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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.196.200

Research Article
Effects of Nettle on Locomotor Activity and Anxiety Behavior in Male Wistar Rats After Pesticide Intoxication

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Abstract

Background and Objective: The nettle is an herbaceous plant belonging to the family of Urticaceae that has been used for centuries against a variety of diseases. Thanks to its high content of nutriments and bioactive compounds. Therefore, this study was conducted to assess the curative effect of nettle decoction on dimethoate-induced changes in locomotors activity and anxiety reaction in male rats. **Materials and Methods:** This experiment was conducted in two stages with 60 healthy male rats that were divided into three groups of 20 rats A, B and C. In the first phase, the treatment groups B and C received dimethoate dissolved in corn oil by gavage at a dose of 100 mg kg^{-1} b.wt., for 5 weeks. During the same period, the control group A received corn oil. Locomotors activity was controlled by the Open Field (OF), anxiogenic behavior was verified by the Elevated Plus Maze (EPM). For the second phase the group A had kept its control position, the group B was maintained without any treatment to remind the extent of intoxication and was served as second control. Group C received a nettle decoction of 2 mL 100 mg^{-1} b.wt., for 30 days. **Results:** The result showed that at the open field level, dimethoate decreased significantly both of the total number of squares crossed by intoxicated rats and the number of elevations. At the EPM level, dimethoate decreased both of the time spent in open arms and the number of entries into the open arms. These results reveal that dimethoate affects the anxiety and mobility of intoxicated rats. On the other hand, the use of nettle decoction as a treatment significantly improved the anxiety level and motor activity of poisoned rats. **Conclusion**: Dimethoate has an anxiogenic effect on intoxicated rats, as well as a remarkable decrease in their locomotor activity. The use of nettle decoction as a treatment has significantly improved dimethoate induced behavioral and motor disorders.

Key words: Anxiety, dimethoate, locomotors activity, *Urtica dioica* L.

Citation: Majda Samih and Ahmed Omar Touhami Ahami, 2019. Effects of nettle on locomotor activity and anxiety behavior in male wistar rats after pesticide intoxication. Pak. J. Biol. Sci., 22: 196-200.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The medicinal plant (*Urtica dioica* L.) used as a natural chelator was present all over the world and in all mountainous regions up to 2400 m. Its diuretic and strenuous effects have been proven, it improved attention and has a positive effect on anxiety and depressive states[1,2]. Some studies reported that the nettle is considered a rich source of vitamins C, A, B1, B2, E, K and minerals (iron, calcium and magnesium), as well as trace elements (Cu, Zn, Mn and Co) and fibers[3-5]. Another study found that nettle contains 26% of protein and 5.09% of calcium[6].

With regard to pesticides, previous studies have shown that organophosphates have affected the quality of our food, as confirmed by a researcher, who stated that in the first world countries, it has been observed that a diet containing fresh fruits and vegetables far outweighs the potential risks associated with the consumption of very low pesticide residues in crops[7]. Pesticides also had an undesirable effect on human health and the environment as reported earlier by Banerjee *et al.*[8], Palaniyappan and Subramaniyan[9] and Sosan and Akingbohungbe[10], who have announced overwhelming evidence that some of these chemicals posed a potential risk to humans and other life forms as well as undesirable side effects to the environment. It is estimated that 2.5 million t of pesticides are applied annually to cultures of the planet. The proportion that comes into contact with the target adverse organisms or that they ingest is minimal. Most researchers estimated it at less than 0.3%, which meant that 99.7% of the substances spilled go elsewhere[11]. The massive use of synthetic insecticides and in particular those belonging to the organochlorine family, constituted between 1945 and 1960, an enormous progress for agriculture and made it possible to ensure sufficient food production for a rapidly growing population[12]. However, with the widespread use of the first synthetic insecticides (especially organochlorine insecticides), there have been clear signs of toxicity and adverse effects on the environment and humans[13,14]. This study did not address the above phenomena, but analyzed the toxic effects that organophosphates may have on the anxious behavior and locomotors activity of mammals, particularly male rats and the search for a cure. Therefore, the objective of this study was to highlight the curative effect of nettle and exploit its properties in an attempt to repair the damage caused to these two parameters as a result of sub-chronic exposure to dimethoate.

MATERIALS AND METHODS

This work was carried out in the Clinical and Cognitive Neurosciences Unit, Biology and Health Laboratory, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco. The total duration of this research was one year and 8 months, from May 2017-December 2018.

Chemicals: Dimethoate was obtained from commercial grade: Dimethoate 50 (active ingredients 500 g by liter). The Dimethoate concentration (50% purity) in commercial grade and was diluted in corn oil.

