Original Research Article

Therapeutic Effects of Nano-Phytosome of Curcumin on Anxiety-like Behaviors, Neuroinflammation and Biochemical Parameters in Rats Exposed to Stress

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ABSTRACT

Curcumin and its nano-phytosome have anti-inflammation and antioxidant properties that may decrease the concentration of metabolic factors, inflammatory parameters and anxiety in stressed rats. However, the effects of administration of of curcumin on inflammation and anxiety have not still been investigated. This study was conducted to scrutinize the metabolic factors, anxiety-like behavior and inflammatory factors in rats exposed to stress and treated with nano-phytosome of curcumin. Eighty female rats were divided into four groups (n=20), including 1 and 2) non-stressed rats treated with saline (negative control) and nanophytosome (N-nano), 3 and 4) stressed rats treated with saline (Positive control) and nanophytosome (P-Nano). The rats in stressed groups were exposed to stress for 13 days and then treated for 21 days. Anxiety-like behavior and inflammatory factors of IL-6, IL-1β, TNF-α and COX-2 and the concentration of glucose and triglycerides were assessed following the treatment with nano-phytosome. The results showed that the induction of stress increased anxiety symptoms (P<0.05), the inflammation (P<0.05) and blood parameters (P<0.05), but the treatment with nano-phytosome of curcumin reduced anxiety symptoms (P<0.05), the inflammation (P<0.05) and blood parameters (P<0.05). Overall, the induction of stress caused the inflammation and anxiety and increased the concentration of glucose and triglycerides. However, the treatment of the rats with nanophytosome of curcumin reduced adverse effects of the stress on inflammation and anxiety paramters. This study showed positive effects of curcumin for decreasing the inflammation and anxiety in the stressed rats, but clinical studies are required for its confirmation on human.

GRAPHICAL ABSTRACT
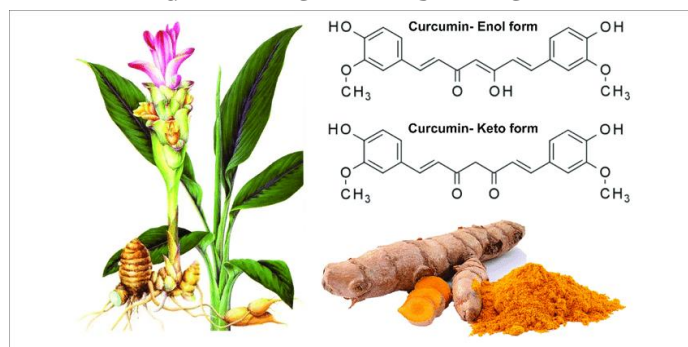
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Introduction

Stress causes mood disorders such as anxiety in adolescence and adulthood [1, 2]. It is defined as a condition in which homeostasis of the body is to be changed [3]. Stress changes physiological and psychological responses in different organs and increases susceptibility to various psychiatric diseases [4]. It also changes immunological and neurobehavioral responses and activates the hypothalamic-pituitary-adrenal axis [5]. In addition, stress causes some neurochemical, hormonal, and behavioral abnormalities in rodents [6]. The chronic mild stress model is commonly used for chronic stress. Studies have reported that chronic stress increases the sensitivity to anxiety and depression disorders by the hypothalamic-pituitary-adrenal axis dysregulation [7]. Stress induces inflammation in animals and humans. Neuroinflammation is known as a potential mechanism that participates in the maintenance of psychiatric disorders and/or resistance to common treatments [8]. Chronic low-grade neuroinflammation has been reported in anxiety and major depression [9]. The inflammatory cytokines of interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) regulate neuropsychiatric diseases [10]. These cytokines cause behavioral deficits such as helplessness, decreased exercise capacity, and intensified asthenia, sleep disturbances, and memory and learning disabilities [11]. Psychiatric diseases and the inflammation resulted from them cause metabolic changes such as the increase in glucose and lipid profile [12].

