Behavioral and neurochemical evidence of deltamethrin anxiogenic-like effects in rats

Evidências neuroquímicas e comportamentais do efeito ansiogênico da deltametrina em ratos

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Abstract

Pyrethroid insecticides are extensively used for pest control around the house, flea prevention for pets, and plant sprays for the home and in agriculture. Deltamethrin (DTM) is a Type II pyrethroid insecticide used to control a variety of insects in agriculture and domestic environments. The present study investigated the possible anxiogenic effects of DTM (1, 3, and 10 mg/kg) in rats using behavioral and neurochemical methods. We assessed general locomotor activity and behavior in the elevated plus maze and open field test. Striatal and hippocampal neurotransmitter and metabolite levels were also measured. DTM (*i*) reduced locomotion and rearing frequency, (*ii*) slightly increased the duration of immobility, (*iii*) reduced the time engaged in social interaction, (*iv*) reduced the percentage of entries into and time spent on the open arms of the elevated plus maze, (*v*) reduced the number of center crossings in the elevated plus maze, (*vi*) Striatal and hippocampal neurotransmitter and metabolite levels were also measured. DTM (*i*) reduced locomotion and rearing frequency, (*ii*) slightly increased the duration of immobility, (*iii*) reduced the time engaged in social interaction, (*iv*) reduced the percentage of entries into and time spent on the open arms of the elevated plus maze, (*v*) reduced the number of center crossings in the elevated plus maze, (*vi*) increased striatal serotonin neurotransmitter and its metabolite, and (*vii*) did not alter motor coordination on the rotarod, grooming duration in the open field test, rectal temperature, or hippocampal neurotransmitter levels. These data suggest that DTM at the present doses and under these experimental conditions presented a similar profile to that of anxiogenic drugs, unrelated with the increased serotonin neurotransmission.

Keywords: Deltamethrin. Pyrethroid. Anxiety. Behavior. Central neurotransmitters.

Resumo

Inseticidas piretróides são amplamente utilizados para controle de pragas, como na prevenção de pulgas em animais de estimação e sprays de plantas para a casa e na agricultura. Deltametrina (DTM) é um inseticida piretróide tipo II usado para controlar uma variedade de insetos na agricultura e ambientes domésticos. O presente estudo investigou os possíveis efeitos ansiogênicos de DTM (1, 3 e 10 mg/kg) em ratos, utilizando métodos comportamentais e neuroquímicos. Foi avaliada a atividade locomotora geral e comportamento no labirinto em cruz elevado e teste de campo aberto. Os níveis de neurotransmissores e metabólitos no estriado e hipocampo também foram mensurados. DTM (i) reduziu a locomoção e a frequência de levantar, (ii) aumentou da duração da imobilidade, (iii) reduziu o tempo de interacção social, (iv) reduziu a percentagem de entradas e tempo gasto nos braços abertos do elevado labirinto em cruz, (v) reduziu o número de cruzamentos no centro do labirinto em cruz elevado, (vi) aumentou neurotransmissor serotonina e de seu metabólito estriatal, e (vii) não alterou a coordenação motora no rotarod, duração do grooming no teste de campo aberto, temperatura retal, ou níveis de neurotransmissores do hipocampo. Estes dados sugerem que DTM nas presentes doses e sob estas condições experimentais apresentaram um perfil semelhante ao de drogas ansiogénicas, não relacionados ao aumento da serotonina estriatal.

Palavras-chave: Deltametrina. Piretróide. Ansiedade. Comportamento. Neurotransmissores centrais.

Introduction

Pyrethroid insecticides are widely used in agriculture and in the home to control a variety of insects and are considered neurotoxic[1]. They can be divided into two classes. Type 1 has no cyano group at the carboxyl a position (a-carboxyl), whereas Type 2 presents this cyano group[2,3,4].

