

ORIGINAL ARTICLE

Quercetin, a Natural Flavonoid, alleviates Fenthion induced Anxiety-like behavior and Depression signs in Male wistar rat

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ABSTRACT

Fenthion is an organophosphorus pesticide with a large spectrum of use in Algeria. Exposure to these compounds may evoke behavioral and cognitive impairments. Besides, the neuroprotection of quercetin, natural flavonol, was evaluated in many works. In this experiment, male wistar rats were exposed to fenthion at a dose of 20 mg/kg during 10 consecutive days, then, behavioral test batteries were used to assess the rats' emotional state. Our results have shown that rats treated with fenthion displayed anxiogenic-like behavior associated with depression signs. However, pretreatment with quercetin alleviated these behavioral responses. Notably, quercetin intake particularly for workers having contact with pesticides, seems to be beneficial against neurotoxicity of organophosphorus.

Key Words: organophosphorus, anxiety, depression, quercetin, rat.

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INTRODUCTION

Organophosphorus compounds (OP) are widely used in pest control and are neurotoxic agents. Their mechanism of action is the inhibition of acetylcholinesterase (AChE) activity in the central and peripheral nervous systems resulting in stimulation of cholinergic synapses [1]. Poor working conditions and unawareness of the potential hazards of OP lead to intoxication that evokes a consistent pattern of physical and neurobehavioral symptoms such as depression, anxiety and cognitive impairments [2]. Workers exposed to pesticides have been reported to manifest physical symptoms, neurobehavioral deficits and emotional disorders [3, 4]. In rats, several studies have also shown that exposure to organophosphorus produces behavioral and cognitive disturbances [5, 6]. Fenthion [O,O-dimethyl-O-(4-methylmercapto-3-5 ethylphenyl)-phosphorothioate] is one of the most well known OP and is a moderately toxic product [1, 2-7]. As other OP, fenthion has been found to be a potent inhibitor of rat behavior [8]. Nevertheless, cognitive and emotional reactivity of rat to this product are still unclear. Interestingly, multiple natural compounds such as diphenyl diselenide and melatonin have been investigated in order to attenuate the neurotoxicity of organophosphorus compounds [9-5]. Thus, the need for a powerful intervention against neurotoxicity of OP remains persistent. Recently, a substantial attention was paid to flavonoids as anxiolytic and antidepressant agents having a prominent pharmacological effectiveness [10, 11]. Among these plant molecules, quercetin is being increasingly used in experimental studies [12]. Quercetin (3,5,7,3',4'-pentahydroxyflavone) is a polyphenolic flavonol molecule that occurs in many fruits and vegetables such as onions, apples, berries, peanuts, soybeans, potatoes, broccoli, grapes, citrus fruits and tea [13,14]. Since it is largely present in the human diet, up to 1g/day average intake of quercetin has been reported [15] which represents from 60 to 75% of the overall polyphenols ingestion [16]. Quercetin scavenges efficiently free radicals and prevents oxidative stress-induced neuronal injuries [17, 18]. Moreover, several experimental investigations showed the potential of quercetin against cognitive deficits in various animal models [19, 20]. The aim of this study was to evaluate the repeated exposure to fenthion on depression and anxiety related behavior in male

wistar rats. We also examined the ability of quercetin pretreatment to prevent the neurobehavioral and emotional changes.

MATERIAL AND METHODS

Experimental protocol

Twenty-eight (28) male Wistar rats obtained from Pasteur Institute (Algiers, Algeria) were housed in transparent cages at a constant temperature (23 ± 1 °C) with a 12 h/12 h light/dark cycle (lights on at 07:30 a.m.). Rats had access to standard rodents chow and tap water ad libitum and weighing 250 ± 10 g at the beginning of the experiment. Rats were randomly divided to four equal groups : group C; control rats received orally corn oil (1ml/kg) and intraperitoneally saline solution NaCl 0.9% (1ml/kg); group Q; received 60 mg/kg of quercetin (quercetin dihydrate, 98% purity powder; Sigma Aldrich Co., Steinheim, Germany) dissolved in 1ml/kg of corn oil ; Group 03 : received 20 mg/kg of fenthion (Lebaycid, EC 500g/l) intraperitoneally dissolved in 1ml/kg of saline solution; Group 04 received quercetin 30 min before fenthion. The dose of fenthion was selected from Virginia [8]. The treatment lasted for 10 consecutive days, then, battery of behavioral testing was done.

