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Neurobehavioral Effects and Oxidative Stress Regeneration By Exirel Pesticide After Low Dose Exposer In *Wistar*rats

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ABSTRACT

Exirel is a second generationanthranilicdiamide insecticide that has biological efficacy against insect pests and can induce the formation of reactive oxygen species (ROS). The main objective of this research is to study the neurotoxicity of an environmental contaminant in albino rats, after Exirel exposure to five concentrations (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1 mg/kg/d) of oral body weight for 90 days, To our knowledge, this is the first directed study to evaluate oxidative stress in rats white *Rattusrattus* of the strain Wistar cause by Exirel.From our results the Exirel induced oxidative damage characterized by a very highly significant decrease in glutathione level (GSH), and the activity of glutathione peroxidase, and catalase in the brain compared to controls, on the other hand we observed an increase in the enzymatic activity of glutathione s-transferase (GST), the MDA level was used as a measure of lipid peroxidation (LPO) in a dose-dependent manner. Behavioral studies containing Classical Open Field Labyrinth and Object Recognition test, we recorded changes in the psychic behavior of rats that manifested themselves in anxiety, disruption of memory and learning disturbances at the level of acetylcholinesterase (AchE) brain, We results showed that exposure to Exirel induced damage and stress in rats.

Keywords
Neurotoxicity; Exirel; Wistar Rats; Oxidative Stress; behviour

Introduction

Pesticides are broad-spectrum substances used in agriculture to prevent. destroy and mitigate pests such as insect pests, rodents and other harmful organisms. Today, pesticides pose a major risk to human health and the environment. the effects on the environment are frequently concerned the degradation of the quality of fresh groundwater. The contamination of natural environments and in the reduction of terrestrial biodiversity. The toxic effects of pesticides on human health understand a variety of areas: some constituents can cause irritating effects on the skin, others can influence liver or lung functions, some carcinogens and the majority of pesticides are neurotoxic especially insecticides**[11][25].** Among the systematic insecticides evolved in recent yearsdiamide insecticides have become the fourth most exploited in the world **[43].** Diamide compounds are the new large class of insecticides and its associate in group 28 of the mode of action category of the Insecticide Resistance Action Committee (IRAC) **[50]**.And are rapidly changing other insecticides such as organophosphorus, pyrethroids, carbamates and neonicotinoids **[13].** Act by activating the ryanodine receptors (RyR) is a non-voltage-dependent calcium channel located on the endoplasmic reticulum that controls the release of intracellular calcium storage necessary for the functioning of muscle cells, which induces an uncontrolled release of calcium reserves that causes muscle contraction and paralysis and promotes death **[47] [7].**

Exirel belongs to Diamides (modulators of ryanodine receptors) is manufactured by the company Dupont de Nemours & Co., Inc., DuPont Electronic Products, 1515 Nichols Road, DaytonOH 45418-2712, suggested to register the insecticide DuPont Exirel of the new active constituent which is cyantraniliprole (100 g/L) **[5].** belongs to the family of anthranilicdiamides and with the following characteristic properties: the name IUPAC 3-bromo-1-(3-chloro-2-pyridyl)-4'-cyano-2'-methyl-6'-(methylcarbamoyl)pyrazole-5-carboxanilide (International Union of pure and applied Chemistry) with an off-white powder color nor odor. The toxicological information base of Exirel (cyantraniliprole), which consists mainly of toxicity studies carried out in rats, mice and dogs, is broad to determine the toxicological profile of Exirel (cyantraniliprole) and to define the risk to human. The insecticide DuPont Exirel (Cyantraniliprole) is an oral toxicant (LD50 > 5000 mg/kg bw) in rats and mice and less toxicity by the dermal and inhalation route (LD50 >5000 mg/kg bw) in rats and mice.5000 mg/kg bw; 4-hour LC50 > 2400 mg / m3) **[5].**

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Analytical methods are available to control cyantraniliprole residues (IN-J9Z38, IN-N5M09, IN-F6L99) in products such as pepper and coffee, tomato and bunches of the grapes under study. Based on the results of the risk assessment EFSA found that the intake of residues resulting from the use of Exirel (cyantraniliprole), it is likely that agricultural practices pose a risk to consumer health through the accumulation of residues over a long time **[17] [18].**

This research aims to study the toxic effects of insecticide diamide which is Exirel (Cyantraniliprole) to evaluate its ability to cause oxidative stress in rats white *Rattusrattus* of the Wistar strain as a biological model received from the Pasteur Institute of Algiers Algeria are nocturnal mammals has been used in behavioral biomedical research and toxicology **[16],** we analyzed chronic toxicity (90 days) and evaluation criteria that included behavioral tests(Classical Labyrinth, Open Field, object recognition test), the variation of enzymatic activities among them the GSH level that plays a role in antioxidant defense and Catalase as a first enzyme against oxidative stress, the GST and GPx then the MDA the biomarker of lipid peroxidation, also the analysis of the neurotransmitter AchE.

Materials and Methods

Chemical and dose selection

The substance used in this study is Exirel, an insecticide in the family of anthranilicdiamides (C H D Brcln O, CAS: 1392493-34-3), the active molecule denoting Cyantraniliprole were obtained by e.i. du pont Canada company agricultural products. The chemical was dissolved in distilled water before each experiment to obtain four concentrations (0.025, 0.05, 0.075 and 0.1 mg /Kg/d) prior to animal handling.
Doses were selected based on recent work; these doses are very close to reality and are likely to contaminate the general population. **[40][17] [8].**

Animals

30 white male rats *RattusRattus* of the *Wistar* strain, weighing from 190g to 260g were obtained from the Institut de Pasteur Algeria (APA), rats are placed in the premises of the Faculty of Nature and Life Sciences at ChikhLaarbiTebessi University at a constant temperature (25 2°C) under a daily light cycle-12-hour darkness with free access to water and specific food.