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Deltamethrin (DTM, [(*S*)-α-cyano-d-phenoxybenzyl-(1*R*,3*R*)-e-(2,2-dibromovinyl)-2,2-dimethylcyclo-propane-1-carboxylate]) is a pyrethroid derivative claimed to be one of the most potent products of Type 2 insecticides. DTM acts by delaying the closure of sodium channels, resulting in a tail current characterized by slow sodium influx during the end of neuronal depolarization[1,5,6].

Exposure to pyrethroid insecticides can induce neurobehavioral effects in rodents and other species, including humans[7,8,9,10]. Aspects of open field behavior and catalepsy[11,12], conditioned behavior[12,13,14], motor activity[15,16], anxiety[11,17] and aggressive behavior[11].

Type II pyrethroids produce a syndrome associated with acute hyperglycemia. These responses are likely linked to the sympathoadrenal medullary system[2,17,18]. Additionally, other studies showed that low doses of DTM, a type II pyrethroid, induced severe neuroendocrine and autonomic responses, reflecting high levels of stress presumably caused by the neurotoxic properties of the insecticide[17]. Type II pyrethroids may act on γ-aminobutyric acid (GABA) receptors as benzodiazepine antagonists[8,19,20].

Considering that pyrethroids can interfere with neurotransmitter systems involved in anxiogenic effects, the present study investigated the effects of DTM, a Type II pyrethroid, in rats using a behavioral model related to anxiety and assessed its possible neurochemical effects. The rectal temperature was measured because thermoregulatory response following acute exposure to many toxic chemicals involves a regulated hypothermic response, characterized by activation of autonomic thermo effectors to raise heat loss and a behavioral preference for cooler temperatures[21].

Materials and methods

Animals

Adult male Wistar rats (Department of Pathology, Faculty of Veterinary Medicine and Zootechny, University of São Paulo, Brazil), weighing approximately 300-310 g and 100 days old, were used. The animals were housed in polypropylene cages (40 x 50 x 20 cm) under controlled temperature (20 ± 2°C) and humidity (70 ± 5%) and a 12 h/12 h light/dark schedule (lights on at 6:00 AM). Food (Nuvilab CR1, species-specific ration) and water (filtered in porcelain) were provided *ad libitum* throughout the study. Each rat was used in only one experiment. The rats were maintained in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animal Resources, National Research Council, USA.

Insecticide

Deltamethrin (DTM; *S*-α-cyano-3-phenoxybenzyl-(*R*)-cis-3-(2,2-dibromovinyl)-2,2-cimethylcyclopropane carboxylate; 10 mg/kg) was purchased from Quimio-Ind. Química S/A, dissolved in glycerol formaldehyde solution (Sigma, St. Louis, MO, USA; a racemic mixture produced by the condensation of glycerol with formaldehyde), and orally administered by gavage in volumes not exceeding 1 ml/kg body weight.

Open field test

General activity was evaluated in the open field test. The device was an arena with 96 cm diameter surrounded by a 25 cm high enclosure painted white and divided into 19 painted black parts. The apparatus was placed in a sound-proof room, 48 cm above the floor, and illuminated with a 40 W light bulb suspended over the center of the field [55 lux]. Each animal was individually placed in the center of the arena, and the following parameters were measured over a period of 5 min: locomotor frequency (i.e., number of squares crossed with the four paws), rearing frequency (i.e., number of times a rat stood erect on its hind legs with its forelegs in the air), and grooming frequency (i.e., washing movements over the head, licking the paws, fur licking, and tail/genital cleaning). A chronometer was used to measure the duration of immobility (i.e., total time in seconds without

spontaneous movements). To minimize the possible influences of circadian changes on open field behavior, control and experimental animals were alternated. The device was cleaned with a 5% alcohol/water solution before placing the animals in it to eliminate the possible bias caused by odors left by the previous rats. Control and experimental rats were intermixed, and the tests were conducted between 8:00 AM and 12:00 PM. Additionally, the animals were previously maintained for at least 90 min in access rooms under the same conditions as the test room.

