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Quercetin, a Natural Flavonoid, Mitigates Restraint Stress Induced Anxiety-Like Behavior in Male Wistar Rat

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Abstract: Restraint stress, experimental model for the study of psychiatric behavior such as anxiety and depression, many things of this stress remain less investigated as the persistence of effect and strategies of repair by pharmacological agent like flavonoid. In this work, male's Wistar rats exposed to restraint stress (3h/day) for fifteen days elicited anxiety like behavior and deterioration of activity and exploratory behavior. These aberrant behaviors appear to be persistent even stopping stress during the next period. Quercetin treatment (50mg/kg, i.p) repaired this change and prevented the persistence of stress induced disorders behavior. Finally, intake of quercetin seems to be beneficial for persons suffering from stressful events.

Key words: Rat · Restraint stress · Quercetin · Anxiety

INTRODUCTION

In life, biological variables of animal organism are in continuous regulated change to maintain the stability of homeostasis. However, some stressors stimulate highly the mechanism of adaptation which allows the development of many physiological disorders [1]. The hypothalamic–pituitary–adrenal (HPA) axis is a key endocrine adaptor against stressors and plays an important role in the pathophysiology of stress-related psychiatric diseases such as depression and anxiety disorders [2]. Hyperactivity of HPA axis induced important secretion of catecholamine and glucocorticoid which are believed to underlie the onset of many physiological changes, among these; the psychiatric disorders such as anxiety and depression are of interest to study. Others mechanism can contribute in the onset of anxiety such as oxidative stress [3]. Many experimental studies are carried out in order to study the anxiety disorder as a negative outcome of stressor event using an experimental model of stress such as restraint stress [4-5]. Exposure to chronic restraint stress in rats has been shown to alter cognitive functions such as learning and memory and have been linked to the pathophysiology of mood and anxiety disorders [6].In this respect, many works were performed to alleviate pharmacologically the disorders behavior related-stress [6-7]. Interestingly, others non pharmacological methods in rats were used to attenuate the anxiety such the enriched environment by music [1]. A substantial attention was paid to flavonoids as anxiolytic and antidepressant agents having a pharmacological effectiveness [8, 9]. Among these plant molecules, quercetin is being increasingly used in studies [10]. Quercetin (3,5,7,3',4'-pentahydroxyflavone) is a polyphenolic flavonol molecule that occurs in many fruits and vegetables such as onions, apples, berries, citrus fruits and tea [11, 12]. Since it is largely present in the human diet, up to 1g/day average intake of quercetin has been reported [13] which represents from 60 to 75% of the overall polyphenols ingestion [14]. Quercetin scavenges efficiently free radicals and prevents oxidative stress-induced neuronal injuries [15, 16]. Based on these data, this study aimed to investigate the persistence of anxiety behavior in male Wistar rat following repeated restraint stress and whether quercetin repairs this aberrant behavior.

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MATERIALS AND METHODS

Experimental Protocol: 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*. The 40 rats were divided into four groups. Control group (B), stressed group (IS), quercetin group (Q) and quercetin+ stress group (Q+IS). Rats of stressed group underwent restraint 3h/day for 15 days. Quercetin group received quercetin (50mg/kg) intraperitoneally dissolved in saline solution (1ml/kg) after the 15 days of stress. Control rats received the vehicle. Behavior response was evaluated on the 5th and 10th of each period of stress and treatment by quercetin.

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 [17]. The OF test was performed to measure changes in exploratory behavior and emotionality. Briefly, the apparatus, as previously described [18] 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. Traveled distance and rearing were measured.

Elevated Plus-Maze Test: The elevated plus-maze (EPM) test is a widely used paradigm to investigate anxiety-related behavior in rats [19]. 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) [20]. 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.

Statistical Analysis: Mintab version 16 was used for statistical analysis. All data are presented as mean ± SEM. P<0.05 was considered statistically significant.