Psychiatric diseases are commonly treated by the help of synthetic drugs [7]. Synthetic plants are commonly used for the treatment of diseases; however, their uses are faced with limitations owing to their unsafe compounds [13]. Medicinal plants are commonly used for the treatment of psychiatric diseases [9]. Studies have shown a positive relation between the consumption of phenolic-rich foods and/or beverages and the prevention of diseases attributed to antioxidant properties of phenolic compounds [6]. The positive effects of medicinal plants and their derivation for decreasing stress have been previously reported [14-18].

Curcumin is an active compound with anti-inflammatory properties that improves lipid metaboilic diorders owing to its effects in regulating signaling mediators such as downregulation of COX-2 properties, mitogen-activated and Janus kinases and inhibiting the production of the TNF-α and interleukins [19]. Curcumin is an active compound; however, its uses are faced with limitations due to its volatile nature. Therefore, it needs better structures for the use in medicine science [20]. Phytosome is a technology used to prepare lipid-compatible molecular complexes and increases nutrients absorption and bioavailability [21].

Regarding the effects of stress on the induction of inflammation, anxiety and modulation in metabolic pathway and side effects of synthetic agents for the treatment of stress, using natural agents is used. Seemingly, nanophytosome of curcumin can decrease the inflammation and anxiety and improve blood profile owing to its anti-inflammatory properties. However, the effects of nanophytosome of curcumin has not been investigated on the metabolic factors, inflammation and anxiety-like behavior in stressed rats. This study is the first attempt to investigate the anxiety-like behavior and some metabolic and inflammatory factors in rats exposed to stress and treated with nano-phytosome of curcumin.

Material and methods

Reagents and Solutions
Both curcumins (EC No. 2072805) were prepared from Sigma-Aldrich Chemical Company (St. Louis, MO).

The prepararion of nano-phytosome of curcumin

The nano-phytosome of curcumin was prepared as reported by Baradaran et al. [20] by using warm ethanol, EYPC and Tween 80. The decrease

in the pressure was used to remove ethanol. The obtained phytosome suspension was submitted to a probe sonication process in an ice bath for 30 minutes in 240 W with a sequence of 1 s of sonication and 1 s of rest by a sonicator (Sonics & Materials, Inc., 20 kHz, Newtown, Connecticut, USA).

Animals
Eighty female Healthy Wistar rats with a weight of 220±20 g were studied. The rats were grouped in clean polypropylene cages and kept in a room with appropriate and controllable ventilation and temperatures and a lighting program of 12 h light/dark. Pelleted diet and drinking water were supplied. The rats were adapted with new environment during 14 days. The rats were divided into four groups (n=20) and each group was divided into four sub-groups (n=5). Forty rats were exposed to stress (stressed) and the rest were not exposed to stress (non-stressed). The rats were grouped as 1) negative control: Non-stressed rats administrated with saline (N-Con), 2) non-stressed rats administrated with 15 mg/kg nano-phytosome (N-Nano), 3) Positive control: Stressed rats administrated with saline (P-Con), and 4) Stressed rats administrated with 15 mg/kg nano-phytosome (P-Nano). The animals were orally administrated with nano-phytosome of curcumin for 21 days after the induction of stress.

The induction of stress
The stress was induced in a bench placed on the opposite side of the animal holding room. The rats were transferred into a transparent tube with a diameter of 5 cm and length of 18 cm for 2.5 h/day for 13 consecutive days [22]. The rats were returned to their environment after the induction of stress. After 13 days and the induction of stress, the rats were administrated with saline and nano-phytosome for 21 days and behavioral tests were conducted.

Behavioral responses

Open field test was conducted by using Masoumi-Ardakani et al.'s [23] report and by using square arena [90 × 90 × 30 [H] cm]. Behavioral parameters of rearing, grooming, time in inner zone and time in outer zone were investigated. Elevated plus-maze was conducted by using walls (30 × 15 × 5 cm) and arranged in line with 2 opposite open arms (30 × 5 cm) as reported by Masoumi-Ardakani et al. [23].

Inflammatory factors
The concentrations of IL-6, IL-1β, TNF-α and COX-2 were assessed in the hippocampus as reported by Lee et al. [24] and their concentrations were reported by enzyme-linked immunoassay (ELISA) and by using commercial kits of Abcam Company (Cambridge, MA, USA).