Treatment Protocol

The rats were subjected to an adaptation period of 2 months, after this period the animals in summer divided into 5 groups contains 6 rats each control group receives the distilled water only, group treated with 0.025 mg/kg/d of Exirel (Cyantraniliprole), group treated with 0.05 mg/kg/d of Exirel (Cyantraniliprole), group treated with 0.075 mg/kg/d of Exirel (Cyantraniliprole), group treated with 0.1 mg/kg/d of Exirel (Cyantraniliprole) (Table 1) by mouth for 90 days between 10.00 and 11.00, from these doses a volume of 100 ul of each prepared solution is administered to the animals, using a micropipette. The treatment conditions are in accordance with international guidelines for the protection of animals used for scientific purposes.

Table 1. Concentration of Exirel administered to the five experimental batches for the study of chronic toxicity

Lot	Identification	Dose mg/kg/D	Administer volume
T	Control	/	100 ul distilled water

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A	Treaty	0.025	100 ul
B	Treaty	0.050	100 ul
C	Treaty	0.075	100 ul
D	Treaty	0.100	100µl

Behavioral testing

Multiple behavioral tests can be used to assess learning, memory and anxiety, Behavior in Animal Models (Classic Labyrinth Test, Object Recognition Test, and Open Field Test). All tests were conducted during the sessions between 8:30 a.m. and 4 p.m., for 6 days.

Classic Labyrinth Test

In order to assess the behavior of a stressful animal exposed over a long period of time and in very small quantities under pesticide-specific conditions Exirel (Cyantraniliprole), The Classic Maze Test (CLT) is a simple way to measure learning ability, memory and anxiety. The CLT uses a square-shaped maze with a starting point and a stopping point. Once the animal is trained, the animal is allowed to see and explore the maze freely for 10 minutes. Meanwhile, all the vertical and horizontal movements of the animal in the maze are recorded. This is a very difficult task because it requires the animal to remember the fastest path between the starting points and the end. In cases where the maze is designed so that the animal only has to walk, it's pretty easy for healthy rats, but for rats exposed to neuro-xenobiotic (drugs, pesticides), there will be disturbances on their way, We cleaned maze after each rat pass by ethanol at 10% **[23].**

Open Field Test

This Open Field test is a measure of behavior was used to evaluate anxiogenic activity, It is characterized by horizontal and vertical movements of animals in transparent glass box, square and open (50×50×40cm) **[54],** with surrounding walls preventing escape **[39]**. The observation of the animals begins a few seconds after the introduction of the animals into the observation cage. It is used primarily to measure motor function, but also to assess anxiety. An anxious animal avoids the centre of the fieldthat is open, and stays at the ring road. Each rat was initially placed in one of the four corners of the Open Field, with the head facing the corner. Its behavior was observed for a few minutes to measure the distance travelled by the rats, between each test the cage is cleaned with 10% ethanol **[54][25].**

Object Recognition Test (NOR)

The object recognition test is used to study declarative memory in rodents. This task assesses the ability of the rat to recognize a new object relative to a familiar object in a known environment (rodent's natural tendency to preferentially explore a new element).

After a phase of habitation at the arena, the rats are treated and placed in a box (40 40cm) and are deposited two identical objects (D: 2 cm, H: 4 cm) the times spent exploring each object are recorded. During the test phase, the animals are in the presence of a familiar object (explored during the training phase) and a new object. An animal that does not have memory problems will spend its time exploring the new object Administration of an amnesiac substance will result in cognitive impairment during training and a memory failure. During the test phase, the animal will explore the familiar object as well as the new object, suggesting that it has "forgotten" its training phase **[46].**

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Rat sacrifice and brain extraction

After the treatment period (90 days), the animals were killed by decapitation. Moreover, removed the brains and rinsed by physiological water with sodium chloride (Na Cl at 0.9%) then weighed with a scale and then kept at -80°C for the determination of oxidative stress parameters such as GSH, MDA, GST, GPx, and CAT in cytosol and assay neurotransmitters such as AchE.

Evaluation of oxidative stress parameters

Reduced glutathione (GSH) measurement

The level of glutathione at the brain level is quantified using the method of **[51]** based on the measurement of the absorbance of 2-nitro-5-mercapturic acid. 0.2ml of 0.25% sulfosalycilic acid solution (SSA), Centrifuge at 1000 turns/min for 5 min, Add 1 ml of tris-HCL+EDTA buffer (0.02M), PH=9.6 then Mix and add 0.025 ml of 5.5`-dithio-bis-2-nitrobenzoic acid (DTNB) to 0.01M dissolved in absolute methanol, and read absorbance at 412 nm.

Determination of glutathione peroxidase (GPx)

The enzyme activity of GPx is measured by the method of **[22],** based on the reduction of hydrogen peroxide (H2O2) in the presence of reduced glutathione (GSH). A volume of 0.2ml of supernatant is recovered in a tube containing 0.4ml of 0.1mM GSH and 0.2ml of 0.067M phosphate buffer, pH 7.8. The mixture is incubated in a 25°C water bath for 05min. 0.2ml of H2O2 1.3mM is added, after 10min 1ml of TCA 1% (tri chloroacetic acid) is added and the mixture is put in the ice for 30min and centrifuged for 10min at 3000t/min. A volume of 0.48 ml of supernatant is placed in a tank to which is added 2.2ml of Na2HPO4 0.32M with 0.32ml of DNTB 1mM. Absorbance is measured at 412 nm each 30 sec for 05min.

Determination of glutathione S-Transferase (GST) activity

The measurement of glutathione S-Transferase (GST) activity is determined using the **[30]** method. It is based on the conjugation reaction between the GST and a substrate, the CDNB (1-Chloro2, 4 di nitrobenzene), The dosage consists in reacting 200µl of the supernatant with 1.2 ml of the mixture CDNB (1mM), GSH (5 mM), The absorbance reading is performed for one minute and every 15 seconds at a wavelength of 340 nm.

Determination of Catalase enzyme activity (CAT)

Catalase activity (CAT) is performed using the method of **[9]**the decay of absorbance is recorded for three minutes by a spectrophotometer for a wavelength of 240nm. The reaction mixture contains: 100µl of the crude enzymatic extract, 50µl of hydrogen peroxide H2O2 at 0.3% and 2850µl of phosphate buffer (50 mM, pH 7.2), the reaction is triggered by the addition of hydrogen peroxide.

