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Combined Effect of Quercetin and Resveratrol Induces Anxiolytic-like Behavior and Improves Brain Antioxidant Capacity in Male Wistar Rat

Bouhali Imed Eddine, Tayaa Hakima and Tahraoui Abdelkrim

Laboratory of Applied Neuroendocrinology, Department of Biology, Faculty of Sciences, Annaba University, Algeria

Corresponding Author: Bouhali Imed Eddine, Laboratory of Applied Neuroendocrinology, Department of Biology, Faculty of Sciences, Annaba University, Algeria

ABSTRACT

Dietary enriched with polyphenols was thought to improve human health and affect positively animal welfare. Besides, the effect of mixture containing polyphenols on emotional response remains unclear. In this study, exposure to mixture of quercetin (60 mg kg^{-1}), natural flavonoid, resveratrol (30 mg kg^{-1}) and non-flavonoid phytolalexin in male wistar rat during ten consecutive days induced anxiolytic-like effect as revealed by the open field and elevated plus maze test. Furthermore, brain GSH level was clearly increased. This study can suggest that use of mixture of quercetin and resveratrol may improve pharmacological impact against pathologies and stress events in life.

Key words: Quercetin, resveratrol, mixture, behavior, antioxidant, rat

INTRODUCTION

Polyphenols are naturally occurring phytochemicals found in fruits and vegetables, exhibiting many therapeutic effects (Basli *et al.*, 2012). Important dietary sources of polyphenols include apples, berries, cocoa, herbs, redwines, seeds, onions and tea (Perez-Jimenez *et al.*, 2010). Foods enriched with these molecules are thought to improve status human health and delay the development of many diseases. Recently, epidemiological studies determined a close link between plant derived food and health benefits, these benefits are mainly related to their containing on polyphenols compounds, such as quercetin and resveratrol (Espin *et al.*, 2007). In addition to improving human health, polyphenols induced neuroprotection, anti-inflammation, antioxidants and alleviates many diseases, such as Alzheimer, ischemia etc. In this respect, Polyphenols with multipotent therapeutic agent was determined (Bhullar and Rupasinghe, 2013). Quercetin, natural flavonoid, resveratrol and non-flavonoid stilben are among molecules known for its promising pharmacological effects including, neuroprotection and antioxidants effects (Toumi *et al.*, 2013; Sharma and Gupta, 2002). Despite these effects, searching to reach a high rate of prevention against pathologies and amplify its pharmacological potentialities remains persistent, in this respects, the use of combined polyphenols to know their others effects or to mitigate more signs of pathologies was recently tested (Arya *et al.*, 2014). Nevertheless, the outcomes of the combined use of quercetin and resveratrol are still unknown. This study attempted to investigate, whether the mixture combined of quercetin and resveratrol could change the behavior responses as well as the brain antioxidants capacities comparatively to their single use in male wistar rat.

MATERIAL AND METHODS

Experimental protocol: Wistar rats obtained from Pasteur Institute (Algiers, Algeria) were housed in transparent cages at a constant temperature ($23\pm 1^\circ\text{C}$) 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 into 4 groups each contains 07 rats. Control (C) group received only corn oil at 1 mL kg^{-1} as vehicule, Quercetin (Q) group received quercetin orally 60 mg kg^{-1} , Resveratrol group (R) received resveratrol orally at 30 mg kg^{-1} , Mixture (M) group, received Quercetin and resveratrol diluted in 1 mL kg^{-1} of corn oil. The treatment lasting for 10 days consecutives days, then, behavioral testing was done and finally, brain was removed after decapitation to biochemical analysis.

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 (Angrini *et al.*, 1998). The OF test was performed to measure changes in exploratory behavior and emotionality. Briefly, the apparatus, as previously described (Saenz *et al.*, 2006) consist of a gray square ($70\times 70\times 40$ 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 (Pellow *et al.*, 1985). The EPM was made of painted wood cross (arms $50\text{ cm long}\times 10\text{ cm wide}$) elevated 50 cm above the floor. Two opposite arms were enclosed by walls ($10\times 50\times 45\text{ cm high}$) and 2 arms were open. The arms extended from a central platform ($10\times 10\text{ cm}$) (Patin *et al.*, 2005). 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 4 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.

