proteins from mitochondrial intermembrane space such as cyt-c which participates in the maturation of caspase-9 with the involvement of apoptotic peptidase activating factor (AIF), these events lead to apoptosome formation, able to activate caspase-9 and 3**(Prieto-Moure et al. 2016 ; Shakeri et al. 2017).**

Histological observation revealed that repeated oral dose of THI resulted in neuronal and glial cells degeneration and vacuolation with congestion of blood vessels in the neuropil of brain. These results are in concurrence with the findings of **Abd-Elhakim et al. (2018),** who reported severe degeneration of neurons and glial cells with vacuolation in neuropil of hipp of rats treated with imidacloprid, similar findings were reported by **Vivek et al. (2020)** after exposure of rats to THI. It is noticed that these histological observations are in agreement with the current study showing the apoptotic effect of THI, whereas the treatment of animals with the hydroalcoholic extract of bitter apricot kernels at 50 mg/kg/day in association with this pesticide resorted the normal architecture and structure of brain, these results demonstrate that this extract have a neuro-protective potential against tissue alterations due to THI neurotoxicity.

CONCLUSION

The results of the current study revealed that chronic exposure to low-dose of THI lead to mitochondrial swelling and membrane permeability injury, accompanied with oxidative stress in this organelle. These mitochondrial damages resulted in releasing of Cyt-c and Cu++into cytosol, besides triggering of apoptosis signalling pathway by up-regulation of Bax rate, caspase-3 and 9, and down-regulation of Bcl2 in both str and hippcell lysates. These organic brain injuries were associated with cognitive and neurobehavioral disabilities in THI-treated rats compared to control group. Conversely, ² treatment with THI associated to the hydroalcoholic extract of bitter apricot kernels prevented imbalance of mitochondrial redox status and membrane permeability disturbances. Consequently, this association mitigated apoptosis signalling pathway and kept intact cognitive and neurobehavioral abilities of animals.

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