

RESEARCH ARTICLE

Crocin Exerts Neuroprotective Effect in Valproate Exposed Rat Model of Autistic Spectrum Disorder by Ameliorating Behavioural and Biochemical Reversions

Sagarika Majhi^{1,2*}, Sokindra Kumar¹

¹Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.

²I.T.S College of Pharmacy, Ghaziabad, Uttar Pradesh, India.

Received: 25th July, 2023; Revised: 21st January, 2024; Accepted: 28th February, 2024; Available Online: 25th March, 2024

ABSTRACT

Autistic spectrum disorder (ASD) is associated with oxidative stress and neuron damage triggered by valproic acid (VPA) in a post-natal rat model of ASD. It was hypothesized that crocin (CRO), may have beneficial effects in ASD due to its antioxidant activities. The study investigated the neuro-behavioural and biochemical modifications of crocin in VPA-induced ASD. Wistar albino rat pups aged 13 days were divided into five groups (06 each). 400 mg/kg VPA subcutaneously on PND 14 successfully induced autism. From PND 14 to 40, crocin was dispensed daily. Different neuro-behavioral markers were examined while the groups were receiving therapy. Animals were sacrificed at the completion of the research for biochemical calculations. Crocin treatment significantly improved behavioral activity and oxidative changes compared to animals treated with the VPA-exposed group. The effects were more pronounced at higher dose of crocin, providing evidence of reversing the valproic acid-induced autistic deficits. Crocin could be a viable option for ASD treatment due to its potential neuronal cytoprotective effects, likely attributed to its antioxidant properties.

Keywords: Autism spectrum disorder, Oxidative stress, Neuroprotective activity, Crocin, Valproic acid, Postnatal day.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.03

How to cite this article: Majhi S, Kumar S. Crocin Exerts Neuroprotective Effect in Valproate Exposed Rat Model of Autistic Spectrum Disorder by Ameliorating Behavioural and Biochemical Reversions. International Journal of Drug Delivery Technology. 2024;14(1):14-22.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Autism spectrum disorder (ASD) is believed to be triggered by exposure to various toxins or alterations in a complex interplay of genetic factors.^{1,2} ASD is a neurological and developmental condition, and it is defined as how people behave, learn, connect, and communicate with others. Because there is a large range in the nature and severity of symptoms that persons with autism experience, the medical condition is referred to as a “spectrum” disorder.³ Poor social reciprocity, restricted interests, lack of focus, hyperactivity, communication impairments,⁴ and repetitive behaviors are some characteristics of autism.⁵ According to some researches, people with ASD frequently struggle with concomitant neurological illnesses, including seizures as well as issues adjusting to novelty and environmental stress.⁶

Usage of sodium valproate (VPA) during gestation may cause a condition called fetal valproate syndrome, which resembles autism in particular ways.⁷ In the same context, valproate fetopathy is a condition that may occur in

children exposed to VPA. This condition links deformities to intellectual impairment. Moreover, VPA is linked to a higher incidence of autistic disease (1 in 36 children).^{8,9} The most compelling data associating VPA exposure to an elevated probability of ASD was found in two published studies: large-scale population-based research and a prospective study.^{10,11} Rodents who receive VPA during pregnancy develop behavioral abnormalities and neurological consequences that are similar to the autism phenotype.¹² Additionally, VPA administration to rodents on post-natal day 14 (PND14) results in autism-like manifestations like impairment of motor and cognitive abilities. The emergence of intrusive behaviours, and persistent deficits in development of social interactions were also observed.¹³ The fact that adults did not experience this effect suggests that PND14 is inside the key developmental window for vulnerability to VPA-induced toxicity to the brain.¹⁴

Dietary polyphenols and plant-based medications have potential as treatments for neurodevelopmental conditions like

*Author for Correspondence: sagarika.majhi@gmail.com

autism. They have been demonstrated to inhibit inflammatory reactions and neuronal cell death while also promoting neurogenesis and neuroplasticity in the brain.¹⁵ *Crocus sativus* L. (Family-Iridaceae) is a tiny perennial plant that yields crocin (CRO). Along with free radical scavenging and anti-apoptotic properties, saffron modulates some the brain's cellular processes, including dendritic morphological remodeling and brain infraction.¹⁶ Crocin has vast pharmacological actions, including anti-inflammatory,¹⁷ and neuroprotective benefits¹⁸. Additionally, crocin has reportedly been linked to the potential ability to act as an antidepressant.¹⁹ Additionally, oral crocin treatment enhanced intestinal barrier performance and gut microbial structure and reduced neuroinflammation, thereby, successfully reduced depression-like behavior in animals.²⁰

Early dietary intervention may be able to influence how the autistic condition develops. The wide range of possible health advantages (neuro-protectives) of carotenoids was the rationale behind the hypothesis that intake of crocin may affect behavioural regressions in rats induced by post-natal VPA treatment. The effectiveness of crocin as neuroprotectant was assessed by neuro-behavioural assessment and antioxidant mechanism.

