Original Article:

The Effect of Crocin on Total Sleep-Deprivation-Induced Amnesia in Male Wistar Rats

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ABSTRACT

Introduction: In recent years, Crocin has been used for its pharmacological functions, such as memory and learning enhancement. The aim of the present study was to assess the effect of Crocin on total sleep deprivation (TSD)-induced amnesia in male Wistar rats. **Materials and Methods:** The water box apparatus was used to induce sleep deprivation followed by Y- maze task as an index of learning and memory (the percentage of time in the novel arm during the retention phase was reported as an index of memory performances). The rats were divided into 12 groups, 8 rats in each group, including four control groups, four sham groups, and four TSD groups. Each group received saline and Crocin at doses of 1, 5 or 15 mg/kg twice a day. **Results:** The findings revealed that TSD for 24 h impaired memory function. In addition, the intra-peritoneal injection of Crocin at all doses (1, 5 and 15 mg/kg) did not change the percentage of time spent in the novel arm of Y-maze in sham of TSD, whereas it abolished the responses induced by the TSD groups. **Conclusion:** The findings showed a close interaction between the Crocin and SD. Based on the findings, Crocin seems to possess a modulatory effect on SD-induced amnesia.

Keywords: Total sleep deprivation (TSD); Crocin; Memory; Y-Maze; Rat

INTRODUCTION

A survey over the present studies shows that sleep affects cognitive and non-cognitive behaviors [1-4]. Sleep has a key role in the metabolic system, mental health and hemostasis throughout life. Sleep is divided into 5 stages, where stages 1 through 4 include non- rapid eye movement (non-REM) and stage 5 includes rapid eye movement (REM). REM stage (low voltage and high frequency waves): Dreams occur at this stage and it is characterized by increased respiration rate and heart rate. REM sleep is also known as paradoxical sleep because the brain waves emit throughout this stage [1, 2, 5]. There are many areas of the brain that control sleep such as frontal lobe (responsible for dreaming), thalamo-cortical (control of non-REM rhythm), nuclei of hypothalamic (control of circadian rhythm and start to sleep), pons (Non-REM and REM cycling), and hippocampo-cortical (responsible for memory consolidation) [6].

Insomnia and sleep disorders are one of the most common health problems around the world. As it affects 30 to 40 percent of ordinary people, this problem can have adverse effects on the health and longevity of individuals. Sleep deprivation and long-term insomnia can be a serious health hazard. Long-term sleep deprivation leads to neuronal death [7], oxidative stress [8], and performance destruction [9] in the hippocampus. Many studies have shown that sleep deficit

(chronic and acute) may lead to impairment in cognitive function such as decision making, vigilance, problem solving, and memory formation [10-14]. Memory is an important component of cognition and a key contributor to life and social interactions. [15, 16]. Moreover, memory is divided into three types: sensory memory, short-term memory, and long-term memory.

In recent years, the use of medicinal herbs has become widespread due to the lack of side effects, while saffron is one of the medicinal plants used for its medicinal properties. Crocus Sativus L. (Saffron) belongs to the iris family that has been used in folk medicine as a expectorant, anti-asthma and sedative [17]. Its active components include: 1-Safranal (classified as a volatile agent and responsible for unique aroma of saffron) [18] 2,3-Crocetin and Picrocrocin (precursor of Safranal and responsible for the bitter flavor of the spice) [19, 20] and 4-Crocin, which is carotenoid chemical compound and responsible for the color of saffron [21]. Moreover, several reports showed that Crocin can alter memory formation [22, 23]. According to animal and human studies, Crocin has various pharmacological effects such as antitumor, antioxidant, improvement of learning and memory, and antiepileptic [24]. The so-far evidence shows negative impact of sleep-deprivation on cognitive function (such as memory) [14] and the positive effect of Crocin on memory. The aim of the present study was to assess the effect of Crocin on total sleep deprivation induced amnesia.

MATERIALS AND METHODS

Animal

Male Wistar rats (weighted 200-230 g) were provided by the Institute for Cognitive Science Studies (ICSS). The subjects were kept in the animal house (in Plexiglas cages in groups of 4) in a room temperature of 22±2 °C under a 12:12-h light–dark cycle; they also had free access to food and water, while all ethical issues were followed during experiments. Each group consisted of 8 animals.