Serum measurements
In the end of the trial, the animals were euthanized after 24 hours fasting (after thiopental anesthesia), trunk blood was collected and serum was stored at -80 °C until the time of analysis.

Data analysis

The data were reported as mean ±SD and analyzed by SPSS software 23.0 (Chicago, IL, USA) by using analysis of variance (ANOVA) and Duncan *post hoc* tests. *p* value<0.05 was considered as significant.
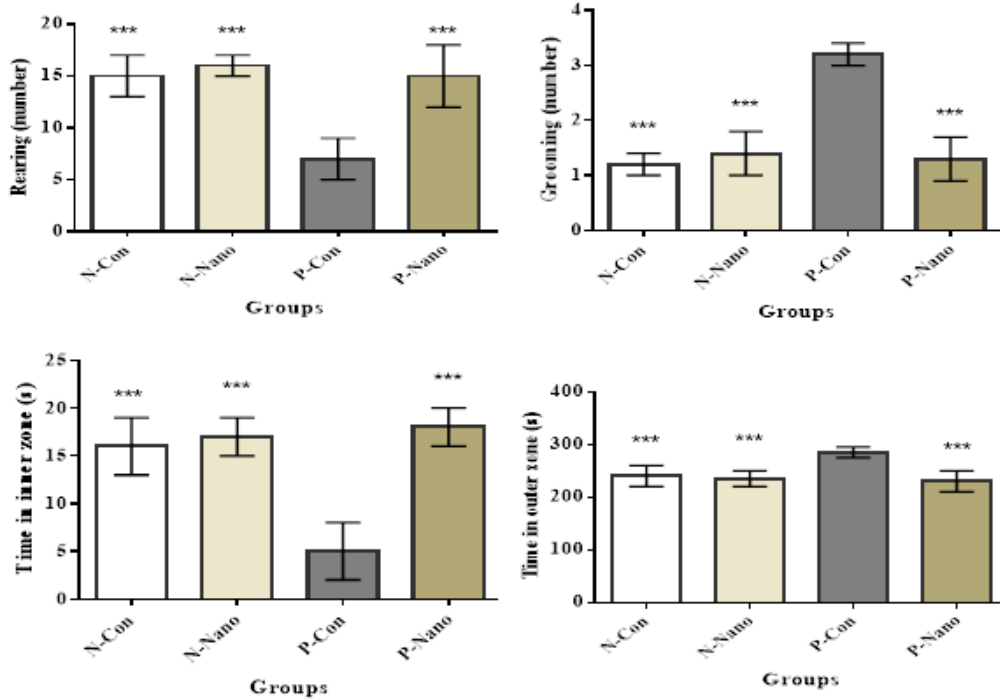
Result and Dissection

Anxiety-like behaviors using open field test
The results for the effects of nano-phytosome of curcumin on anxiety-like behaviors using open field test in stressed and non-stressed rats are shown in Figure 1. The results showed that the induction of stress decreased rearing ($P<0.05$) and time in the inner zone ($P<0.05$), and increased grooming ($P<0.05$) and time in outer zone ($P<0.05$) (N-Con *vs* P-Con). The results also showed that the administration of nano-phytosome did not have significant effects on anxiety-like behaviors using open field test in

non-stressed rats (*P*>0.05) (N-Nano *vs* N-Con). The administration of nano-phytosome increased rearing (*P*<0.05) and time in inner zone (*P*<0.05), and decreased grooming (*P*<0.05) and time in outer zone (*P*<0.05) (P-Nano *vs* P-Con). In sum, the induction of stress increased anxiety-like behaviors using open field test and the administration of nano-phytosome decreased anxiety-like behaviors in stressed rats.
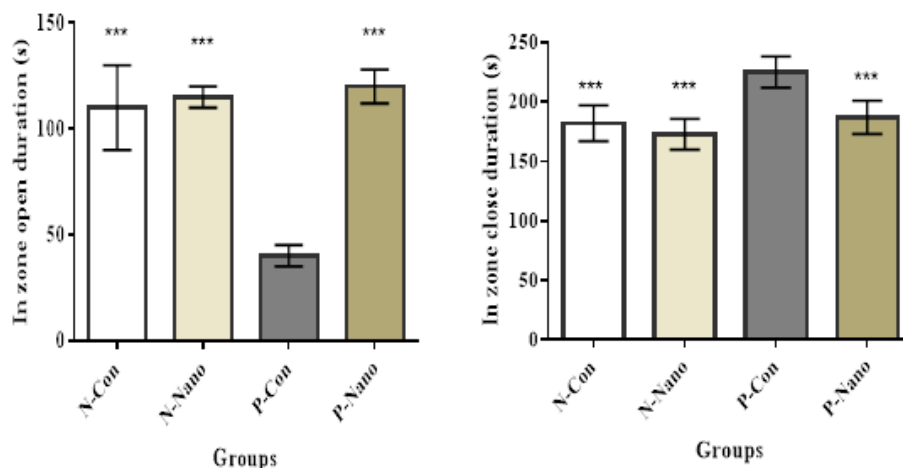