Forty male rats were divided into four groups (n = 10 rats per group) that received the following treatments: DTM (1, 3, 10 mg/kg) or glycerol formaldehyde (1 ml/kg). Four sessions were performed, i.e, the rats were observed in the open field 30-35, 60-65, 90-95, and 120-125 min after the treatments.

Rotarod test

In this test, the rats were placed with all four paws on a 2.5 cm diameter bar, 25 cm above the floor, which turned at 12 rotations per minute (rpm). Thirty naïve rats were used for this test. The rats were habituated to the apparatus in five daily sessions of 2 min each (14 rpm) prior to the test. The training session consisted of 4 days of trials of 60 s each. Initially, 30 rats were used for this experiment, but only 15 rats remained on the rotarod for 60 s for all four consecutive sessions and were selected for use in the test. After selection, these rats received the respective treatments, i.e. glycerol formaldehyde (1 ml/kg), or DTM (1, 3, or 10 mg/kg). The tests were performed 30, 60, 90, 120, and 180 min after the treatments. The percentage of rats that remained on the rotarod during each 60 min test was recorded. The apparatus was washed with a 5% ethanol solution prior to each behavioral test. Control and experimental rats were intermixed, and the tests were conducted between 8:00 AM and 12:00 PM.

Elevated plus maze

The elevated plus maze was constructed of wood, with two open arms (50 x 10 cm) and two closed arms of the same size, surrounded by 40 cm high walls. The entire apparatus was elevated 50 cm above the floor. Forty naïve male rats were divided into four groups (n = 10 rats per group) and received the following treatments: DTM (1, 3, and 10 mg/kg) and glycerol formaldehyde (1 ml/kg). One hundred twenty minutes after the treatments, each rat was placed in the central square (10 x 10 cm), and the number of entries into each type of arm (with all four paws defining an entry) and time spent on the open and closed arms were recorded during 5 min. The percentage of entries was calculated for each arm. The apparatus was washed with a 5% ethanol solution prior to each behavioral test. Control and experimental rats were intermixed, and the tests were conducted between 8:00 AM and 12:00 PM.

Social interaction test

Eighty naïve male rats were divided into four groups (n = 20 rats per group to obtains 10 pairs of the same treatment) and singly housed for 5 days prior to testing. The social interaction test was performed in the open field apparatus, similarly to File[22,23]. Two days before the test, each rat was individually subjected to 10 min familiarization sessions in the test arena. On the next day, the rats were paired by weight (i.e., no more than 10 g difference), and social interaction was observed for 10 min. Two hours after the administration of DTM (1 mg/kg) or glycerol formaldehyde (1 ml/kg), the paired rats were placed in the center of the test arena to evaluate social interaction. The total time (in seconds) spent by the test pairs in active social interaction (i.e., sniffing, following, grooming, kicking, boxing, biting, and crawling under or over the partner) was recorded for 7.5 min. The apparatus was washed with a 5% ethanol solution prior to each behavioral test. Control and experimental rat pairs were intermixed, and the tests were conducted between 8:00 AM and 12:00 PM.

Rectal temperature

Rectal temperature was measured with an Impulstron® thermometer connected to a metallic material

sensor lubricated with Vaseline. The first measurement was taken prior to and 120 min after the respective treatments, at the deltamethrin peak of effects on open field behaviors. The thermometer was cleaned with a 5% ethanol solution prior to each temperature measurement. Control and experimental naïve rats were intermixed, and the measurements were taken between 8:00 AM and 12:00 PM to prevent interference caused by circadian variations.