Determination of Lipid Peroxidation

The product of malondialdehyde lipid peroxidation (MDA) was measured by the method of **[20]**the principle of MDA in acid and hot medium with thiobarbituric acid, to form a pink pigment. In short, 375µl of supernatant were mixed with 150µl of TBS solution and 375µl of TCA-BHT solution (TCA 20%, BHT 1%), after mixing is Vortexed and centrifuged at 1000t/min for 10min and then sampled 400µl of the supernatant to which is added 80µl of HCL and 320µl of the tris-TBA solution is then incubated in the bainmarie at 80°C for 10minutes. The absorbance of the supernatant was measured at 530 nm.

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Determination of acetylcholinesterase (AChE) activity

Acetylcholinesterase (AChE) activity was measured using the method **[19]**to provide the enzyme with a substrate of acetylthiocholine, whose hydrolysis releasesthiocholine and acetic acid. 100µl of supernatant are added to 100µl of DTNB (0.1M, pH 8) then 1ml of tris buffer (0.1M, pH 7) after 5min rest and add 100µl of acetylthiocholine substrate after reading at 412nm every 4min for 20min.

Data Analysis

The results are obtained from a single-criterion test present in form (SE ± mean), and to better visualize the results obtained the graphic representation chosen is that of the histograms using Microsoft office 2013. The Graph Pad Prism (Version 7) software was used and a unidirectional analysis of variances (ANOVA) with a significance rate of $\alpha \leq 0.05$.

Results

Behavioral study

the results of this study on behavioral testing of wistar rats after long-term Exirel exposure as shown in Fig 01, 02, 03.

Classic Labyrinth

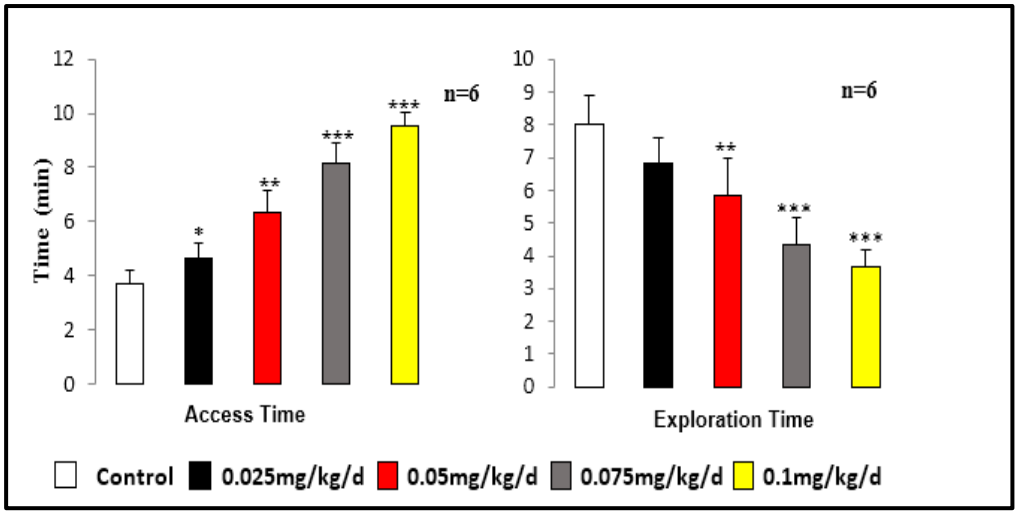

Fig. 01 Changes in access time and exploration time of control and treated rats (Classic labyrinth test) (n = 6). Mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001).

figure 01 shows a very highly significant increase (p≤0.000) in time access and a very highly significant decrease in time exploration (p≤0.000) of rats treated at different doses by Exirel compared to control rats.

Open Field Test

Figure 02 shows a very significant decrease (p≤ 0.000) in the number of entry into the centre of treated rats compared to controls.However, statistical analysis showed a very significant increase (p≤ 0.000) in the number of entering the periphery of treated rats as controls.A very highly

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significant decrease in the number of cells crossed in treated rats compared to controls.A very high increase significant ($p \leq 0.000$) immobility time in treated rats compared to controls.