Figure 1: The results for the effects of nano-phytosome of curcumin on anxiety-like behaviors using open field test in stressed and non-stressed rats. The stress and the administration of nano-phytosome of curcumin changed anxiety-like behaviors. Superscripts (***) show significant differences between positive control with other groups at P<0.001

Anxiety-like behaviors by elevated plus-maze test
The results for the effects of nano-phytosome of curcumin on anxiety-like behaviors by elevated plus-maze test in stressed and non-stressed rats are shown in Figure 2.
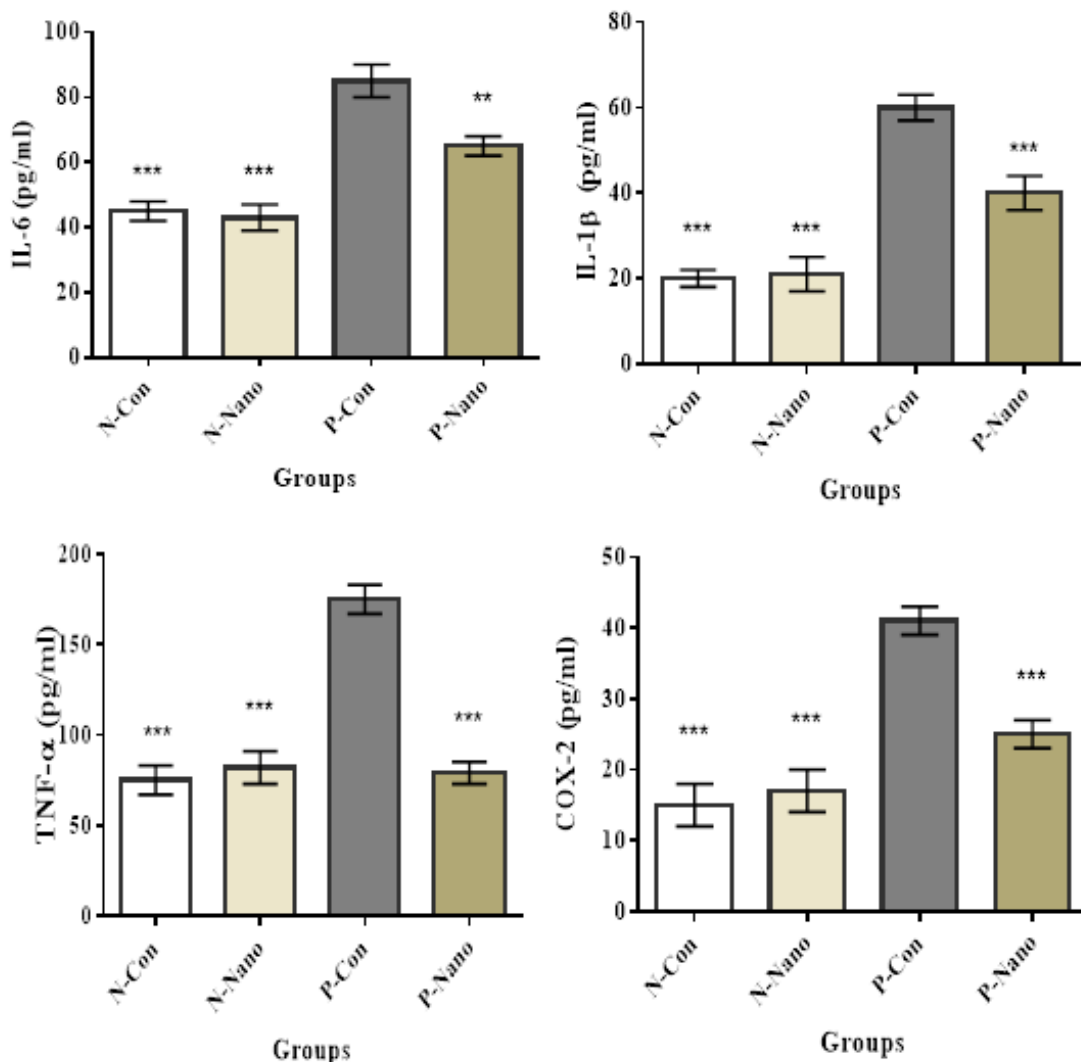