Determination of neurotransmitter and metabolite levels

Twenty-seven six naïve male rats were divided into four groups (n = 9 rats per group) that received DTM (3 or 10 mg/kg) or glycerol formaldehyde (1 ml/kg). Two hours after the treatments, the rats were sacrificed by decapitation under ice-cold hypothermia. The brains were collected and dissected on dry ice and prepared as previously described De Souza Spinosa et al.[24]. The striatum and hippocampus were dissected and separated within 3 min on an ice-cold plate, weighed, and homogenized (Polytrons) in 0.1M perchloric acid. A volume of 20 µ/mL of tissue (wet weight) of this solution was used for the analyses. To precipitate the proteins completely, the homogenates were left overnight in a refrigerator (4ºC) and then centrifuged (Eppendorf) for 60 min. The supernatants were analyzed by high-performance liquid chromatography (HPLC, Shimadsu, model 6A) with a C-1 column (Shimpak-ODS), electrochemical detector (Shimadsu, model 6A), sample injector, and integrator (Shimadsu, model 6A Chromatopac). The levels of the following monoamines and their metabolites were measured: dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), norepinephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG), homovanillic acid (HVA), serotonin (5-hydroxytryptamine [5-HT]), and 5-hydroxyindolacetic acid (5-HIAA).

Statistical analysis

The results are expressed as mean ± SD. Bartlett's test was used to analyze the parametric data. Two-way analysis of variance (ANOVA) was used to analyze the open field and rotarod data. A one-way ANOVA followed by Bonferroni´s multiple comparison test was used to evaluate differences between groups in the elevated plus maze, rotarod test, social interaction test and neurochemical data. The t-test was used to analyze the differences in rectal temperature. In all cases, the results were considered significant at $p < 0.05$. The statistical analyses were performed using GraphPad Prism software, version 5 (GraphPad, San Diego, CA, USA).

Results

Figure 1 shows the general locomotor activity of the rats treated with different doses of DTM in the open field. With regard to locomotor frequency, significant effects were found for treatment ($F_{3,144}$ = 7.26, p = 0.0001) and session ($F_{3,144}$ = 67.34, $p < 0.0001$), with no interaction between factors ($F_{9,144}$ = 1.41, p = 0.189). The Bonferroni test showed that the 10 mg/kg dose decreased locomotor frequency in the 120 min session compared with the glycerol formaldehyde group. A similar result was found in the 10 mg/kg DTM dose from the 60 to 120 min sessions. With regard to rearing frequency, significant effects were found for treatment ($F_{3,144}$ = 7.64, p= 0.004) and session ($F_{3,144}$ = 20.65, p = 0.0004), with no interaction between factors ($F_{9,144}$ = 0.68, p = 0.727). The Bonferroni test showed that the 3 and 10 mg/kg doses decreased rearing frequency in the 120 min session compared with the glycerol formaldehyde group. With regard to the duration of immobility, significant effects were found for DTM treatment ($F_{3,144}$ = 3.04, p = 0.030) and session ($F_{3,144}$ = 27.35, $p < 0.0001$), with no interaction between factors

($F_{9,144}$ = 1.06, p = 0.395). The Bonferroni test showed that 3 mg/kg DTM increased the duration of immobility in the 120 min session compared with the control and glycerol formaldehyde groups. No effects