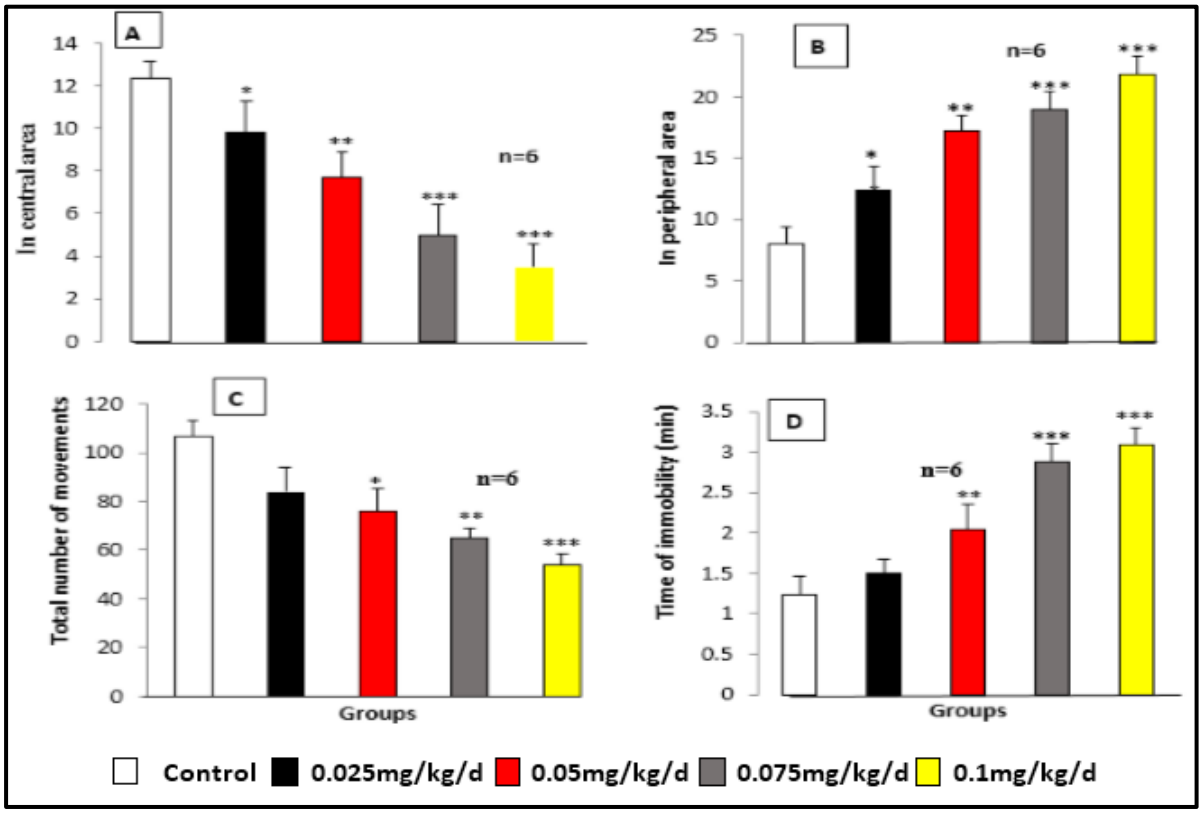

Fig. 02 Variation in Open Field test data in control and treated rats after 90 days of treatment
A.ICA B. IPA C. TNM D. TI

Object recognition test

Treated rats spent less time exploring the new organism than a family object, unlike control groups that did not remember the familiar object. The statistical study shows that there is a very highly significant ($p \leq 0.000$) Fig 03.

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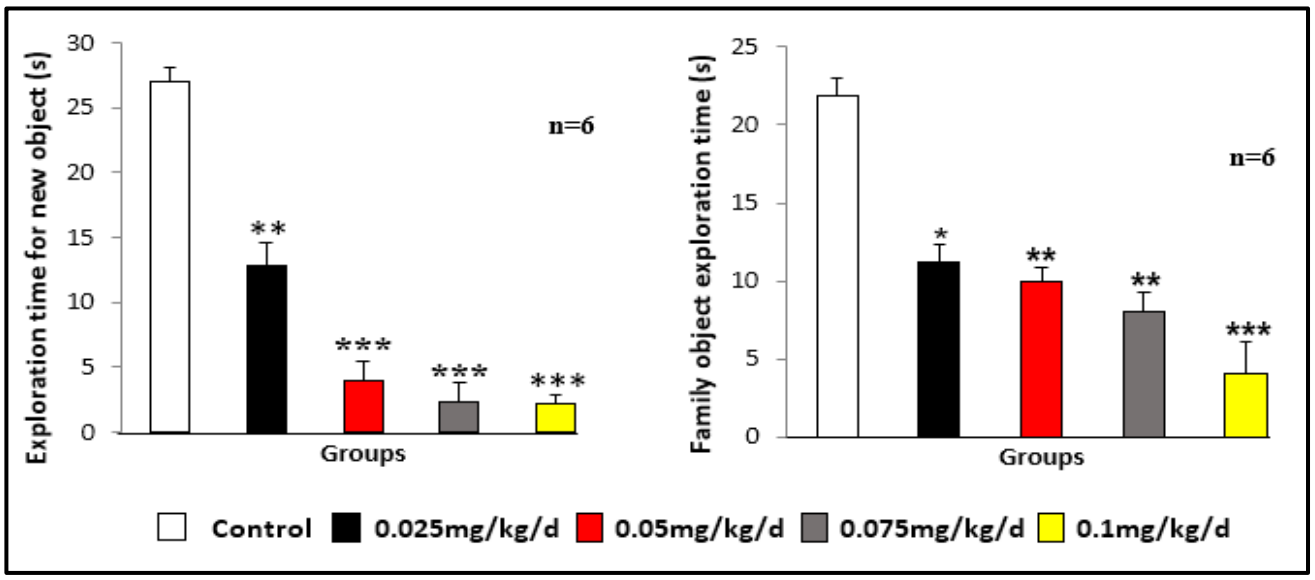

Fig. 03 Variation in Object recognition test data in control and treated rats after 90 days of treatment

AchE Activity

The results in **Figure 04** show a significant decrease ($p \leq 0.05$). inAchE levels in the brains in batches treated with Exirel at a dose of 0.1mg/kg/d compared to controls.

However, there was a highly significant decrease ($p \leq 0.01$) in AchE levels in batches treated with Exirel at a dose of 0.075 mg/kg/d compared with controls, whereas in the other groups (0.025 mg/kg/d, 0.05 mg/kg/d) no statistical significance compared controls.

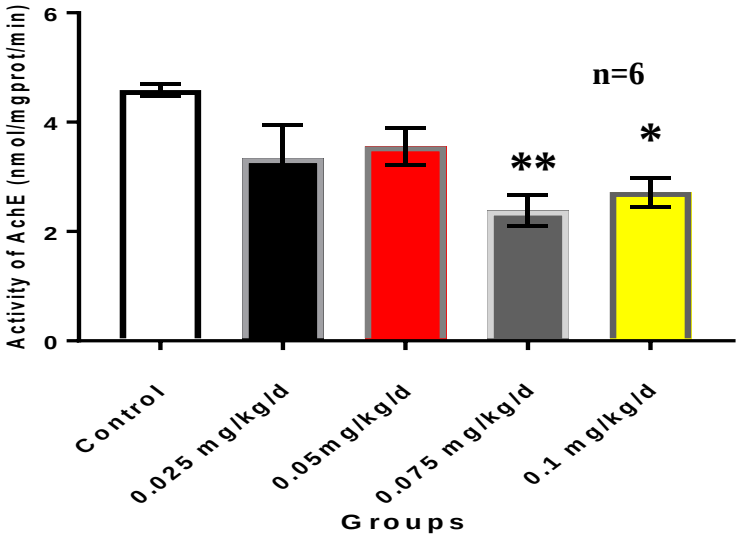

Fig. 04 Change in AchE activity in the brain after 90 days of Exirel treatment
Mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001).