Figure 2: The results for the effects of nano-phytosome of curcumin on anxiety-like behaviors by elevated plus-maze test in stressed and non-stressed rats. The stress and the administration of nano-phytosome of curcumin changed anxiety-like behaviors. Superscripts (***) show significant differences between positive control with other groups at P<0.001

The results showed that the induction of stress changed anxiety-like behaviors (comparing stressed vs non-stressed control rats) ($P<0.05$). The administration of nano-phytosome of curcumin changed anxiety-like behaviors by elevated plus-maze test in stressed rats ($P<0.05$). In sum, the induction of stress increased anxiety-like behaviors using elevated plus-maze test and the administration of nano-phytosome decreased anxiety-like behaviors in stressed rats.

Neuroinflammation responses
The results for the effects of nano-phytosome of curcumin on neuroinflammation in stressed and non-stressed rats are shown in Figure 3. The results showed that the induction of stress increased the concentration of inflammatory factors ($P<0.05$) (P-Con vs N-con). The results revealed that the administration of nano-phytosome of curcumin did not change inflammatory factors compared with control non-stressed rats ($P>0.05$), but its administration decreased the inflammation in stressed rats ($P<0.05$). In sum, the induction of stress increased neuroinflammation and the administration of nano-phytosome decreased it in stressed rats.
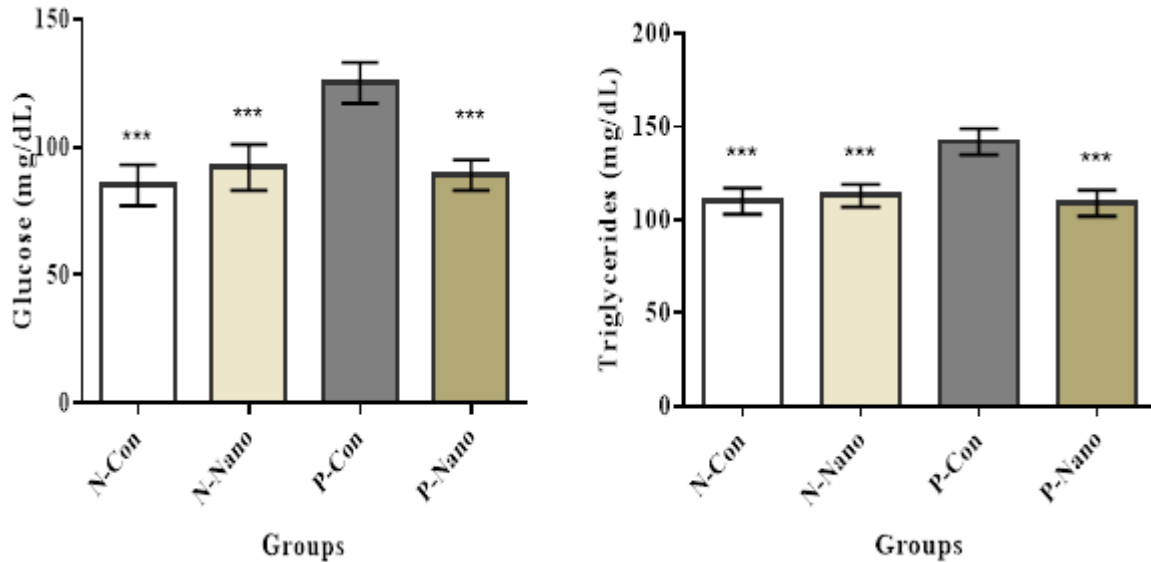