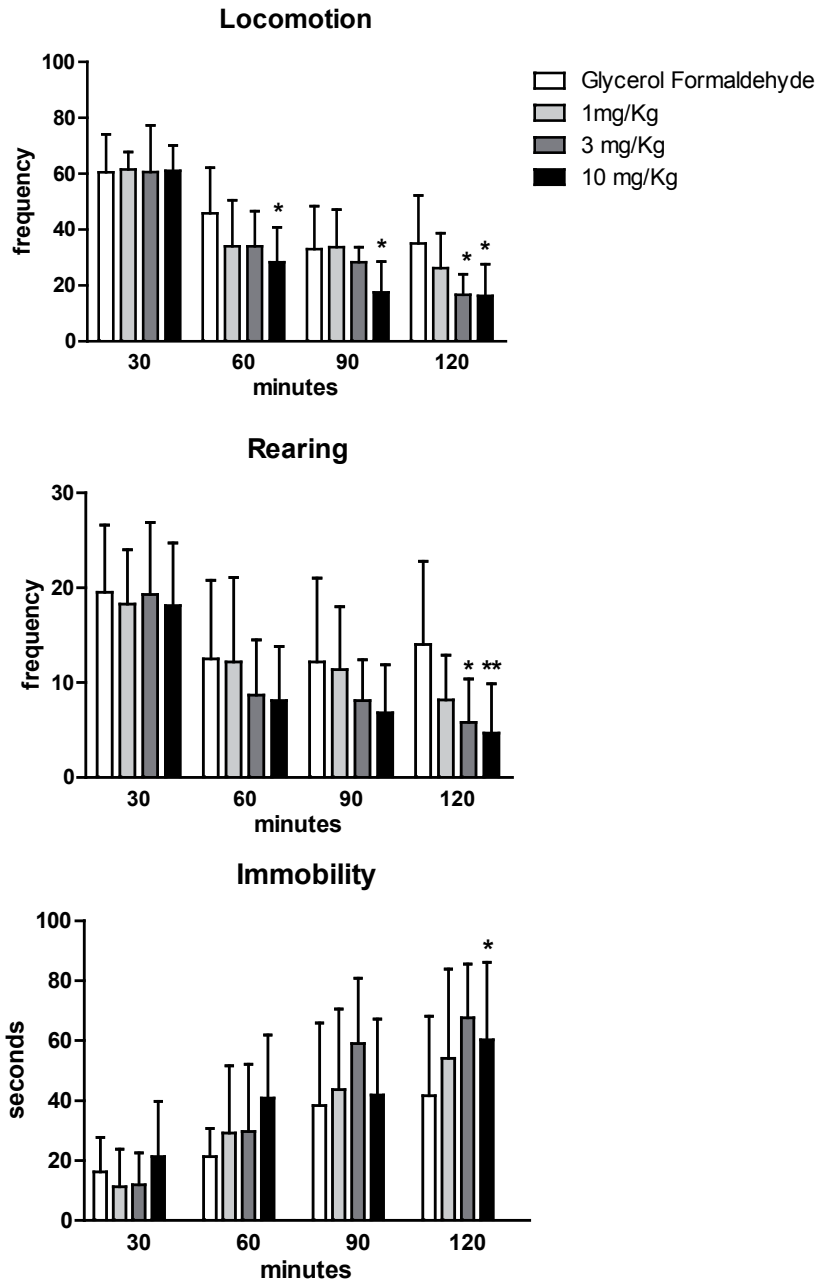

Figure 1 - Open field behavior—(A) locomotion, (B) rearing, (C) immobility—in rats treated with 1, 3, or 10 mg/kg DTM and observed 30, 60, 90, and 120 min after treatment. The data are expressed as mean ± SD. n = 10/group. *p < 0.05, compared with glycerol formaldehyde group (two-way ANOVA followed by the Bonferroni multiple comparison test)

were detected in rotarod performance between rats after DTM treatment (data not shown).

Figure 2 shows that DTM treatment reduced activity in the elevated plus maze compared with the control group. DTM reduced the percentage of entries into and time spent on the open arms ($F3,39$ = 3.430, p = 0.027, and $F_{3.39}$ = 6.893, p = 0.0009, respectively) and reduced the number of center crossings ($F_{3,39}$ = 5.491, p = 0.003). The Bonferroni p*ost hoc* test showed that, relative to control group, 3.0 and 10 mg/kg DTM 120