Sleep Deprivation apparatus

In this study, a sleep-deprivation water box apparatus was used (Borje Sanat Azma Co, Tehran, Iran). The apparatus composed of a water tank, made of Plexiglas, which was divided into four equal boxes (30cm*30cm*50cm), with each box having two platforms that moved up and down in the water; the speed of movement was set at 1.5 cm/s. Each platform motion cycle required 20 seconds. During this period, each platform remained stable over the surface of the water (at 12.5 cm) for 20 sec at the highest position (holding time). This process continuously reaped for 24h. Rats avoided facing with water; in doing so, they tended to remain awake. In previous reports, the behavioral observation during a 10 h sleep deprivation period documented that animals tended to remain awake 100% of the time in the water box [25, 26].

Y-maze apparatus

Memory performances were evaluated by using a two-trial arm discrimination task in a Y-maze [27] based on the innate tendency of rodents to explore novelty [28] . The Y-maze apparatus in this study was made of Plexiglas with three arms that symmetrically placed at 120° angle from each other. Each arm of the Y-maze was 40 cm long, 15 cm wide, and 35 cm high; further, each arm was marked with a different black and white pattern for discriminating arms from each other. Briefly, the test consisted of two trials (training and test), separated by an inter trial interval of 1 h. During the training trial, rats were allowed for 10 minutes to visit two arms (familiar arms 1 and 2) of the Y-maze; on the other hand, during the test trial, they had free access to the three arms for 8 minutes, i.e. the familiar arms and the ‘‘novel arm” that was previously closed. The time spent in each arm was recorded and the percentage of time in the novel arm during the retention phase was reported as an index of memory performances. The apparatus was cleaned with 5% alcohol and was allowed to dry between sessions. The memory has been assessment by Y-maze task 24 hr after TSD.

Drug injection

In this study, Crocin (Sc-217957A, Lot # K 1815) powder was supplied by Santa Cruz Biotechnology Company (USA). The Crocin was dissolved in saline (Na Cl 0.9%) and injected intra-peritoneal (I.P.) mg/kg of body weight.

Design

Rats were divided into 12 groups, 8 rats in each group: four non-sleep deprived groups, four sham groups (to eliminate the possible stress effects of environmental novelty, rats were assigned to sham groups undergoing similar situation in turned-off apparatus), and four sleep-deprivation groups. Each group received saline and Crocin at doses of 1, 5, and 15 mg/kg twice a day, first time at the beginning of sleep-deprivation process, and second time after 12 h. The memory was assessed by Y-maze task 24 hr after TSD.

Statistical analysis

Given the normality of distribution and homogeneity of variance in data (using Kolmogorov–Smirnov goodness of fit test), results were statistically evaluated using the one- and two-way analysis of variance (ANOVA), in which mean±SEM represented the possible difference outcomes between the experimental groups and their corresponding controls. Further analyses for paired-group comparisons were carried out using the post-hoc Tukey's test. In all comparisons, $P<0.05$ represented statistical significance. The post-hoc analysis results are shown in figures.

RESULT

One-way ANOVA and Post-Hoc analysis revealed that intra-peritoneal injection of Crocin at dose of 15 mg/kg increased the percentage of time spent in the novel arm of Y-maze [$F(3, 28) = 4.87$, $P=0.008$] (Fig. 1, left panel). Moreover, Crocin did not alter locomotion in all doses. [$F(3, 28) = 0.309$, $P=0.818$].

Two-way ANOVA followed by Post-Hoc analysis indicated that Crocin did not alter the percentage of time spent in the novel arm of sham-sleep deprivation group (Fig. 1, middle panel [sham effect: $F(1, 56) =9.665$, $P<0.003$; drug effect: $F(3, 56) =5.961$, $P<0.001$; and sham-drug interaction effect: $F(3, 56) =1.728$, $P=0.172$]. Correspondingly, similar analysis indicated that Crocin in all applied doses did not alter the locomotor activity (Fig. 2, middle panel [sham effect: $F(1, 56) =1.719$, $P=0.195$; drug effect: $F(3, 56) =1.027$, $P=0.388$; and sham-drug interaction effect: $F(3, 56) =0.753$, $P=0.525$].