MATERIALS AND METHODS

Experimental Protocol

The experimental protocol (Table 1) was carried out at Subharti Medical College, Meerut, involving thirty male albino rats (wistar strain; 30–40 g; 2-week-old pups). Housed in a controlled environment (12-hour day-night cycle) and humidity (55–65%). The animals were provided standard laboratory food and unlimited access to water. Experimental animals were sanctioned by the Institutional Animal Ethical Committee of Subharti University (1204/PO/Re/S/08/CPCSEA/22-01). Adhering to power analysis and prior literature survey for selecting the appropriate number of animals, the study was carried out from May-June 2023.

Post-Natal Valproic Acid Induced ASD

Following acclimatization, the young animals were placed into five groups of six rats each. Animals were fed with standard pellets. The rat pups' weights will be recorded on the first day of the experiment. Rat pups received a subcutaneous injection of 400 mg/kg valproic acid (VPA) on PND 14 and were treated daily with crocin from PND 14 to 40. Neurobehavioral, biochemical, and histological assessments were conducted at specified intervals following the outlined timeline¹³ (Figure 1).

General & Behavioural Parameters

Body weight measurement and other parameters were conducted for each animal according to the established timeline.

Repetitive Behaviour Assessment

Marble Burying Test

On husk bed, twenty marbles, each measuring one centimeter in diameter, were spaced equally apart. The animal was allowed

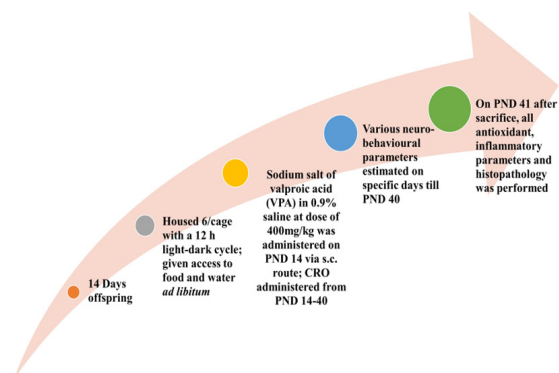


Figure 1: Timeline and treatment protocol of crocin (CRO) in VPA-exposed wistar rats

half an hour on the bed after the partition was removed. Marble that was complete, two thirds, and half buried were scored three, two, and one points, accordingly.²¹ The marble-burying score was the allocated variable's description on PND 35.

Negative Geotaxis Test

From post-natal days 16 to 18, a rat was positioned on an inverted grid, elevated above a soft surface to assess negative geotaxis. Each rat was positioned with its head pointed down the descent and looking downward along a 45° inclination. The animal's performance (facing up the slope) was graded manually for 180° turn in less than 30 seconds.²²

Mid-air Righting Reflex

The ventral side of the mouse was released from 30 cm above a cushioned surface on PND 17–19. If the animal lands on all four paws within a given amount of time, the score is considered favorable.²²

Analgesic activity

Each animal was placed on a heated plate (55 ± 0.3°C) on PND 30. The delay in the initial hind-paw reaction was observed through licking of paws within thirty seconds.²³

Rota Rod Test

Every animal from PND 24-26, was hooked up to a rod revolving at 40 revolutions per minute (rpm). The length of time (maximum of 5 minutes) it took each animal to maintain balance was used to calculate the persistence time.²⁴

Open Field Test

Using an actophotometer (INCO Pvt Ltd, Ambala), each rats' patterns of motion were captured on PND 20 and 40. During evaluation, the equipment had been placed in a ventilated, sound-attenuated, and dimmed room. A photobeam breakdown was used to characterize locomotor activity. By counting the number of beams pauses throughout a 20-minute session divided into two 10-minute periods was assessed.