RESULTS

Effect of Repeated Restraint Stress on Body Weight Evolution: Effect of Quercetin: As depicted in Fig. 2. Repeated restraint stress induced significant (p<0.001)

Fig. 1: *Experimental protocol: stress and strategy of repair*

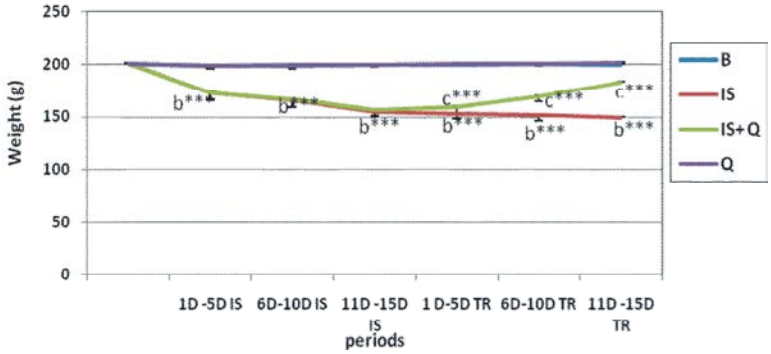

Fig. 2: Change in the body weight (g) of male Wistar rats during the periods of application of restraint stress and treatment. b: IS vs basal, c :I S vs IS+Q, (n=10,* p <0.05, **p<0.01, ***p<0.001).

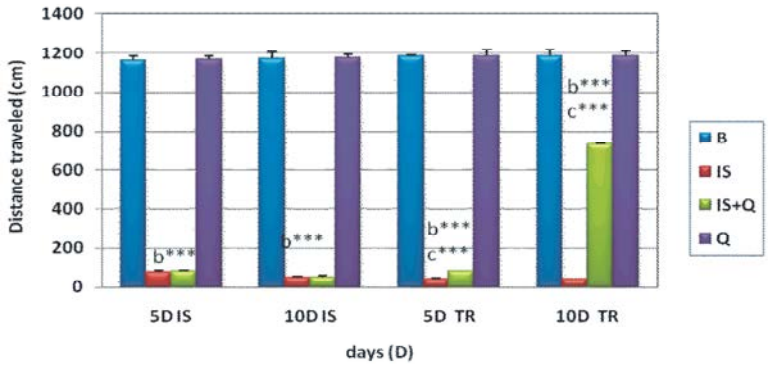

Fig. 3a: Change of the distance traveled (cm) in the Open Field test in male Wistar rats during the periods of application of restraint stress and treatment. b: IS vs basal, c :IS vs IS+Q, (n=10,*p<0.05, **p<0.01, ***p<0.001). ***p<0.001).

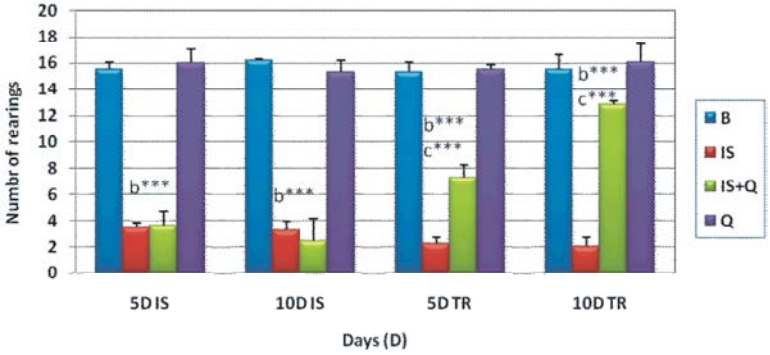

Fig. 3b: Change of the number of rearing in the Open Field test in Wistar rats during the periods of application of restraint stress and treatment. b: IS vs basal, c :I S vs IS+Q, (n=10,*p<0.05, **p<0.01, ***p<0.001). ***p<0.001).

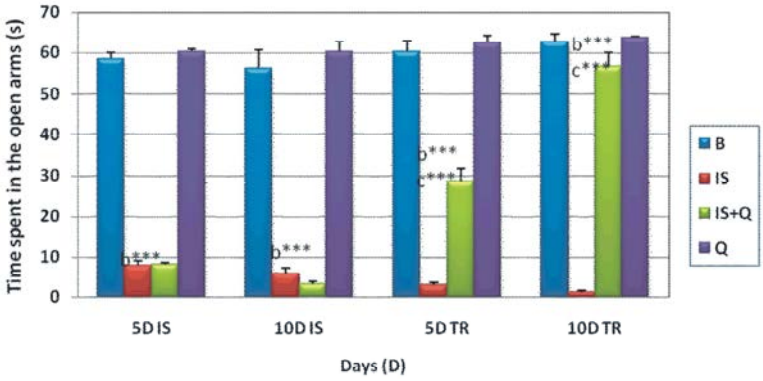

Fig 4.a. Change of the time spent in the open arms (s) in the Plus Maze test in male Wistar rats during the periods of application of restraint stress and treatment. b: IS vs basal, c :IS vs IS+TR, (n=10,*p<0.05, **p<0.01, ***p<0.001). ***p<0.001).

reduction in body weight as compared to control group. This loss sustained even after stopping of stress during the fifteen days. Quercetin treatment prevents significantly (p<0.001) the continuous pondered reduction.