Similar analysis demonstrated that Crocin at all doses abolished the amnesia induced by sleep deprivation (Fig. 1, right panel) [sleep effect: $F(1, 56) =0.625$, $P=0.433$; drug effect: $F(3, 56) =4.999$, $P<0.004$; sleep-drug interaction effect: $F(3, 56) =3.670$, $P<0.017$]. Furthermore, data analysis demonstrated that, intra-peritoneal injection of Crocin at doses of 1, 5 and 15 mg/kg did not alter the number of novel arm visits among groups (Fig. 2, right panel) [sleep effect: $F(1, 56) = 0.756$, $P=0.388$; drug effect: $F(3, 56) =0.887$, $P=0.453$; sleep-drug interaction effect: $F(3, 56) =0.252$, $P=0.860$].

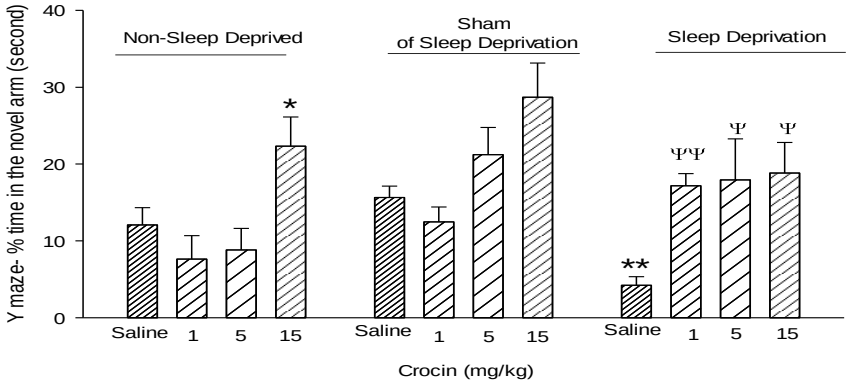

Figure1. The effects of Crocin on impairment of memory induced by total sleep deprivation (TSD). Twelve groups of animals were divided into three sets of four groups (left set is in normal condition, while middle and right sets were placed in sham of TSD and TSD condition, respectively). All animals received saline (1 ml/kg) or Crocin (1, 5 and 15 mg/kg). Data are expressed as Mean±S.E.M for eight animals per group. *$P<0.05$ and **$P<0.01$ as compared to saline/control group. (□$P<0.05$ and □□$P<0.01$ as compared to the respective groups in the left panel)

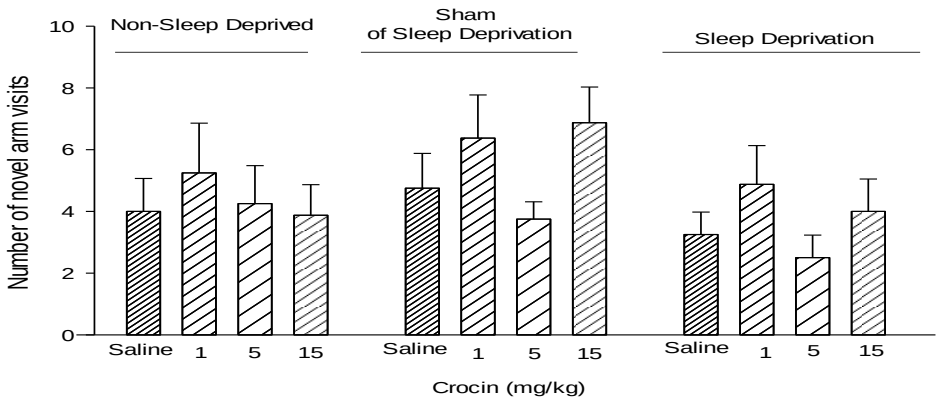

Figure 2. The effects of Crocin on the number of novel arm visits (Locomotor activity). Twelve groups of animals were divided into three sets of four groups (left set is in normal condition, while middle and right sets were placed in sham of TSD and TSD condition, respectively). All animals received saline (1 ml/kg) or Crocin (1, 5 and 15 mg/kg). Data are expressed as Mean±S.E.M for eight animals per group.

DISCUSSION

The amnestic effects of TSD

The present findings demonstrated that 24 hours of sleep deprivation notably impaired the memory process according to previous study [29]. However, others studies indicated that cognitive performance was maintained after 25–35 hours of sleep deprivation [30,31]. The divergent findings in these studies may be explained by the uneven loads between different subtests as well as by uncontrolled practice effect. Sleep appears to be important for memory processing in two major ways. First, sleep is important before learning to prepare the brain to effectively acquire new information. Second, sleep is important following learning to facilitate the consolidation (i.e. stabilization) and integration (i.e. assimilation) of newly learned information into existing memory structures [32, 33]. It seems that most recent studies have investigated the effect of SD on learning process.