Behavioral test

Open field test

The open field (OF) can be considered as a non-conditioned anxiety test based on the creation of a conflict between the exploratory drive of the rat and its innate fear of exposure to an open area [21]. The OF test was performed to measure changes in exploratory behavior and emotionality [5]. Briefly, the apparatus, as previously described [22] consist of a gray square (70 cm x 70 cm x 40 cm) divided into 16 equal squares that had been drawn in the floor of the arena. Each rat was placed in the arena individually, and allowed to freely explore it for 5 min. Upon completing the task, the rat was removed from the arena by the experimenter and returned to the home cage. After each test, the apparatus was cleaned with an alcoholic solution followed by wet and dry paper towels to avoid transfer of olfactory cues between animals. Time spent in peripheral and central areas was measured.

Elevated plus-maze test

The elevated plus-maze (EPM) test is a widely used paradigm to investigate anxiety-related behavior in rats [23]. The EPM was made of painted wood cross (arms 50 cm long x 10 cm wide) elevated 50 cm above the floor. Two opposite arms were enclosed by walls (10 cm x 50 cm x 45 cm high) and two arms were open. The arms extended from a central platform (10 x 10 cm) [24]. The open arms in the maze that we use do not have a railing, but addition of a 3–5 mm high railing on the open arms of the plus maze has been used with success to increase open arm exploration. The rat was placed in the center of the apparatus facing one of the open arms, for a free exploration of 5 min. Entry into an arm was defined as the animal placing all four paws on the arm. After each test, the rat was returned to its home cage and the maze was cleaned with an alcoholic solution followed by wet and dry paper towels, prior to the next trial. Time spent in open and closed arm was measured

Forced swimming test

Forced swimming test was performed according to the protocol of Porsolt et al [25]. The rats were placed individually in glass aquarium (height: 54.0 cm; length: 34.0 cm; width: 30.0 cm) filled with water to a depth of 40.0 cm (24.0 ± 1 C°). The procedure consists of a pre-swimming test and swimming test separated by 24h. During the pre-swimming procedure, rats were placed in the aquarium for 15 min and then were removed from the aquarium, dried with towels, and placed in a warmer enclosure then returned back to their home cages. The aquarium was emptied and cleaned after every two testing sessions. Twenty-four hours later, rats were retested for 5 min (300 s) under identical conditions. Immobility time was measured during the 5 min test period. Rats were considered to be immobile when floating motionless or making only the movements necessary to keep their heads above the water surface.

Statistical analysis

Mintab version 13 was used for statistical analysis. All data are presented as mean \pm SEM. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Parameters of elevated plus maze

Exposure to fenthion decreased significantly ($p < 0.001$) the time spent in the open arms when compared to the control group. However, pretreatment with quercetin in F+Q increased significantly the time spent in open arms ($p < 0,001$) when compared to fenthion group (Figure 01). In addition, fenthion group spent longer time in closed arms ($p < 0.001$) when compared to the control group. Moreover, pretreatment with

quercetin in F+Q decreased significantly ($p < 0.001$) the time spent in closed arms when compared with F group.