Reduced glutathione (GSH) assay: The GSH content was estimated according to the method of Ellman (1959). Briefly, 1.0 mL of supernatant was precipitated with 1.0 mL of 4% sulfosalicylic acid for 1 h at 4°C . Samples were then centrifuged at $1,200\text{ g}$ for 15 min at 4°C . One milliliter of supernatant was then mixed with 0.2 mL of 5-5 dithio-bis-nitrobenzoic acid (DTNB, 0.01 M) and 2.7 mL of phosphate buffer (pH 8.0). Immediately, the absorbance of the reaction product was measured at 412 nm . The results were expressed as $\text{nmol GSH mg}^{-1}\text{ protein}$.

Statistical analysis: All results were expressed as the Means \pm Standard Error of the Mean (SEM). Mutiples comparisons between groups were carried out using one way ANOVA following by tukey test (Minitab).

RESULTS

As depicted in Fig. 1a, treatment with combined quercetin and resveratrol increased significantly ($p < 0.01$) time spent in open arms comparatively to others groups. In the same way, the time of spent in closed arms was decreased significantly (Fig. 1b) ($p < 0.01$). In relation to open field test, As depicted in Fig. 2a and 2b the time spent in periphery was significantly ($p < 0.05$) decreased, however, no difference was noted on the time of spent in centre. As depicted in Table 1, concentration of GSH was significantly ($p < 0.05$) increased comparatively to control group.

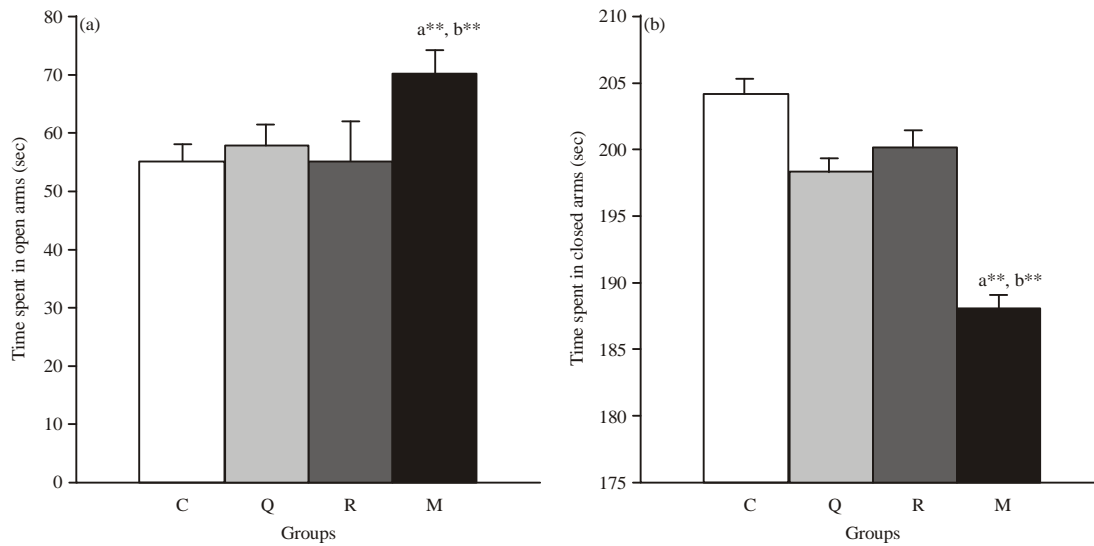


Fig. 1(a-b): Time spent in (a) Open and (b) Close arms (sec), $n = 07$, $**p < 0.01$, a: vs control and b: vs groups