Cognitive Rigidity Assessment

Elevated Plus Maze

It consists of two open and closed arms, to study anxiety in the animals. For five minutes, rats were individually positioned on

Table 1: Study Protocol of VPA exposed experimental animals

Groups	Drug Administered (PND)	Dose (mg/kg)	Route
I. Control	0.9% NaCl (14–40)	1-mL/kg	Per oral
II. VPA	VPA (14)	400	Sub-cutaneous
III. Crocin (<i>per se</i>)	Crocin (14–40)	100	Per oral
IV. VPA + Crocin I	VPA (14) + Crocin I (14–40)	400 50	Sub-cutaneous Per oral
V. VPA + Crocin II	VPA (14) + Crocin II (14–40)	400 100	Sub-cutaneous Per oral

VPA, Valproic acid and CRO, Crocin I (50mg/kg) II (100mg/kg); PND, Postnatal Day.

the central maze, facing open arm. Observations included the duration spent in open arm and the duration taken to enter it.²⁵

Morris Water Maze

Based on the earlier research that autistic patients frequently have a memory deficiency,²⁶ the impact on memory was assessed on PND 38-40. The rectangular pool filled with tap water, had four regions and innocuous milk was placed on top. One quadrant's water surface had the detachable platform submerged. The position of the submerged platform has to be memorised by each animal utilizing certain visual hints placed around. The length of time it takes to find and ascend the submerged stage was calculated.²⁴

Social Impairment Assessment

• Social interaction test

Impaired interaction is the main characteristic of ASD.^{27,28} This study used a three-chambered apparatus to measure social relationships on PND 35. The rat was momentarily acclimated to the centre compartment prior to the sociability phase. Unfamiliar rats were put on one side, leaving the other unoccupied, during the sociability phase. The "Sociability Index" (SI) was computed as follows:

$$SI = \frac{\text{Time spent in stranger chamber}}{\text{Time spent in empty chamber}}$$

• Social preference test

In this phase, a fresh rat was introduced to the vacant chamber and, the stranger chamber now considered the familiar chamber. The time the test animal played out in each compartments was recorded. The "Social Preference Index" (SPI) was calculated perquation.²⁹

$$SPI = \frac{\text{Time spent in new chamber}}{\text{Time spent in familiar chamber}}$$

• Dissection, homogenization & Biochemical assessment

Animals were euthanized on PND 41, and their brains were extracted out. A 10% w/v tissue homogenate was prepared using 0.1 M phosphate buffer (pH 7.4). After centrifugation (4°C) at 3000 rpm/10,000g for 15 minutes, the supernatant was collected to study oxidative stress indicators. UV

spectrophotometric analysis at 750 nm was used to estimate the total protein in the brain³⁰. Further, the brain's malondialdehyde (MDA), reduced glutathione (GSH), catalase, superoxide dismutase (SOD) and nitrate/nitrite levels level³⁰ were estimated through standard procedures.

• Statistical Analyses

The Mean \pm standard deviation (SD) will be utilized for depicting the result statistically. t-test (paired), analysis of variance (ANOVA) was practised to identify the statistical significance, subsequently followed by a tukey post-hoc test.

RESULTS

Exposure to VPA did not result in a notable delay in weight change from PND14 to 40. On PND 35, in line with a previous study, the VPA group exhibited increased digging and burying behavior compared to the control group ($^{###}p < 0.001$) (Figure 2). CRO I and II treatments significantly reduced ($*p < 0.05$, $**p < 0.01$) marble burying as compared to the VPA group. However, the comparison of CRO *per se* and control group displayed no significant differences. Additionally, CRO I and II treatment depicted comparable results to the control group.

On PND 16, 17, and 18, the VPA-exposed group took longer to re-orient on inclined plane than control group ($^{###}p < 0.001$). Compared to the control, re-orientation time did not change substantially in the CRO *per se* group. CRO I and II treatment resulted in a substantial decline ($*p < 0.05$ and $**p < 0.01$, respectively) in time required for re-orientation (Figure 3).

Comparing the VPA-exposed to control group, demonstrates a substantial increase in righting reflex time on PND 17, 18 and 19 ($^{##}p < 0.01$) (Figure 4). The righting reflex time was not affected by CRO *per se* treatment. A dosage-dependent substantial reduction in CRO I and II group ($*p < 0.05$ and $**p < 0.01$) was observed as compared to VPA-exposed group to land on the platform.

On PND 30 thermal nociception study revealed that VPA exposed group has significantly greater latency to withdrawal ($^{###}p < 0.001$) (Figure 5). The latency did not change appreciably upon treatment with the dosage of CRO *per se*. After receiving CRO I and II ($**p < 0.01$, $^{***}p < 0.001$), there was a reduction in the latency to foot withdrawal relative to VPA-exposed group, respectively.

The coordination of movements on rotarod (Figure 6) on PND 24 and 25 ($^{##}p < 0.01$) and 26 ($^{###}p < 0.001$), for VPA exposed group was significantly reduced. Animals administered with CRO *per se* and control group had a similar latency to fall. When compared to the VPA exposed group, the groups administered with a low dosage of CRO had substantial protection on PND 24-26 ($*p < 0.05$). However, on PND 24-25 ($*p < 0.05$) and 26 ($**p < 0.01$), administration with a high dosage of CRO lengthened the duration to fall from the rotarod.