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Antioxidant enzymes activities

Evaluation of MDA levels

Figure 05 shows a very highly significant ($p \leq 0.000$) increase in MDA levels in Exirel-treated rats (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1mg/kg/d) compared to controls.

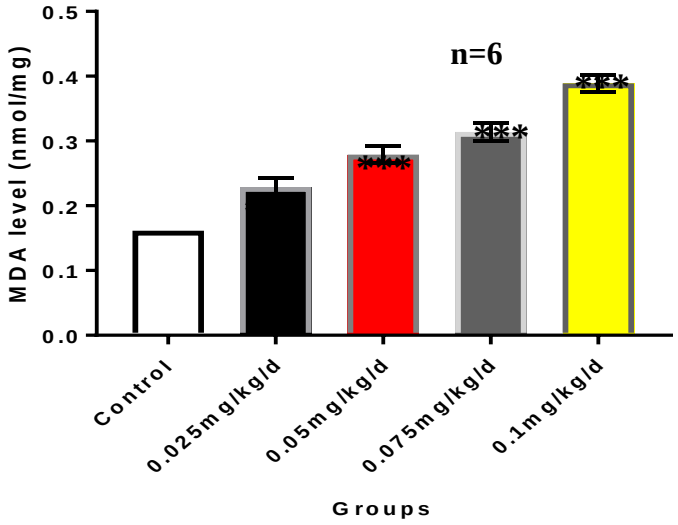

Fig. 05 Variation in MDA content in the brain in control and treated groups
Mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001).

Evaluation of GSH level

According to **Table 02** and **Figure 06**treatment of rats with Exirel at different doses (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1mg/kg/d) results in a very highly significant decrease ($p \leq 0.000$) in glutathione levels (GSH) in the brain compared to controls.

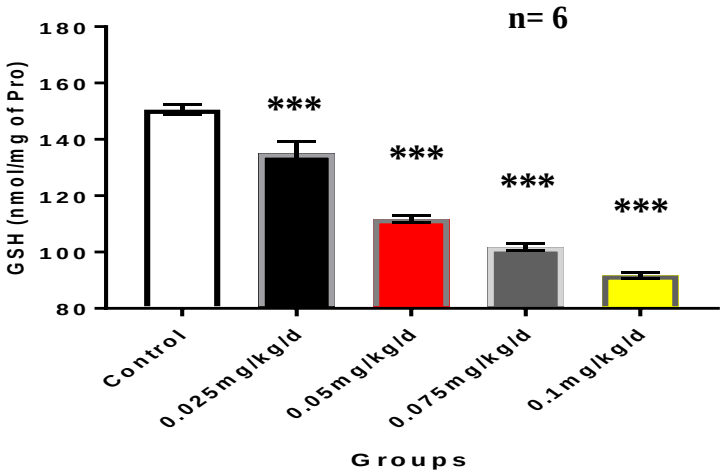

Fig 06. Change of GSH levels in the brain of control rats and rats treated with Exirel after 90 days of treatment
Mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001).

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Evaluation of the enzymatic activity of GP_X

Treatment of rats with Exirel at doses (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1mg/kg/d) causes a very highly significant decrease ($p \leq 0.000$) in the enzyme activity of glutathione peroxidase (GPx) in all treated groups compared with the control groups (**Tab2, fig 07**).

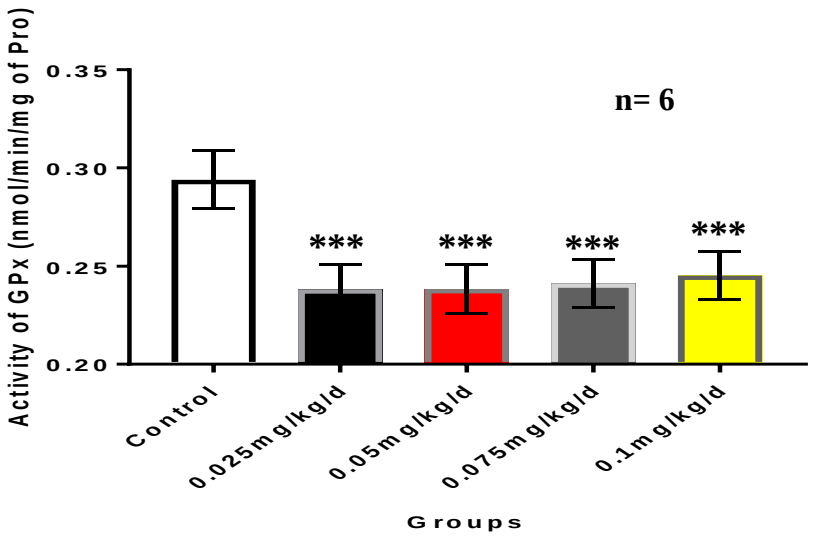

Fig 07. Activity of GPx in the brain of control rats and rats treated with Exirel after 90 days of treatment Mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001).

Evaluation of the enzymatic activity of GST

Table 02 and **Figure 08** show the variation in enzymatic activity of GST due to the administration of Exirel, which causes a very highly significant increase ($p \leq 0.000$), observed in the treated groups (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1mg/kg/d) compared control rats.

Evaluation of the enzymatic activity of catalase

Table 02 and **Figure 09** show the enzymatic variation of catalase in the brain, the results obtained show a very highly significant decrease ($p \leq 0.000$) of catalase in rats treated with Exirel (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1mg/kg/d) compared to the control rats.