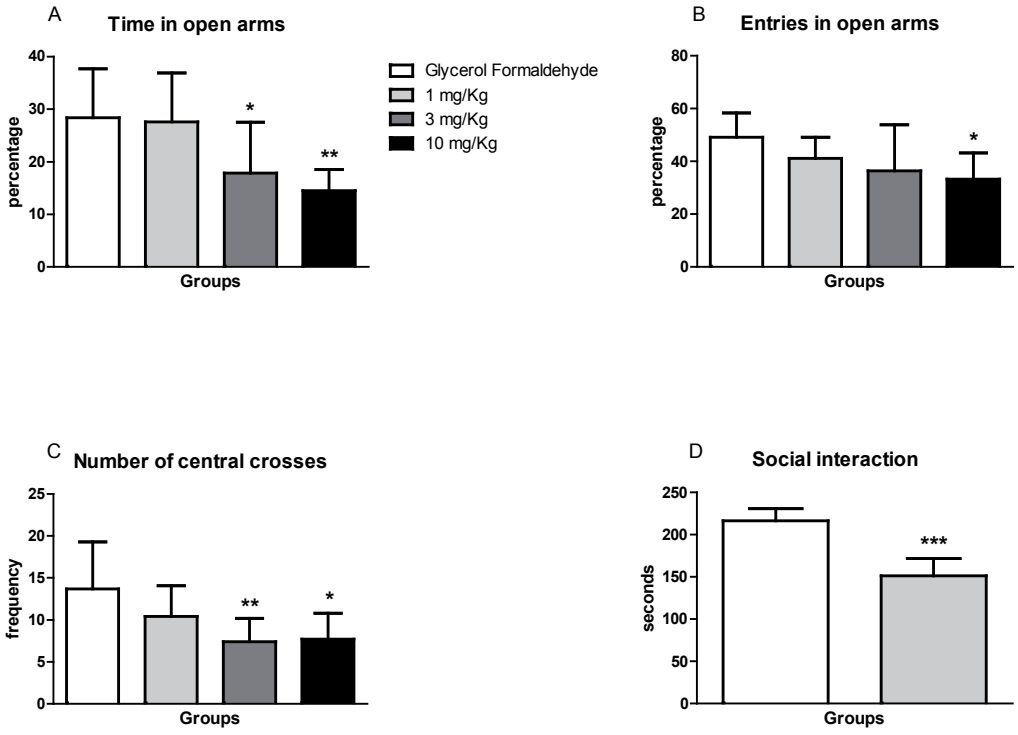

Figure 2 - Time in open arms (A), entries in open arms(B) and number of crossings (C0 in the elevated plus maze of rats treated with 1 mg/kg, 3 mg/kg, or 10 mg/kg DTM and observed 120 min after treatment. (D) Social interaction in rats treated with 1 mg/kg DTM. The data are expressed as mean ± SD. n = 10/group. $^{*}p < 0.05$, $^{***}p < 0.0001$, compared with glycerol formaldehyde group (one-way ANOVA followed by the Tukey-Kramer multiple comparison test)

min ($p < 0.05$) prior to the test reduced the time spent on the open arms. Also, it was observed a reduced percentage of open arms entries but only after 10 mg/Kg treatment. The number of center crossings in the 3 and 10 mg/kg DTM groups were reduced compared with the glycerol formaldehyde group.

The social interaction (Figure 2D) was different between groups with 1 mg/kg DTM decreased compared with the control group (Student t test, $p < 0.0001$).

DTM treatment did not alter rectal temperature in any of the groups (36.0 – 36.2°C).

The evaluation of the effects of DTM on striatal neurotransmitter and metabolite levels showed that 5-HT ($F_{2,26}$ = 13.93, $p < 0.0001$) levels of both DTM groups were increased in relation to control group and the 5-HIAA levels were increased in rats of the higher DTM dose ($F_{2,26}$ = 4.43, p = 0.022) while no differences were detected in the 5-HIAA/DA ratio. No other striatal neurotransmitter or metabolite levels were significantly modified by the treatment (Table 1). The treatments also did not significantly alter hippocampal neurotransmitter levels (Table 2).