The exploration conducts of animals on PND 40, showed that the CRO *per se* group did not exhibit significantly different behaviors of exploration from the control group. However, the group treated with VPA showed significantly more counts in the 0-10 mins ($^{###}p < 0.001$) and 10-20 mins ($^{###}p < 0.001$)

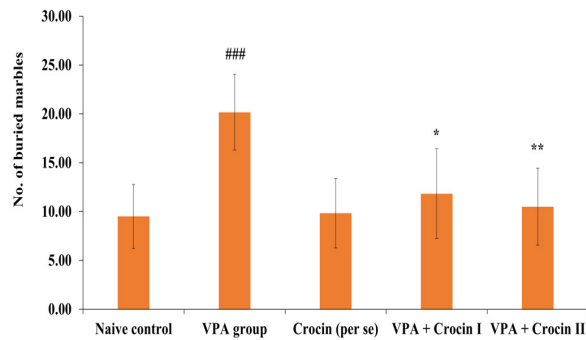


Figure 2: Effect of Crocin on marble burying behaviour in VPA-exposed wistar rats on PND 35 [Control compared with VPA exposed group (### $p < 0.001$); VPA exposed group compared with CRO I and II (* $p < 0.05$, ** $p < 0.01$)].

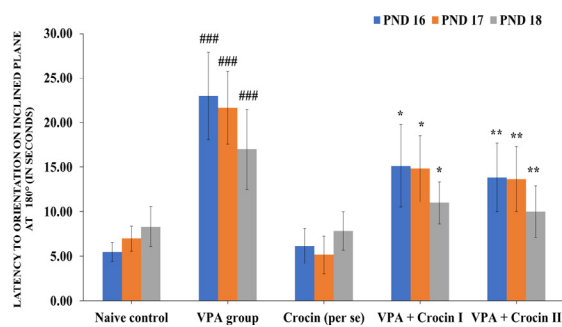


Figure 3: Effect of Crocin on latency to re-orientation in VPA-exposed wistar rats on PND 16, 17 and 18 [Control compared with VPA exposed group (### $p < 0.001$); VPA exposed group compared with CRO I and II (* $p < 0.05$, ** $p < 0.01$)].

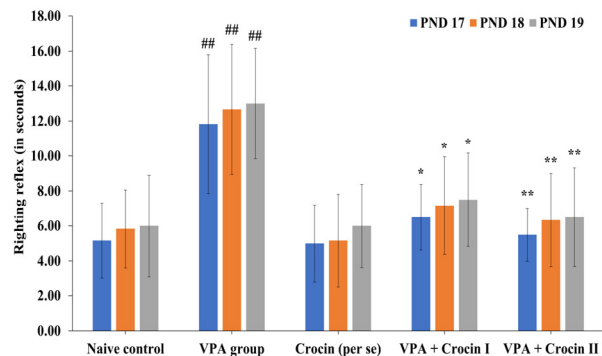


Figure 4: Effect of Crocin on righting reflex in VPA-exposed wistar rats on PND 17-19 [Control compared with VPA exposed group (### $p < 0.01$); VPA exposed group compared with CRO I and CRO II (* $p < 0.05$, ** $p < 0.01$)].

intervals. The results (Figure 7) also showed that both dosages of CRO mitigated the hyperlocomotion activity by reducing the number of counts in the 0-10 mins (** $p < 0.01$) and 10-20 mins (** $p < 0.01$) duration.

As compared to control, the VPA group dramatically reduced the visits (### $p < 0.01$) and the time duration invested (### $p < 0.001$) in open arm (Table 2). The CRO *per se* alone resulted in a non-significant result. In contrast to the VPA exposed group, the number of visits and time spent

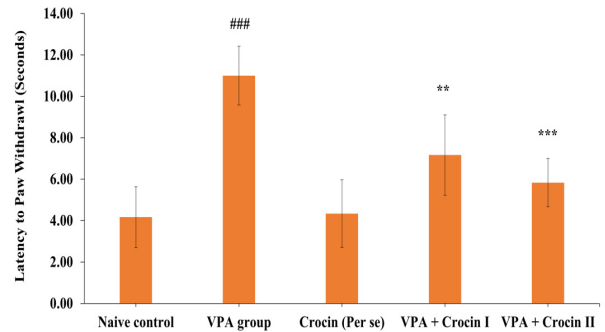


Figure 5: Effect of Crocin on paw withdrawal latency in VPA-exposed wistar rats on PND 30 [Control compared with VPA exposed group (### $p < 0.001$); VPA exposed group compared with CRO I and CRO II (** $p < 0.01$, *** $p < 0.001$)].