Effect of Restraint Stress on Open Field Parameters (Distance Traveled and Rearing): Effect of Quercetin: As depicted in Fig. 3a, b. restraint stress suppress significantly (p<0.001) the locomotor activity as revealed by the distance traveled when compared to control group

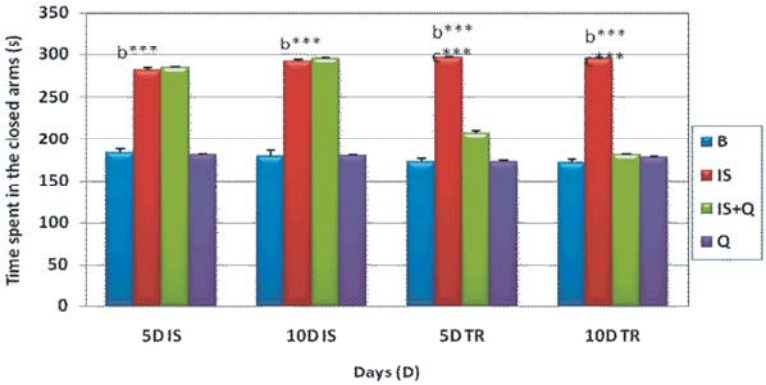

Fig. 4.b: Change of the time spent in the closed arms (s) in the Plus Maze test in male Wistar rats during the periods of application of restraint stress and treatment. b: IS vs basal, c :I S vs IS+Q, (n=10,*p<0.05, **p<0.01, ***p<0.001). ***p<0.001).

and sustained less active even after stress. Interestingly, Quercetin treatment enhanced significantly (p <0.001) this behavior. The same analysis for the number of rearing state which indicated of exploratory behavior.

Effect of Quercetin on Elevated plus Maze Parameters (Time Spent in Arms): Effect of Quercetin: As depicted in Fig. 4.a.b. Restraint stress elicited a significant (p <0.001) decrease on the time of spent in open arms even after stopping stress, consequently the time of spent in closed arms were increased. Quercetin treatment increase significantly (p <0.001) the time of exploring the open arms.

DISCUSSION

Anxiety is one of the major mental disorders affecting a large number of the population, which disturbs normal physiological equilibrium of the body by producing adverse effects on the nervous, endocrine, biochemical and immune systems [21]. Exposure to acute and repeated restraint stress was reported to induce anxiety behavior [4-5]. However, the investigating of many conditions of stress intensity depending period may lead to understand more the responses associated anxiety and whether this reaction could persist later. According to our results, restraint stress reduced time of spent in open arms indicating of the onset of anxiety response. In addition, the decrease in the traveled distance and rearing numbers represents a decrease in the exploratory capacity. In our experiment, anxiety symptoms continue even within the fifteen days after stopping the application of restraint stress, suggesting the presence of that alteration on neurotransmission, neuronal plasticity and antioxidant capacity of brain. The same observation for the reduction of body weight which may refer to the decrease in food intake and the consumption of reserve organism. Many mechanisms of actions was established to explain stress related anxiety such as oxidative stress, release of glucocorticoids and alteration in Gabaergic and serotonin system [22, 23]. The main attention of science was paid to the strategies which enhance this aberrant behavior, such as chronic exercise, music therapy and bioactive molecules [24, 25]. In our work, quercetin attenuate the evolution of these symptoms. Several experimental investigations showed the potential neuroprotection of quercetin against cognitive deficits in various animal models [26, 27]. It is reported that quercetin produces behavioral effects through modulation of neurotransmitter systems like GABA and serotonin [28], which are also implicated in anxiety and depression. In addition, strong link was suggested between antioxidant capacity of quercetin and its anxiolytic and antidepressant effect [22]. In summary, the use of quercetin was effective to inhibit the evolution of sickness behavior of anxiety and associated locomotors abnormalities.

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