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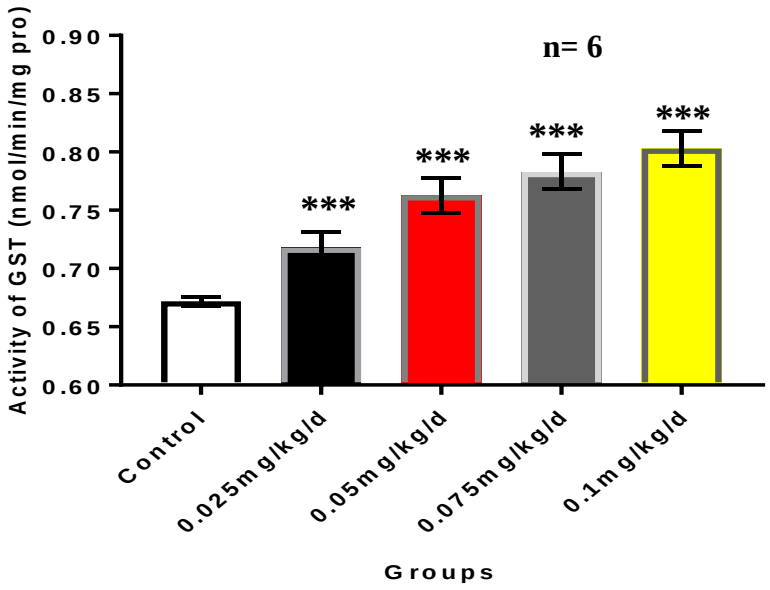

Fig 08. Change of the GST activity in the brain of control rats and rats treated with Exirel after 90 days of treatment Mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001).

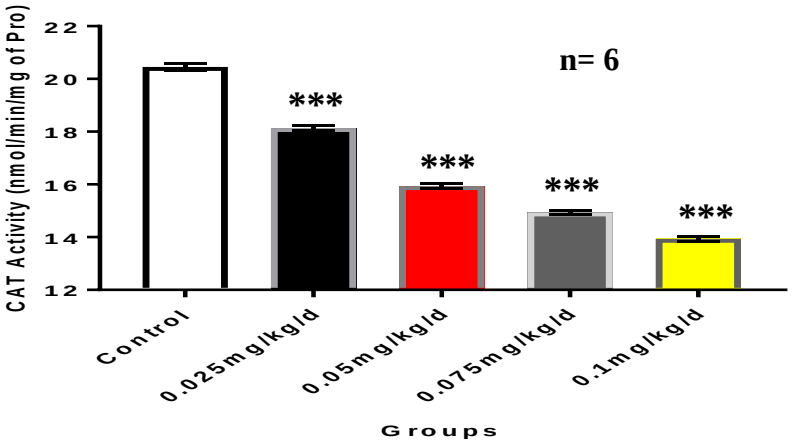

Fig 09. Change of the CAT activity in the brain of control rats and rats treated with Exirel after 90 days of treatment Mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001).

Table2. Effect of Exirel on antioxidant enzyme activities in the brains of control and experimental rats after 90 days of treatment

Parameters	**Control**	**0.025mg/kg/d**	**0.05mg/kg/d**	**0.075mg/kg/d**	**0.1mg/kg/d**
GSH *(µmol/mg pro)*	***±150,551,67***	***135,04±4,15****	***111,68 ±1,16****	***101,68 ±1,16****	***91,68 ±1,16****
GPX *(µmol/min/mg pro)*	***0,29 ± 0,01***	***0,23 ± 0,01****	***0,23 ± 0,01****	***0,24 ± 0,01****	***0,24 ± 0,01****
GST *(µmol/min/mg pro)*	***0,67 ± 0,003***	***0,71 ±0,013****	***0,76 ±0,014****	***0,78 ±0,014****	***0,80 ±0,014****
CAT *(µmol/min/mg pro)*	***20,45 ± 0,13***	***18,14 ±0,08****	***15,94 ± 0,08****	***14,94 ± 0,08****	***13,94 ± 0,08****

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Discussions

Exirel Insecticide is an anthranilicdiamide insecticide is a new class of insecticide used to control insect pests in fruit crops, the results of our study show that Exirel causes oxidative stress in male wistar rats after chronic exposure. Our work to test the ability of this insecticide to induce oxidative stress in male rats by toxicological study and is the first study by the fact that few studies are interested in the appearance of toxic effects of this insecticide on mammals.

Our results show that exposure to Exirel at different concentrations (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1 mg/kg/d) for 90 days, causes several brain-level adverse effects by studying behavioral enzyme parameters.

Effects of Exirel on animal behavior

This is the first work to investigate the effect of exirel on the behavior of rats at different doses by exposing them to multiple tests, saving standard values for future studies.The set of behavioral tests on animals presents a main approach to strengthen our understanding of the brain structure and function behavioral testing plays a central role in understanding neurodevelopmental and behavioral effects **[29].**The classical labyrinth test is one of the most essential behavior patterns for memory and learning anxiety **[31].**

About the classical labyrinth test, theresults obtained in this study show that repeated oral exposure to Exirel at different doses (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1 mg/kg/d) causes an increase in arrival time and time spent and a highly significant decrease in exploration time in the medium in rats treated with Exirelcompared to control rats, this behavioral change explains negative emotions like loss of learning and memorization also increases anxiety **[26].**Anxiety is one of the disorders that effect of a large number of population, which leads to disturbances of the physiological balance of the body such as nervous, endocrine systems... **[28]**.These results are in line with recent work **[26]** following chronic exposure of *wistar* rats by the pesticide deltamethrin (0.32 mg/kg/d) which causes a sharp decrease in memory and an increase in anxiety. In addition, the onset of anxiety and disorders of locomotors activity and memorization can illustrate with changes in neurotransmitter systems (glutaminergic, gabaerogic, serotoninergic and dopaminergic)**[45]**, so learning and memory are largely affected by the stress generated by these pesticides such as the Exirel. Acute or chronic stress experiences may be more significant to memory deficiencies than non-stressful cases**[48][27].**For the Open field test, record a highly significant increase in the locomotors activity of rats in the peripheral and a highly significant decrease in the activity of rats in the center an increase in the time of immobility in a way highly significant for 10min compared to control rats. This change showed that the lowest doses of the Exirel cause depressive behavior **[49]** through increased anxiety, nervousness, fear and loss of memory and inability to think... Previous studies have done a lot of research and found that the progressive increase of mitochondrial ROS, as well as the onset of disorders of neural flexibilities in the brain disturbs memory, learning, and locomotion in rats **[11][42] [28]** already cited. Other study of the effects of treatment with Malathion to measure anxiety and depressive behaviors this study found that the administration of repeated doses of Malathion Modified locomotor activity in rats in the open field test, thus motor alteration following chronic exposure by Malathion **[4].** From these studies, chronic exposure of pesticides such as Exirel induces neurostimulation that disrupts locomotion, memory and learning**[27].**With respect to the object recognition (NOR) test, control subjects exploring both objects in the same way, while those receiving the Exirel show a decrease in operating time for familiar objects and new objects, these results reveal that the pesticide the Exirel alters spatial memory and