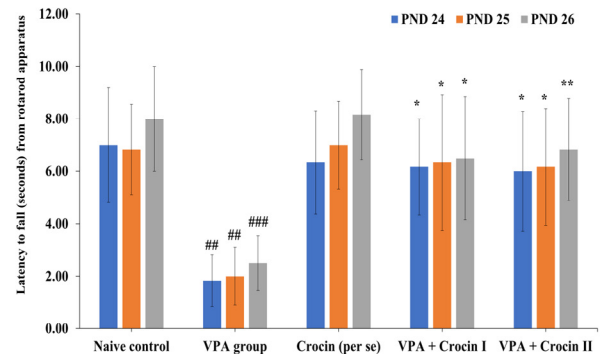


Figure 6: Effect of Crocin on latency to fall from rota-rod in VPA-exposed wistar rats on PND 24, 25 and 26 [Control compared with VPA exposed group (### $p < 0.01$, *** $p < 0.001$); VPA exposed group compared with CRO I and CRO II (* $p < 0.05$, ** $p < 0.01$)].

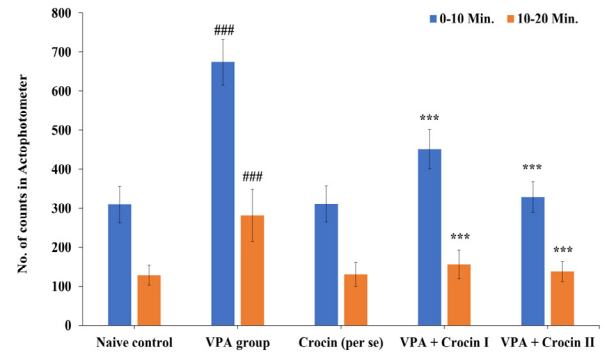


Figure 7: Effect of Crocin on locomotor activity in VPA-exposed wistar rats on PND 40 [Control compared with VPA exposed group (### $p < 0.001$); VPA exposed group compared with CRO I and CRO II (** $p < 0.01$, *** $p < 0.001$) at 0-10 mins and 10-20 mins interval].

were considerably higher after intervention with CRO I (open arm entries, * $p < 0.05$; time spent, ** $p < 0.01$) and CRO II (open arm entries, * $p < 0.05$; time spent, *** $p < 0.001$). Also, the CRO I and II treated rats showed a substantial reduction in closed-arm visits (* $p < 0.05$, ** $p < 0.01$) and the amount of time spent (** $p < 0.01$, *** $p < 0.001$) as compared to the VPA exposed group.

Rats were allowed to roam the maze and locate the immersed platform within three separate trials daily. The

Table 3a: Effect of crocin on MDA and NO levels in VPA and CRO treated groups

Groups	Antioxidant Parameters (Mean \pm SD)	
(PND 41)	MDA (nM/mg of protein)	Nitrite (μ g/mg protein)
Control	0.650 \pm 0.508	12.20 \pm 5.22
VPA group	2.731 \pm 1.013####	30.47 \pm 8.96####
Crocin (per se)	0.663 \pm 0.449	12.67 \pm 4.98
VPA + Crocin I	1.615 \pm 0.530*	17.58 \pm 6.88*
VPA + Crocin II	1.014 \pm 0.251***	14.88 \pm 5.37**

VPA, Valproic acid and CRO, Crocin I (50mg/kg) II (100mg/kg); PND, Post-natal Day; MDA, Malondialdehyde [Control compared with VPA exposed group (#### p < 0.001); VPA exposed group compared with CRO I and CRO II (* p < 0.05, ** p < 0.01 and *** p < 0.001)].

rat. In VPA exposed groups the social proximity for familiar rat was significantly higher (#### p < 0.001), (Figure 9b) and was quantitatively declined (#### p < 0.001) in the CRO treated groups. In contrast to control rats, VPA exposed exhibited a lower sociability preference index (*** p < 0.001). CRO significantly altered (*** p < 0.001) this effect at both doses.

Rats exposed to VPA exhibited significantly higher levels (#### p < 0.001) of total nitrite and MDA than the control group. The MDA and NO levels were unaffected by treatment with CRO *per se* group, which had an impact akin to that of control group. Rats exposed to VPA exhibited reduced levels on the aforementioned parameters upon receiving CRO treatment. The MDA and NO levels in the low (* p < 0.05) and high dose (*** p > 0.001, ** p > 0.01) CRO treated groups depicted statistically significant reduction (Table 3a).