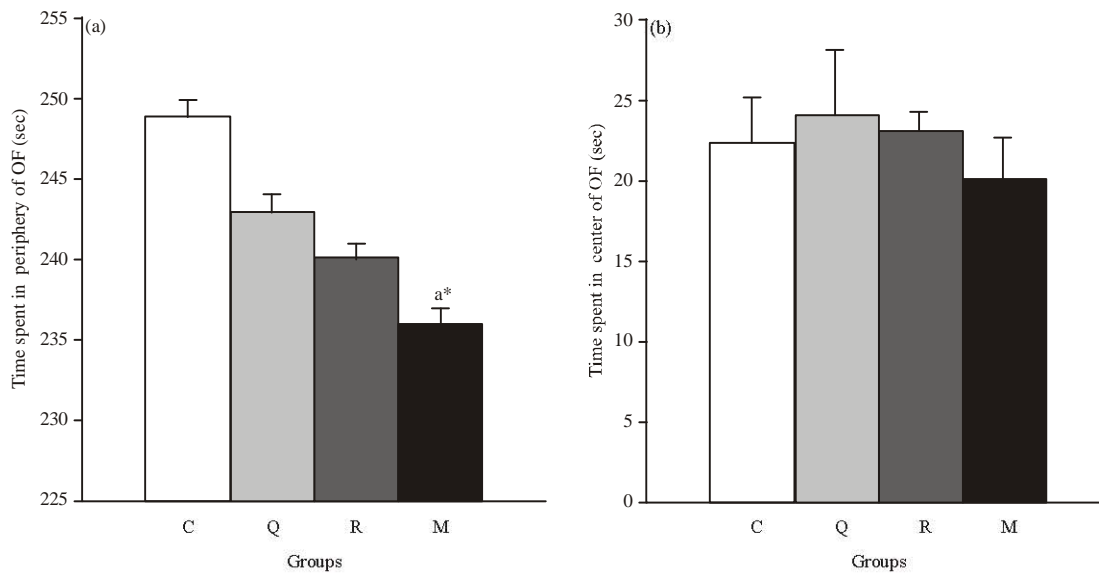


Fig. 2(a-b): Time spent in (a) Periphery and (b) Center (sec), $n = 07$, $**p < 0.05$, a: vs control

Table 1: Brain GSH content in treated groups

Groups	GSH (nmol mg ⁻¹ prot)
C	20.25±1.09
Q	24.25±1.8
R	24.75±2.2
M	25.25±2.21**

n = 07, *p<0.05, **p<0.01, ***p<0.001, a: vs control, b: vs groups

DISCUSSION

Our results shown that combined effects of quercetin and resveratrol promoted behavioral responses associated with increase in GSH level. According to EPM, the rats treated with mixture of quercetin and resveratrol increase the time of spent in open arms, this finding lead us to assume that this mixture promoted anxiolytic-like effect. In relation with the Open field test, a significant decrease was noted on time of spent in the peripheral area indicating that rats avoid thigmotaxic behavior, this point corroborate the result of EPM. The effect of quercetin and resveratrol, as alone administered was shown to induce anxiolytic effect and antidepressant-like effect. However, these responses depend on the dose and experimental conditions (Jung and Lee, 2014; Damian *et al.*, 2014). This anxiolytic-response was not strong in quercetin and resveratrol, when singly administers, which may be due the experimental conditions. The appearance of anxiolytic-effect in mixture may be due to the pharmacological properties of mixture. In fact, it has been demonstrated that mixture of quercetin and resveratrol increase the bioavailability of these molecules, even for resveratrol by improving the intestinal absorption (Lund and Pantuso, 2014). In addition, this behavior may due to modulation of GABAergic system (Jung and Lee, 2014). These behaviors responses were correlated with increase in brain GSH level. The most important endogenous antioxidant defense systems are composed of the thiol-containing tripeptide glutathione and small thiol-containing proteins such as thioredoxin, glutaredoxin and peroxiredoxin. Glutathione is found at millimolar concentrations in most cells and is the major contributor to the redox state of the cell. Glutathione is synthesized enzymatically by Glutamyl Cysteine Synthetase (GCS) and glutathione synthetase, with the former being the rate-limiting enzyme (Lu, 1998). One important task for cellular glutathione is to scavenge free radicals and peroxides produced during normal cellular respiration, which would otherwise oxidize proteins, lipids and nucleic acids. One mechanism operating to counteract oxidative damage involves transactivation of genes encoding enzymes that participate in glutathione metabolism and synthesis (Hayes and Pulford, 1995). Many worker investigated the antioxidants effect of quercetin and resveratrol (Shin *et al.*, 2010; Zhu *et al.*, 2008). Interestingly, The relationship between the antioxidant and anxiolytic abilities of quercetin and resveratrol is strengthened by several lines of evidence showing that dietary antioxidants can improve cognitive functions and prevent stress-induced neurobehavioral disorders (Chakraborti *et al.*, 2007; Sharma and Gupta, 2002). In this respect, some properties persist even in mixture conditions, which may increase pharmacological potentialities of quercetin and resveratrol. Finally, the use of mixture strategies may alleviates, even to prevent, many pathological disorders, this point need to be investigate in others works.

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