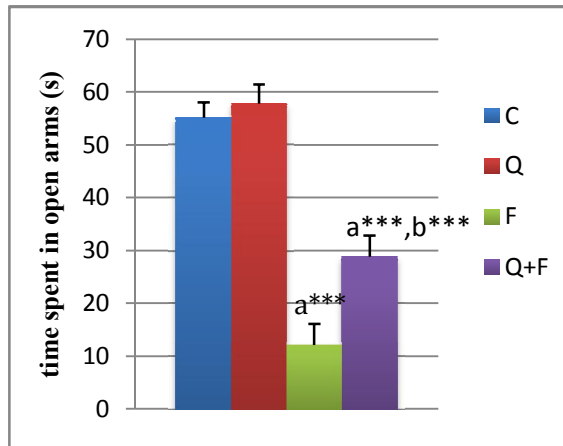


Figure 1a. Time spent in open arms

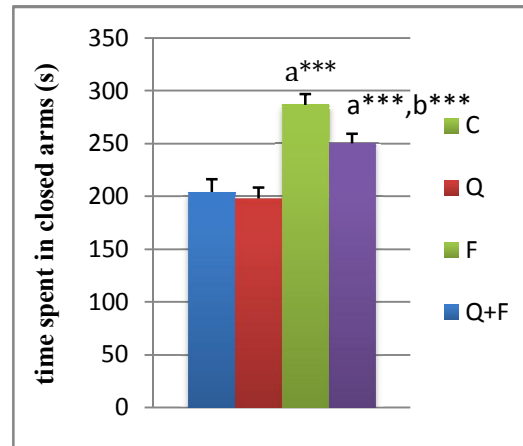


Figure 1b. Time spent in closed arms

a : comparison vs control ; b : Q+F vs F (n=07, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Parameters of open field

Fenthion group showed a significant decrease ($p < 0.001$) in the time spent in central area when compared to control group. However, quercetin pretreatment in F+Q increased significantly ($p < 0.01$) the time spent in central area when compared to fenthion group. Furthermore, F group spent longer time in peripheral area ($p < 0.001$) when compared to control group. Nevertheless, F+Q showed a significant decrease ($p < 0.01$) in the time spent in closed arms (Figure 02).

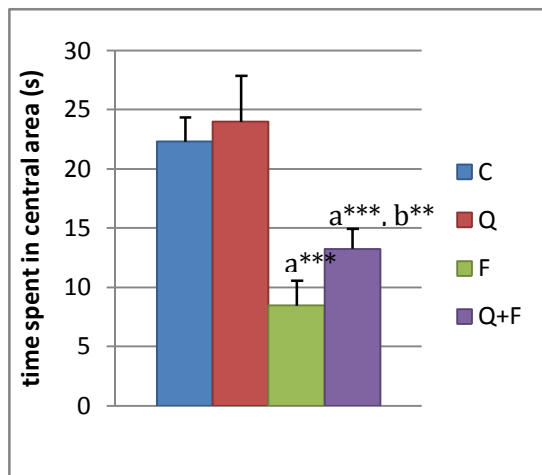


Figure 2a. Time spent in central area

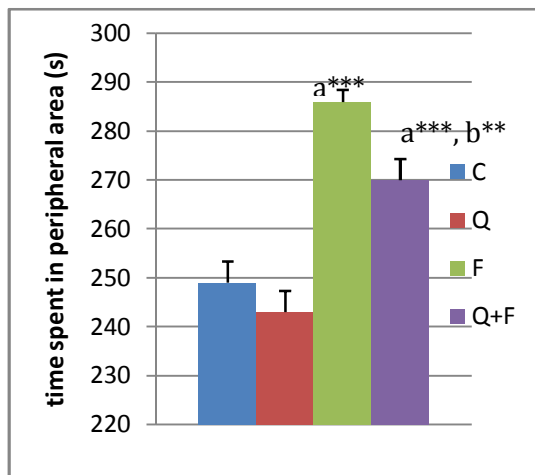


Figure 2b. Time spent in peripheral area

Parameter of Forced swimming test

Exposure to fenthion increases significantly ($p < 0.001$) the immobility time in forced swimming test when compared to control group. Interestingly, quercetin showed an important decrease in immobility time when compared to control group. Moreover, pretreatment with quercetin decreases significantly ($p < 0.001$) the immobility time when compared to F group (Figure 03).