On PND 41, rats treated with VPA exhibited a decline (#### p < 0.001) in the measure of GSH, SOD, and catalase, indicating significantly high amount of free radical production (Table 3b). Antioxidant levels were not significantly impacted by CRO *per se* treatment; however, groups who received CRO I showed moderate level of GSH (** p < 0.01), SOD (* p < 0.05) and catalase (* p < 0.05). CRO II depicted a high level of GSH (*** p < 0.001), SOD (** p < 0.01) and catalase (*** p < 0.001) than VPA exposed group.

DISCUSSION

The research confirmed a strong protective response against symptoms induced by VPA in the animals treated with CRO. When considering the context of maturation growth and development, we found that CRO depicted a non-significant gain in weight. It has been proposed that crocin might be used to treat CNS diseases. The outcomes showed that crocin treatment might lessen the quantity of marbles buried. Crocin may operate as an antagonist of 5-HT_{2C} receptors, while the precise pharmacological mechanism for how it affects repeated behavior is yet not understood.³¹ Because of its significant antioxidant activity, crocin displays neuropsychiatric effects³².

The development and functionality of the cerebellum are linked with motor skills. Animals exposed to VPA exhibited

Table 3b: Effect of crocin on GSH, SOD and Catalase levels in VPA and CRO treated groups

Groups	Antioxidant Parameters (Mean \pm SD)		
(PND 41)	GSH (μ g /mL of protein)	SOD (U/mg of protein)	Catalase (U/mg of protein)
Control	45.21 \pm 9.38	22.40 \pm 6.51	26.69 \pm 4.42
VPA group	11.44 \pm 6.69####	4.81 \pm 3.02####	9.50 \pm 2.73####
Crocin (per se)	44.66 \pm 8.67	22.38 \pm 8.80	25.84 \pm 7.48
VPA + Crocin I	33.16 \pm 8.58**	16.01 \pm 2.72*	18.27 \pm 3.82*
VPA + Crocin II	38.70 \pm 9.85***	18.82 \pm 4.99**	23.07 \pm 2.83***

VPA: Valproic acid and CRO: Crocin I (50 mg/kg) II (100 mg/kg); PND, Post-natal Day, GSH, Glutathione; SOD, Superoxide dismutase [Control compared with VPA exposed group (#### p < 0.001); VPA exposed group compared with CRO I and CRO II (* p < 0.05, ** p < 0.01 and *** p < 0.001)].

considerably delayed righting reflexes, an unsteady gait, notable challenges in navigating static beams, and impaired motor coordination on the rota rod. The most popular method for evaluating the progression of motor skills in rats is called negative geotaxis, which measures their capacity to navigate on an inclined surface.²⁴ The longer time needed for re-orientation on the slanted surface in animals treated with VPA may be due to the negative impact of VPA on the hippocampal and cerebellar maturation.¹⁴ Administration of CRO improved the motor deficiencies in mid-air righting and trimmed the re-orientation time from PND 17 onward. The primary bioactive ingredient in saffron, crocin, is beneficial to the neurological system. Its neuroprotective action demonstrates crocin's effects on reflexive motor function³³ and anti-depressive behaviors.^{34,35}

Mid-air righting is a systematic process used to evaluate motor coordination, shown on PND 13. Motor deficits are demonstrated by the decline in the proportion of animals that can right themselves in mid-air after exposure to VPA.³⁶ Given that the occurrence of righting in mid-air is significantly associated with cerebellar maturation,^{33,37} CRO may have improved the motor performance by protecting cerebellar structures.

The cerebellum is primarily responsible for the regulation of muscle coordination, and autism is plainly manifested as motor inefficiency, which is easily measured with a rota rod apparatus.^{38,39} It is well-established that exposure to VPA damages the cerebellum, which might explain why a lesser number of rats were retained on the rotating rod. However, the latency to fall off the rotating rod was prolonged in CRO-treated rats. The CRO-treated rats had improved motor activity as seen by a decrease in the inability of the muscles to coordinate, which may have been brought about by protecting the cerebellum tissue from damage. The results confirm earlier studies that found CRO to be protective against behavioral defects in rodents (anxiolytic, improving motor function).^{40,41}

Drugs that treat anxiety are evaluated for effectiveness using protocols such as the elevated plus maze and open field.