Discussion

The present results showed that DTM administration reduced locomotor and rearing frequency mainly in the 120 min session. Therefore, activity in the elevated plus maze, social interaction, rectal temperature, and neurotransmitter levels were evaluated 120 min after treatment. Additionally, the 3 and 10 mg/kg doses of DTM

Table 1 – Effects of DTM administration on the striatum neurotransmitter's levels and its metabolites. Data are presented as means±SD

Neurotransmitters/Metabolites (ng/g of tissue)	Glycerol Formaldehyde	DTM (3mg/kg)	DTM (10MG/kg)
DA	10946.0±2106.5	12139.7±2037.0	11802.4±1632.0
DOPAC	1063.2±149.5	976.3±317.6	1084.6±192.3
HVA	729.8±207.8	847.8±279.7	787.9±149.7
DOPAC/DA	0.099±0.016	0.083±0.027	0.092±0.013
HVA/DA	0.068±0.024	0.070±0.025	0.068±0.017
NA	26.4±8.1	32.8±14.4	28.8±9.0
MHPG	142.6±24.1	137.8±30.2	129.9±26.7
MHPG/NA	6.3±1.9	4.9±2.0	4.9±1.3
5-HT	812.3±81.4	1117.2±197.9*	1109.1±112.7***
5-HIAA	662.7±136.9	793.3±265.4	901.8±169.7*
5-HIAA/5-HT	0.812±0.124	0.700±0.153	0.824±0.194

DA- dopamine; DOPAC- 3,4-Dihydroxyphenylacetic acid; HVA-homovanillic acid; NA-noradrenaline; MHPG= 3-Methoxy-4-hydroxyphenylglycol ; 5-HT-serotonin; 5-HIAA- 5-Hydroxyindoleacetic acid. * p< 0.02, *** p < 0.0001 compared to respective glycerol formaldehyde group, N= 9 rats/group

Table 2 - Effects of DTM administration on the hippocampal neurotransmitters levels. Data are presented as means±SD

	Glycerol Formaldehyde	DTM (3mg/Kg)	DTM (10mg/kg)
DA	709.5±289.8	625.4±445.7	624.9±239.0
DOPAC	359.6±58.9	356.5±117.6	357.5±96.3
HVA	1580.6±286.5	1306.0±398.3	1187.6±378.7
DOPAC/DA	0.6±0.21	0.7±0.4	0.7±0.2
HVA/DA	2.5±0.8	2.8±1.8	2.4±1.2
NA	754.7±166.1	738.1±206.1	701.7±131.3
MHPG	391.2±53.8	346.7±62.2	349.0±60.8
MHPG/NA	0.5±0.1	0.5±0.1	0.5±0.1
5-HT	N.D.	N.D.	N.D.
5-HIAA	6207.5±606.6	5617.7±613.4	5839.9±713.5

DA- dopamine; DOPAC- 3,4-Dihydroxyphenylacetic acid; HVA-homovanillic acid; NA-noradrenaline; MHPG= 3-Methoxy-4-hydroxyphenylglycol ; 5-HT-serotonin; 5-HIAA- 5-Hydroxyindoleacetic acid. N.D. = Not detected, N= 9 rats/group

altered these parameters. Both the elevated plus maze and social interaction test revealed an anxiogenic-like profile of the pesticide. Striatal 5-HT levels increased after treatment with 10 mg/kg DTM without affecting the 5-HIAA and 5-HIAA/5-HT ratio. No effects were observed in the rotarod test, on rectal temperature, or on hippocampal neurotransmitter levels.

In the present study, glycerol formaldehyde was used to dissolve DTM. Laurent et al.[25] demonstrated the absence of any toxic effects of glycerol formaldehyde, and the only effect observed after administration of a high dose (2000 mg/kg) was narcosis.

The open field test results suggest that locomotor activity decreased at 90 min (10 mg/kg) and 120 min (3 and 10 mg/kg), specifically with regard to locomotor frequency. Rearing frequency decreased only at 120 min (3 and 10 mg/kg). Diminished locomotor activity is consistent with numerous previous reports on the acute effects of pyrethroids. Several authors demonstrated reduced locomotor activity produced by exposure to different types of pyrethroids, including DTM[3,15,26,27,28,29]. Interestingly, the decreased locomotor and rearing frequencies were followed by an increased duration of immobility. Particularly,

DTM produces dose-dependent decreases in locomotor activity[10,27,30].