recognition and learning of rats. In another study the treatment of mice by OP daizinon presents a slight distinction between new and familiar objects during the object recognition test (NOR), in this context of lesions at the perihinal cortex and hippocampus induces behavioral changes because these latter regions play important roles in spatial memory and recognition of objects therefore damage that affects these regions of the brain causes less recognition and memory damage. In addition, exposure of *Wistar* male rats by Exirel or OPs such as diazinon, CPF and parathion may be due to inhibition of activity in the hippocampus resulting in oxidative stress and inflammatory responses **[52] [3] [44].**

Behavioral testing also plays a central role in understanding neuro-developmental and long-term behavioral effects, Although research on the pesticide is insufficient and very limited, it has greatly affected the behavior of rats due to the results obtained.

Effect of Exirel on oxidative stress parameters

Toxic accidents due to pesticides certainly associated the increase in the generation of reactive species of oxygen at the level of the brain that causes inflammation, chronic and acute diseases such as diabetes, neuropathology and cancer also Alzheimer's disease**[38] [41].** The antioxidant system recognized as the first line of defense of the body against free radicals**[32].** Glutathione (GSH) is a non-enzymatic endogenous antioxidant present in the cytoplasm, nucleus and mitochondria with high cellular concentrations, and the first defense against oxidative stress**[2].**Several important roles of glutathione are the reduction of ROS with the formation of gl utathione disulfide (GSSH) and the conjugation of GSH thus reduces the elimination of xenobioti c**[26].** Here, the concentration of encephalic GSH decreased in dose- dependent and significant manner ($p \leq 0.000$) in Exirel treated rats (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1 mg/kg/d) compared to controls during 90 days of treatment. These results are consistent with another study **[37] [2] [1]**which found a decrease in GSH levels in the brains of rats by different pesticides (Malathion and Deltamethrin). The decrease because of the increase in super oxide and free radicals. The decrease because of the increase in super oxide and free radicals. Results of a study on the toxicity of acetamiprid insecticide in albino male rats showed a significant decrease in GSH in the brain due to high levels of super oxide and free radicals (GSH is converted to GSSG) (oxidized glutathione)**[26].**Another study showed that glutathione and enzymatic work bound to glutathione in combination to capture free radicals such as reactive oxygen species (ROS) and/or neutralize their toxic oxidizing effect, Once chronic oxidative stress attacked some tissues, as proposed in toxicity by pesticides, This would cause buffer changes by greatly increasing GSSG levels due to the inability of cells to restore GSSG to GSH effectively as normal. Thus, the GSH/GSSG ratio would also decrease**[33].**The first line of defense against oxidative stress consists of the antioxidant enzyme GPx, CAT which transform superoxide anions into hydrogen peroxide and then into water and molecular oxygen**[53].**According to **Table 02** and **Fig. 02**, the treatment of *wistar* rats with Exirel resulted in a very highly significant decrease (p 0.000) in the enzyme activity of glutathione peroxidase (GPx) in the brain for all concentrations tested against control rats. GPx among antioxidant enzymes that control the content of ROS (which converts H2O2 into oxygen and water but also oxidation of hydroperoxides of unsaturated fatty acids) and ensures cells against alterations by toxic doses of diamides (Exirel)**[26].**Based on our results, the accumulation of ROS content**[13]**may cause a decrease in the enzymatic activity of cerebral GPx in rats treated with Exirel, due to overproduction of hydrogen peroxide **[26]**and as an exceeded antioxidant capacity after exposure to diamide insecticides**[13]**Previous studies that conducted toxicity studies on various insecticides such as Malathion and chlorpyrifors**[53] [1]**reported that decreased enzyme activity (GPx) results

from the depletion of antioxidant cellular defense mechanisms against ROS generated by these pesticides also biotransformation (Malathion) catalyzed by cytochromes P450 resulted in significant concentrations of ROS that may exceed neutralization of endogenous antioxidant enzymes.Glutahione-S-transferase (GST) is a multifunctional enzyme of a multigenic family present in all the body involved in the detoxification of endogenous (intracellular metabolites) and exogenous substances (drugs, pesticides, heavy metals, etc.), this enzyme plays a role in the conjugation of electrophilic compounds integrated into cell biotransformation **[53].** However, catalyzing the conjugation of GSH with certain substrates has a phase in the formation of less toxic and water-soluble components this step is the key to metabolic detoxification is followed by elimination **[26][6]** and also involved in the defense against oxidative damage and peroxidants whose lipids and nucleic acids are the target **[53]**.