It is thought that post-natal intervention with VPA causes anxiety and increased apprehension, which is similar to the symptoms seen in individuals with autism²⁴ VPA can damage the neurological anatomy of the brain, altering related activities and impacting fear-related neural networks, particularly the amygdala, leading to anxiety-related behavior.⁴² After CRO treatment, the duration of time spent and entries into open arms significantly increased, suggesting a significant reduction in fear and anxiety. The analysis of post-CRO treatment indicates well-regulated stereotypical locomotion and enhanced exploratory behavior.^{43,44}

Inadequate neural circuits in the hippocampus are responsible for the lack of spatial organization abilities & learning behavior shown in autistic children.⁴⁵ It was discovered that hippocampal lesions affected a person's ability to navigate mazes.¹³ Memory loss is another effect of oxidative stress brought on by VPA-induced neurotoxicity. Additionally, giving VPA to PND 14, may result in dendritic atrophy of the hippocampus's memory-processing structures. The delay to reach the concealed stage in Morris water maze was enhanced in the current investigation by the injection of VPA.⁴⁶ Crocin displayed a reduction in escape latency and distance traveled. It also improved the animal's presence in the maze's target quarter. Several previous investigations have shown that CRO and other equivalent herbal substances can enhance learning and memory. Administration of CRO enhanced the navigation abilities of VPA-treated animals, which may be due to its ability to mitigate cognitive impairment.¹⁸

In line with earlier findings, rats treated with VPA showed decreased social activity, which may have been caused by a reduced potential for social behavior and the expression and understanding of intra-specific stimuli.^{13,22} VPA can cause behavioral alterations in rats that are thought to resemble autism's social abnormalities. Previous research indicates that crocin suppresses glutamatergic synaptic communication in brain and decreases extracellular glutamate concentrations.⁴⁷ Crocin also reduced behavioral consequences linked to NMDA receptor hypofunction, evoking a functional relationship between crocin and the glutamatergic system.⁴¹ CRO's neuroprotective, anti-anxiety, and glutamate release prevention properties may contribute to its capacity to mitigate the negative consequences underlying behavioral changes.

A significant factor contributing to neuronal abnormalities in ASD is increase in oxidative stress. Post-natal exposure to VPA triggers the generation of various oxidants and free radicals, coupled with a reduction in reduced glutathione. Such process leads to lipid peroxidation, degradation of protein, and eventual neuronal degeneration, particularly affecting the vulnerability of neurons during early developmental stages.⁴⁸ VPA-induced autism is linked to both oxidative and nitrosative damage. Lipids, proteins, and DNA damage during early stages of neuronal formation due to reactive oxygen species (ROS), which increases the vulnerability of the neuron to oxidative stress and disturbs the neurodevelopmental process.⁴⁹ Crocin may function as a neuroprotective antioxidant, and these findings are in line with prior field investigations on this

compound.^{34,50} In the current investigation, we found that total nitrite and MDA levels increased while glutathione levels declined, supporting the hypothesis that ASD caused by VPA is influenced by oxidative stress. Due to its antioxidant properties, CRO treatment drastically lowered the levels of TBARS and rectified the impaired oxidative stress indicators.^{51,52} Microglial activation is believed to be a rapid cellular reaction to inflammation. Cytotoxic mediators, like NO, are produced when microglia are activated, and these substances may help the nervous system grow. Elevated nitrite concentrations have been connected to autism. Oxidative stress was lessened when MDA and nitrite levels were lowered using CRO treatment.^{53,54} According to the study, crocin shows great promise in preventing behavioral abnormalities and oxidative stress brought on by VPA. Because of its remarkable antioxidant properties, it is a viable therapeutic option for ASD treatment.

CONCLUSION

The outcomes strongly indicate that crocin possesses robust potential in safeguarding against oxidative stress and behavioral impairments induced by VPA, attributed to its noteworthy antioxidant capabilities. By mitigating the consequences of anxiety and motor coordination deficits triggered by VPA, crocin demonstrated a protective effect on neurons. This therapeutic approach may yield positive outcomes and prove more efficacious in autism treatment, given its restoration of oxidative balance. The study's results provide promising data, justifying further investigation of the bioactive component's mechanism of action and exploring the ASD etiology for potential treatment avenues involving crocin.

ACKNOWLEDGMENT

None

AUTHORSHIP CONTRIBUTION

Sagarika Majhi - Writing original draft, Investigation and Data analysis. Sokindra Kumar - Supervision.

REFERENCES

1. Rossignol DA, Bradstreet JJ. Evidence of Mitochondrial Dysfunction in Autism and Implications for Treatment. *Am J Biochem Biotechnol* [Internet]. 2008 Jun 30 [cited 2023 Apr 25];4(2):208–17. Available from: <https://thescipub.com/abstract/ajbbsp.2008.208.217>
2. Alshammary RAA, Jouda J, Al-Salihy AAS. Some Immunological and Physiological Parameters Levels in Iraq Children with Autism Spectrum Disorders. *International Journal of Drug Delivery Technology*. 2023 Sep 25;13(03):955–60.
3. Autism Spectrum Disorder - National Institute of Mental Health (NIMH) [Internet]. [cited 2024 Jan 10]. Available from: <https://www.nimh.nih.gov/health/topics/autism-spectrum-disorders-asd>
4. Volkmar FR, Pauls D. Autism. *Lancet* [Internet]. 2003 Oct 4 [cited 2023 Apr 25];362(9390):1133–41. Available from: <https://pubmed.ncbi.nlm.nih.gov/14550703/>
5. Gerlai R, Gerlai J. Autism: A target of pharmacotherapies? *Drug Discov Today* [Internet]. 2004 Apr 15 [cited 2023 Apr 25];9(8):366–74. Available from: <https://pubmed.ncbi.nlm.nih.gov/14550703/>