Decoction preparation: About 100 g of fresh nettle including roots were washed, cut roughly and left in 1 L of water for 24 h, the whole was boiled for 30 min under the lid. After cooling, the decoction was filtered. This filtrate was prepared daily.

Animals and treatment: Sixty healthy adult Wistar male rats received from the local breeding colony of the Faculty of Sciences of Kenitra in Morocco, weighing between 157 and 166 g were used in this study. The animals were housed in groups at temperature 21±2°C and 12-h light/dark cycle with *ad libitum* access to food and water. After 10 days of acclimatization, the animals were divided into 3 groups A, B and C. Group A for control, B and C for daily treatment with dimethoate dissolved in corn oil by gavage at a dose of 100 mg kg^{-1} b.wt., for 5 weeks. The control received corn oil. At the end of the first experiment, locomotors activity was verified at the Open Field highlighted already[15]. This test was widely validated in the measurement of motility disorders, anxiety and exploration in intoxicated rats[16]. Anxiety behavior has been tested in the Elevated Plus Maze (EPM) the most popular tests of all currently available animal models of anxiety[17]. The results of the behavioral tests of B and C groups were recorded. To accomplish the second phase, the animals in group B were detained without any treatment but they had free access to food and water, this group served as a second control. The group C received nettle decoction by gavage for 4 weeks at a daily dose of 2 mg/100 mg. The final results were compared to the control A and to those of B.

The procedures on the animals were carried out in accordance with the recommendations of the Internal Ethics Committee of the Ibn Tofail University, Kenitra. This procedure was examined and approved by the Committee.

Statistical analysis: All data are expressed as an Average±S.E.M. (Standard Error of Mean). Repeated measurements and one-way analyses of variance (ANOVA) are used to analyze the difference body weight. The values of $p<0.05$ are considered statistically significant.

RESULTS

Effect of Urtica Dioica L. on anxiety behavior in the elevated plus maze (EPM): The results of treatment with nettle and the effect on the anxiety level of rats that is expressed by the number of entries into the open arms of the EPM are shown in Fig. 1. Dimethoate has an anxietogenic effect expressed by a statistically significant decrease in the number of entries into open arms in intoxicated rats ($p<0.001$). In the treatment trial, rats receiving nettle decoction showed a considerable improvement in their anxiety level compared to poisoned rats ($p<0.01$).

The level of anxiety estimated by the time spent in open arms is illustrated in Fig. 2. The time spent in open arms decreased in poisoned rats, this decrease is statistically highly significant $p<0.001$, which confirms the anxiogenic effect of dimethoate.

The treatment of nettle has significantly improved the level of anxiety in poisoned rats ($p<0.01$).

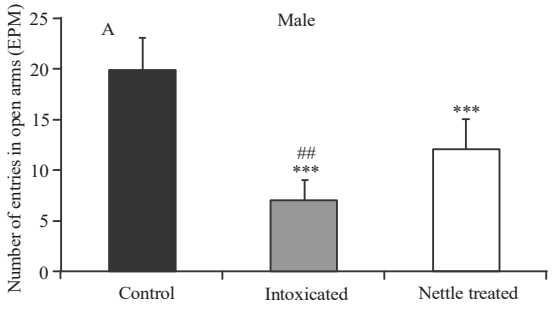

Fig. 1: Effect of *Urtica dioica* L. on the anxiety levels in EPM (number of entries)

Number of entries into open arms is expressed as an average±mean standard error (SEM) (***$p<0.001$), comparison between the 2 treated groups ($^{\#\#}p<0.01$), comparison between the dimethoate intoxicated group and the plant treated group (1 factor ANOVA/ Tukey *post-hoc* analysis

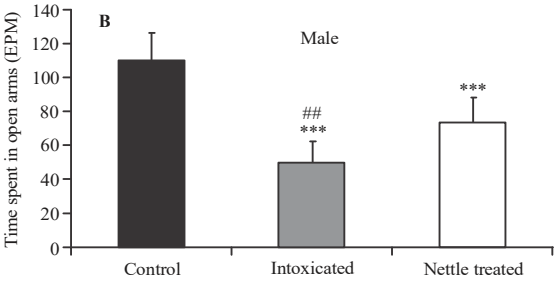

Fig. 2: Effect of *Urtica dioica* L. on anxiety levels in EPM (spent time)

The time spent in open arms are expressed as an the average±mean standard error (SEM) (***$p<0.001$), comparison between the 2 treated groups ($^{\#\#}p<0.01$), comparison between the dimethoate intoxicated group and the plant treated group (1 factor ANOVA/ Tukey *post-hoc* analysis.