Given the explanation about the selective effect, SD affects the function of some brain regions and thus, disrupted cognitive function depends on neurophysiological perspective [34]. Horne et al. proposed prefrontal vulnerability hypothesis for the effect of SD on cognitive function [35]. Based on this theory, SD decreases the cognitive function that depends on the prefrontal cortex and cognitive function such as divergent thinking, language, executive function, and creativity.

Much of the work on the effects of sleep deprivation on memory has focused on declarative memory, which involves memories that are consciously accessible and either involve autobiographical memory for actual events in one's past (episodic memory) or memories for facts and general knowledge (semantic memory). These types of memory are highly dependent upon the hippocampus and medial temporal lobe structures during initial encoding. For instance, Drummond and colleagues found that 35 h of sleep deprivation resulted in significantly impaired verbal learning relative to rested wakefulness [37].

When looking for potential sources, there is a longstanding agreement that the ascending nuclei in the reticular activating system are crucial for a normal sleep-wake cycle and are thus most likely involved in SD [38]. The reticular activating system consists of several noradrenergic, dopaminergic, and cholinergic nuclei that project throughout the cortex and have a central role in attention and arousal [39, 40], for example removal or blockage of cholinergic neurons reduces or disrupts the N1 and P300 components in encephalographic recordings. The cholinergic neurons innervate the thalamocortical network, which plays a central role in the generation of cortical rhythms; this cholinergic activity is likely related to the modification of cortical rhythms as observed during a sleep cycle and after SD.

4-2-The Effects of Crocin on Memory Retention

In the present study the findings showed that, Crocin could improve amnesia induced by SD in different doses. Other studies indicated that saffron extract attenuated morphine-induced memory impairment on passive avoidance learning in mice [43] and prevented ethanol-induced inhibition of hippocampal Long-Term Potentiation (LTP), a form of activity-dependent synaptic plasticity that may underlie learning and memory. This effect of saffron was attributed to Cronin [44]. Another study showed that saffron and its active constitutes, Crocin and Safranal could improve the impaired memory induced by hyoscine [45] and cognitive performance in Morris water maze task in Streptozocin (STZ) - lesioned rats [46].

The previous studies proposed several mechanisms for carotenoids from saffron; for instance: 1- Crocin was the most potent antioxidant that combats ischemic stress-induced neuron death by increasing glutathione levels [47, 48]. In an animal study, chronic restraint stress rats that received saffron extract or Crocin had significantly higher levels of lipid peroxidation products, higher activities of antioxidant enzymes and lower total antioxidant reactivity capacity [23]. 2-another study postulated that Crocin may prevent FA-induced neuronal damage in the hippocampus and restores weight loss in NO-dependent manner. 3- It showed that Crocin attenuated acrolein-induced tau hyper phosphorylation and oxidative stress in rat cerebral cortex via modulating MAPKs signaling pathways [49]. According to different memory impairment models, the impacts of Crocin on memory enhancement might be related to the antioxidant, anti-hyperglycemic, and anti-hypoinsulinemic properties of this compound [22, 50]. In addition, beta amyloid induced-memory deficit was inhibited via anti apoptotic and anti-oxidative effects of Crocin [51]. 4- Ghasemi et al. showed that antidepressant effects of saffron and Crocin in long term administration could be related to the effect on the expression of some proteins that are involved in depressive disorders including CREB and BDNF [52]. The results are in agreement with the findings of other studies showing the antidepressant effects of saffron aqueous extract as well as showing that Crocin could be attributed to the increase in the protein and mRNA levels of BDNF, CREB, and p-CREB in rat hippocampus [52]. The critical role of CREB in neuronal survival and plasticity has also been identified [53]. It is suggested that actions of neurotransmitters and neurotrophic factors on adult neurogenesis could be regulated by cAMP-CREB cascade [54]. The physiological modulation of neuronal excitability by CREB can affect learning and memory processes through at least three non-exclusive mechanisms

CONCLUSION

Our data suggest an intimate interaction between the Crocin and SD. Based on the above finding, Crocin seems to possess a modulatory effect on SD-induced amnesia.

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"The authors declare no conflict of interest"

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