In the present study, oral DTM administration reduced locomotor activity but not motor coordination. Pham Huu et al.[31] observed a decrease in motor coordination after 0.5-1.5 g/kg DTM, and Manna et al.[32] found the same effect after 14.5–145 mg/kg DTM administration in rats. The lack of effects observed here may be related to the lower dose used in the present study.

Bhattacharya and Mitra[33] showed that anxiogenic drugs dose-dependently reduce locomotion and rearing behavior in rats in the open field. Therefore, the decreased open field activity after DTM administration may have been a consequence of increased "anxiety" and not motor behavior in general. In this respect, decreased locomotor and rearing frequencies were observed with an increased duration of immobility. However, the lack of effects on motor coordination supports the hypothesis that DTM does not interfere with motor function but interferes with emotional parameters.

The elevated plus maze is a useful animal model for studying anxiolytic drugs. When rats were placed in an elevated plus maze for the first time, its behavior is largely based on its "anxiety" level. Rats treated with anxiolytic drugs, such as diazepam, tend to exhibit less anxiety-like behavior, spending more time in the open arms[33,34]. DTM reduced the percentage of time spent on the open arms, suggesting an anxiogenic-like effect. The number of entries into the open arms also decreased. These effects of DTM are similar to those observed with anxiogenic drugs[17,34,35,36]. The lowest levels of center crossings may be a consequence of a decreased general spontaneous activity, but in rodents increased levels of anxiety leads to hypoactivity in the open field[37].

In the social interaction test, 1 mg/kg DTM reduced the time spent engaged in social interaction. These results confirm our hypothesis that DTM induces anxiety-like behavior. This conclusion is based on the results with anxiolytic and anxiogenic drugs used in the social interaction test, i.e., a reduction in the time engaged in social interaction indicates an anxiogenic effect of the drug, whereas an increase in this parameter indicates an anxiolytic effect[23,38,39].

Thermoregulation in rats is an important factor for evaluating the toxic effects of pyrethroids. One of the effects of Type I pyrethroid exposure is hyperthermia, possibly attributable to extensive muscular activity[40]. In contrast, type II pyrethroids, such as DTM, can produce hypothermia[41]. The three doses used in the present study did not modify rectal temperature, suggesting that the present doses do not induce severe toxicity.

DTM increased the striatal levels of 5-HT (3 and 10 mg/kg) and of 5-HIAA. No differences were detected between the 5-HIAA/5-HT ratios of all groups. These data suggest that the synthesis and metabolism of serotonin were increased but not its activity. Central 5HT is involved in anxiety[42,43]. The role of serotoninergic system on anxiety brain areas are inconsistently[44]. Low levels of this neurotransmitter in the synaptic clef is correlated with anxiogenesis[44,45,46,47]. Striatal lesions in serotonin innervation leads to moderate anxiogenic effects in the elevated plus maze[44]. On the contrary, in several elevated mazes as animal models of anxiety, serotoninergic agonists present anxiolytic activity[48]. So, we suggest that the increased anxiety levels here observed in both, elevated plus maze and social interaction behavioral models, were not correlated with the increased activity in serotoninergic system.

No alterations in dopamine or its metabolites, DOPAC and HVA, were observed either in the striatum or hippocampus. Moreover, no alterations in norepinephrine or its metabolites were found. These data confirm our hypothesis that motor function did not critically impact the effects of DTM treatment.

In conclusion, the present data suggest that DTM exerts effects that may be similar to anxiogenic drugs, possibly through interference with serotonin neurotransmission. These results are important because we show that the level of exposure which increases the anxiety is very low. In this sense, high levels of anxiety can have serious implications in important aspects of humans and animal health.

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