For the purposes of our study, the activity of this enzyme (GST) was measured in brain cells after stress induced by Exirel**. Fig 3** and **Tab 2** showed very significant cytosolic GST enzyme activity ($p \leq 0{,}000$) in rats treated with Exirel at different concentrations (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1 mg/kg/d), the results of our study Following previous studies in land glass and fish, rats**[53][26][6][14] [43]**whichrevealed that exposure to the Xenobiotic family such as CPF and Malathion, deltamithrin may induce an increase in cytosolic activity of GST, The induced GST activity can illustrate the role of this enzyme in protecting against the toxicity of lipid peroxidation induced by xenobiotic also explained the high levels of concentration of xenobiotic present in the environment or the induction of stress oxidative causing an increase in ROS production, These probable explanations may indicate that the diamide tested induced oxidative stress.The mechanism of correlation of Exirel with CAT remains poorly understood; however, information on the effects of diamides such as Exirel on biomarkers has remained limited to aquatic organisms and on pests **[13] [43].** The enzyme catalase of the endogenous antioxidant system direct to neutralize the ROS localized mainly in the peroxysome that catalyzes the reduction of high H2O2 oxygen and water contents, thus preventing oxidative damage at the peroxysome level. Indeed, catalase is considered one of the main sensitive biomarkers of oxidative stress induced by chemical pollution influencing the environment **[10][26][6] [1].**The results of our experiment showed a very highly significant dose-dependent decrease in brain cytosolic catalase this for all tested concentrations of diamideExirel compared to controls. This result indicated that Exirel may cause an increase in H2O2 so oxidative stress state also this decrease related to excessive production of ROS caused by Exirel which impaired the ability to suppress both enzymes (CAT and SOD) these results in accordance with the tests of**[26]**on rats which after 90 days of treatment induces a decrease in the enzymatic activity of catalase**[34]**on fish exposed to Del after 28 d showed a decrease in catalase activity, and**[43]**on earthworms were exposed to cyantraniliprole for 7 and 14 days also causes a decrease in activity of and enzyme.Other authors revealed in experiments tested the effect of certain types of pesticides on quail (Coturnix japonica)**[14]**and Daphnia magna**[13]** showed an increase in the enzymatic activity of the brain catalase, this high CAT activity results in a decrease in the production of hydrogen peroxide (H2O2) in cells and prevents the creation of oxidative cellular damage also can be caused to a repair mechanism to remove excess H2O2 during decreased GPx activity.Cell membranes contain polyunsaturated fatty acids that are sensitive to the action of free radicals that trigger the peroxidation of membrane lipids. Malondialdehyde (MDA) the final product of lipid peroxidation (which are an initial oxidation of a number of lipid molecules) is used as a marker to identify oxidative tissue damage caused by pesticides **[2] [15].** The current study showed that **fig05. Table2** treatment with Exirel in four concentrations (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1 mg/kg/d) very highly significantly increases the MDA (lipid peroxidation) level in

rats treated in comparison controls, The results of this study were consistent with previous research on different pesticides **[1] [37]** have reported that malathion increases lipid peroxidation in brain tissues this increase may be explained by the higher production of reactive oxygen and nitrogen species, in addition the increase in MDA levels may be the consequence of inhibition of antioxidant enzymes by pesticide-induced free radicals **[2].**Another study on rats showed that chlorpyrifos causes lipid peroxidation by causing overproduction of MDA (Ceylon T and al. 2021), the elevation of lipid peroxidation by the effect of pesticides is very troubling for all tissues such as nervous tissues that can cause the destruction of the membrane barrier function to cell lysis and finally to cell death because lipid peroxidation it has an effect the reduction of fluidity and the increase of the ion permeability of the membrane which plays an essential role in energy metabolism and neuronal conduction **[15].**After chronic exposure to environmental xenobiotics in determined doses is able to damage brain functions and disrupt the learning and memorization of the animal organism, neurotransmitters is among the neuronal molecules susceptible altered by oxidative stress by triggering neurobehavioral disorders. Acetylcholine among the neurotransmitter that monitor movement, the decrease in the latter causes disturbances in muscle contraction, memory loss, weak concentration. This neuromediatoris degraded by acetylcholine esterase (AchE) at the synaptic cleft into two molecules: acetate and choline**[24].**Studies of the toxic effects of diamides such as Exirel on neurotransmitters remained limited to mammals, We noticed the results of our study AchE activity **(Fig04**) which showed a significant decrease in the brain after administration of Exirel at several concentrations (0.025 mg/kg/d, 0.05 mg/kg/d, 0.075 mg/kg/d, 0.1 mg/kg/d) orally for 90 days in rats, this significant decrease observed in batches treated with doses (0.025 mg/kg/d, 0.075 mg/kg/d, 0.1 mg/kg/d) but the two lots remain(0.025 mg / kg / d, 0.05 mg / kg / d) there was a non-significant decrease compared to controls, the results of this study were consistent with previous research**[15] [37] [14]** have revealed that inhibition of AChE activity is an indicator of neurotoxicity in chronic exposure induced by various pesticides such as Malathion and imidacloprid, chlorpyrifos in rats, mice and fish. Another study on organophosphorus pesticides (OP) showed that chronic exposure to OP causes prolonged inhibition of AChE is consequently the appearance of anxiety and depression, neurofibrillary degeneration which responsible for several neurological diseases, including Parkinson's Disease, seizures, depression and Alzheimer's disease **[21],** the decrease in ACHE could modulate motor activity, learning capacity and memory**[35].** Studies on OP and carbamates showed that exposure to carbaryl (5 or 20 mg/kg) causes a significant decrease in AChE activity compared to controls**[36],** so our results are consistent with our previous study on rat behavior.

Conclusion

In this research, the toxic effects of the novel insecticide Exirelwere investigated using several biomarkers. Based on our results, the administration of Exirel at different concentrations (0.025mg/kg/d, 0.050mg/kg/d, 0.075mg/kg/d, 0.1mg/kg/d) of body weight in adult male rats induced behavioural disturbances. (open field test, maze test, object recognition test), a hypoactivity of AChE which affects the normal level of acetylcholine (Ache) and therefore reduce the capacity of this neuromediator included memorization (Alzheimer’s disease).Also causes stress results from increased levels of ROS, increased GST activity and may induce lipid peroxidation, as indicated by high levels of MDA in all treatment concentrations assessed. a depletion of the glutathione detoxification system, a decrease in the activity of glutathione peroxidase,catalase.

Finally, this research found that Exirel increases the risk of chronic toxicity in male rats at certain doses of the insecticide.

Limitations and Future Studies

This research is the first study of the insecticide Exirel in rats, and through our findings we have found that it has very serious toxic effects on the nervous and behavioral system.In future, it will be desirable to develop this research by: Look for biomarkers of neurodegenerative diseases such as Alzheimer's, Quantification of cytochromes, enzyme parameters of mitochondria such asGP_{Xm} , GST_m…, study the protective effect of natural antioxidants against toxicityinduced by Exirel in rats or other experimental models.

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