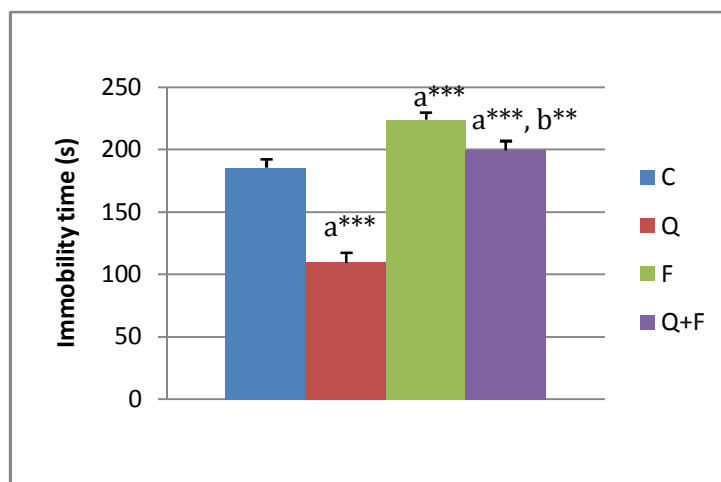


Figure 3. Immobility time (s)

DISCUSSION

Organophosphate insecticide exposure has been reported to be associated with emotional disorders such as depression and anxiety [26, 27, 28]. In its pathological form, anxiety is a maladaptive state that impairs the ability of an organism to respond optimally to its environment [29]. Rats treated with fenthion showed a decrease in the time spent in open arms and an increase in time spent in closed arms. The decreased venturing into the open arms in the elevated plus maze is typically interpreted as an increase in anxiety [30]. We think for example of gabaergic system that its perturbations are involving in anxiety disorder [31, 32]. Cholinergic system is also known to play a modulatory role in the control of anxiety [33, 34]. According to this point, the stimulation of cholinergic receptors may induce anxiogenic responses [35, 34]. In literature, many studies showed anxiogenic-like effects of OP such as diazinon and malathion [36-5]. Open field test is also used to measure anxiety level and emotional reaction [37], therefore, anxious rats tend to spend more time in the corners and the periphery of the device rather than in the center [38]. Our results have shown that treated rats spent longer time in the periphery. These findings corroborating the previous observation are those obtained in the elevated plus maze test. The Forced Swimming Test, test of antidepressants efficacy, represents a stressful and aversive situation which the rat can't escape, and produces immobility or behavior despair [39, 40]. The immobility of animals is interpreted as a lack of will to survive, and is considered a sign of depression. The measurement of immobility time in this test lead us to assess the level depression [39-41]. Our results showed an increase of immobility time in rats treated with fenthion which confirms its depressive effect. The mechanism of depression is quite complex [42]. Although psychobiological researches on depression have traditionally concentrated on the neurotransmitters, noradrenaline and serotonin, dysfunction of cholinergic transmission is also involved in the pathophysiology of depression [43,44]. Several studies have reported the depressive effect of OP in rats, low dose of malathion was able to induce depressive response [36-9]. Moreover, workers in agriculture have been reported to manifest depression signs [45, 46]. In our study, quercetin has clearly modulated the emotional deficits including anxiety and depression. Several experimental investigations showed the potential neuroprotection of quercetin against cognitive deficits in various animal models [47,48-20]. It is reported that quercetin produces behavioral effects through modulation of neurotransmitter systems like GABA, and serotonin [49], which are also implicated in anxiety and depression. It's logical to suggest that quercetin prevents anxiety and depression induced following pesticide exposure through these pathways. In conclusion we can say that fenthion has as potential anxiogenic and depressive-like effects and quercetin pretreatment was able to alleviate these behavioral responses. Other investigations are needed to understand the mechanism underlying the neurotoxicity promoted by fenthion and the neuroprotection of quercetin.

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