Figure 3: The results for the effects of nano-phytosome of curcumin on neuroinflammation in stressed and non-stressed rats. The stress and the administration of nano-phytosome of curcumin changed inflammatory factors in the hippocampus. Superscripts (***) show significant differences between positive control with other groups at P<0.001

Blood parameters

The results for the effects of nano-phytosome of curcumin on blood factors in stressed and non-stressed rats are shown in Figure 4. The results revealed that the induction of stress increased the the concentration of blood factors ($P<0.05$) (P-Con vs N-con). The results showed that the administration of nano-phytosome of curcumin did not change blood factors compared to control non-stressed rats ($P>0.05$), but its administration decreased the blood factors in stressed rats ($P<0.05$). In sum, the induction of stress increased blood factors and the administration of nano-phytosome decreased it in stressed rats.

Figure 4: The results for the effects of nano-phytosome of curcumin on blood parameters in stressed and non-stressed rats. The stress and the administration of nano-phytosome of curcumin changed blood parameters. Superscripts (***) show significant differences between positive control with other groups at *P<0.001*

This study was conducted to evaluate the anxiety-like behavior and inflammatory factors in rats submitted to stress and treated with nano-phytosome of curcumin. The results showed that stress increased sera parameters, anxiety-like behavior and neuroinflammation, but the administration of nano-phytosome of curcumin reduced sera parameters, anxiety-like behavior and neuroinflammation. Stressful factors change the physiological homeostasis in the organisms and cause changes in neurobehavioral profile in the course of adaptational processes. It causes significant changes in the pathophysiology of psychiatric disorders such as anxiety [25], and changes neurological functions in central and peripheral levels by activation of hypothalamus pituitary-adrenal axis. Stresses affect brain activity through the induction of long-term changes in the multiple neural systems [26]. It participates in anxiety-and depression-like behaviors in the animals [27]. Open field test is used to study the effect of anxiolytics on behavioral parameters in animals. The results showed that the administration of nano-phytosome of curcumin had positive effects on decreasing anxiety-like behavior. It means that anxiety-like behavior acts as an anxiolytic agent. It did not show positive effects on non-stressed rats. Nano-phytosome of curcumin shows its effects in abnormal conditions such as inflammation. Parallel to our findings, previous studies have reported chemical compounds of plants extract containing flavonoids, alkaloids, phenolic acids, saponins and tannins have beneficial effects against a wide range of central nervous system disorders [28,

29]. Studies have reported a relation between neuroinflammation and anxiety-like behavior in the rats [24]. The results showed a decrease in neuroinflammation factors parallel with anxiety-like behavior, so that the rats with lower concentrations for neuroinflammation factors showed lower anxiety-like behavior. On the other hand, natural products such as active compounds provide opportunities for preparing the novel drugs and covering the compounds provides an appropriate condition for increasing their efficiency. The exact mechanism of nano-phytosome of curcumin is unknown. It was reported that GABAA receptor agonists are anxiolytic and the medicinal plants act through GABAA pathway [23]. The nano-phytosome of curcumin may act by GABAA pathway. The nano-phytosome of curcumin showed anti-inflammatory response that could be attributed to its antioxidant activity [19]. It also regulates the action of some signaling mediators such as downregulation of COX-2 properties, mitogenactivated and Janus kinases, and blocks the production of the TNF-α and interleukins [13]. Studies have also linked the increase in glucose and lipids with stress and depression [12]. Seemingly, the administration of curcumin prevents peroxidation that results in the decrease of glucose and lipid and improves the serum concentrations of glucose and lipid.

Conclusion

In conclusion, stress has negative effects on inflammation and metabolic parameters and induces anxiety; however, the treatment with nano-phytosome of curcumin reduced anxiety-like behaviors possibly through the modulation in neuroinflammation parameters in stressed rats. The use of nano-phytosome of curcumin for decreasing anxiety and inflammation is suggested following its confirmation by clinical studies.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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