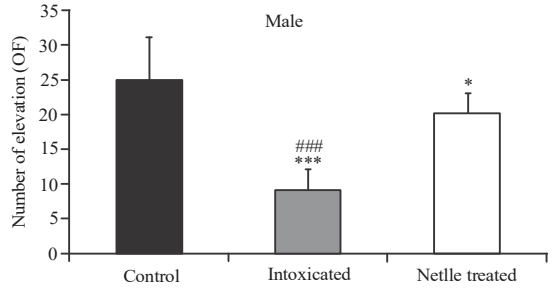

Fig. 3: Effect of *Urtica dioica* L. on the anxiety level in OF (elevation number)

The number of elevations is expressed as an average±average standard error (SEM)(***$p<0.001$; *$p<0.05$), comparison between the 2 treated groups ($^{\#\#\#}p<0.001$), comparison between the dimethoate intoxicated group and the plant treated group (1 factor ANOVA/ Tukey *post-hoc* analysis)

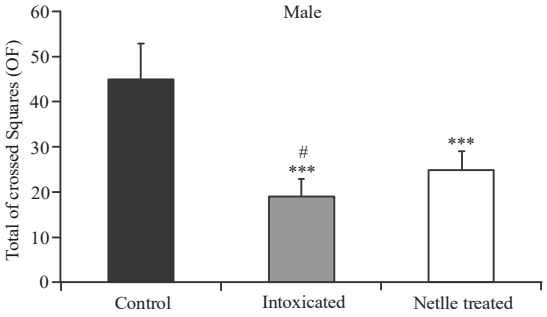

Fig. 4: Effect of the *Urtica dioica* L. on locomotor activity in the Open Field

The number of total squares is expressed as an average±average standard error (***$p<0.001$), comparison between the 2 treated groups ($^{\#}p<0.05$), comparison between the intoxicated group and the nettle treated group (1 factor ANOVA/ Tukey *post-hoc* analysis)

Effect of *Urtica dioica* L. on anxiety behavior in the open field (OF): The level of anxiety expressed by the number of elevations in the open field and the nettle treatment trial are illustrated in Fig. 3. In rats poisoned with dimethoate, the number of elevations was significantly reduced compared to controls ($p<0.001$), The treatment with nettle decoction resulted in a significant increase in the number of elevations.

Effect of *Urtica dioica* L. treatment on spontaneous locomotor activity in the Open Field of: The dimethoate intoxication induces a highly significant reduction in the number of total crossed squares by rats in the Open Field $p<0.001$ compared to control rats. The treatment of poisoned rats by *Urtica dioica* L. was slightly improved the deficit in locomotor activity as shown in Fig. 4 ($p<0.05$).

DISCUSSION

The results showed that dimethoate induces an anxiogenic effect in the treated rats, these results are in agreement with those obtained by a researcher who reported that chlorpyrifos, an organophosphorus causes an anxiogenic effect in the treated rats with an acute dose[18].

It has been reported in previous studies that organophosphorus (OPs) act primarily on the peripheral nervous system, because the blood-brain barrier protected the central nervous system. However, because of their liposolubilities and small size, OPs can cross this barrier and cause neurotoxic effects[19], such as impaired memory, anxiety and depression[20].

These results can be explained by the alteration of the serotoninergic and dopaminergic, glutamatergic and GABAergic systems. Indeed, these neurotransmitter systems played an important role in the onset of anxiety. Several studies showed the involvement of serotonin levels and these associated proteined in mood and anxiety disorders[21]. These results also showed that dimethoate alters the locomotor activity of intoxicated rats; a decrease in the total number of cells crossed is recorded. These results are consistent with those[22,23].

With regard to the results obtained from the nettle treatment. An improvement in the level of anxiety was noted. Nettle has, therefore; been shown to be anxiolytic. These results are consistent with previous work that showed the anxiolytic effect of both aqueous[24] and alcoholic[25] extracts of nettle.

CONCLUSION AND FUTURE RECOMMENDATIONS

This study found that *Urtica dioica* L. resulted in a significant improvement in locomotors activity and anxiety response in intoxicated rats. Although the results of these parameters did not reach the values recorded by the control rats, but they are largely acceptable compared to those of the group which group B animals have not received a decoction. However, it would be preferable to support these results with a clinical study.

In addition, this experimentation served as a starting point for a trial that required some improvements such as isolation of the active ingredient from nettle, increased duration of treatment of rats and testing of other extraction methods.

SIGNIFICANCE STATEMENT

This study highlighted the use of herbal products against the involvement of dimethoate in modulation of motor activity and anxiety of rodents, this experiment will encourage future research to reveal more secrets of medicinal plants which can be more effective for long-standing health problems. The introduction of new alternatives using natural sources may be beneficial in reducing adverse effects. Herbal products are today the symbol of safety as opposed to synthetic drugs. If the doses will be analyzed adequately these plants will not pose any danger to human health.

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