- gov/15081964/
6. Llana DC, DeLuke S V., Batista M, Crawley JN, Christodulu K V., Frye CA. Communication, interventions, and scientific advances in autism: a commentary. *Physiol Behav* [Internet]. 2010 Jun [cited 2023 Apr 25];100(3):268–76. Available from: <https://pubmed.ncbi.nlm.nih.gov/20093134/>
 7. Ardinger HH, Atkin JF, Blackston RD, Elsas LJ, Clarren SK, Livingstone S, et al. Verification of the fetal valproate syndrome phenotype. *Am J Med Genet* [Internet]. 1988 [cited 2023 Apr 25];29(1):171–85. Available from: <https://pubmed.ncbi.nlm.nih.gov/3125743/>
 8. Data & Statistics on Autism Spectrum Disorder | CDC [Internet]. [cited 2024 Jan 10]. Available from: <https://www.cdc.gov/ncbddd/autism/data.html>
 9. Moore SJ, Turnpenny P, Quinn A, Glover S, Lloyd DJ, Montgomery T, et al. A clinical study of 57 children with fetal anticonvulsant syndromes. *J Med Genet* [Internet]. 2000 [cited 2024 Jan 10];37(7):489–97. Available from: <https://pubmed.ncbi.nlm.nih.gov/10882750/>
 10. Christensen J, Grnøborg TK, Srøensen MJ, Schendel D, Parner ET, Pedersen LH, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA* [Internet]. 2013 Apr 24 [cited 2024 Jan 10];309(16):1696–703. Available from: <https://pubmed.ncbi.nlm.nih.gov/23613074/>
 11. Bromley RL, Mawer GE, Briggs M, Cheyne C, Clayton-Smith J, García-Fiñana M, et al. The prevalence of neurodevelopmental disorders in children prenatally exposed to antiepileptic drugs. *J Neurol Neurosurg Psychiatry* [Internet]. 2013 [cited 2024 Jan 10];84(6):637–43. Available from: <https://pubmed.ncbi.nlm.nih.gov/23370617/>
 12. Williams G, King J, Cunningham M, Stephan M, Kerr B, Hersh JH. Fetal valproate syndrome and autism: additional evidence of an association. *Dev Med Child Neurol* [Internet]. 2001 Mar 1 [cited 2023 Apr 25];43(3):202–6. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8749.2001.tb00188.x>
 13. Wagner GC, Reuhl KR, Cheh M, McRae P, Halladay AK. A new neurobehavioral model of autism in mice: pre- and post-natal exposure to sodium valproate. *J Autism Dev Disord* [Internet]. 2006 Aug [cited 2022 Jun 28];36(6):779–93. Available from: <https://pubmed.ncbi.nlm.nih.gov/16609825/>
 14. Yochum CL, Dowling P, Reuhl KR, Wagner GC, Ming X. VPA-induced apoptosis and behavioral deficits in neonatal mice. *Brain Res* [Internet]. 2008 Apr 8 [cited 2023 Apr 25];1203:126–32. Available from: <https://pubmed.ncbi.nlm.nih.gov/18316065/>
 15. Majhi S, Kumar S, Singh L. A Review on Autism Spectrum Disorder: Pathogenesis, Biomarkers, Pharmacological and Non-Pharmacological Interventions. *CNS Neurol Disord Drug Targets* [Internet]. 2023 Apr 30 [cited 2023 Jul 21];22(5):659–77. Available from: <https://pubmed.ncbi.nlm.nih.gov/36915952/>
 16. Skladnev N V., Johnstone DM. Neuroprotective properties of dietary saffron: more than just a chemical scavenger? *Neural Regen Res* [Internet]. 2017 Feb 1 [cited 2023 Jan 5];12(2):210–1. Available from: <https://pubmed.ncbi.nlm.nih.gov/28400798/>
 17. Elsherbiny NM, Salama MF, Said E, El-Sherbiny M, Al-Gayyar MMH. Crocin protects against doxorubicin-induced myocardial toxicity in rats through down-regulation of inflammatory and apoptic pathways. *Chem Biol Interact* [Internet]. 2016 Mar 5 [cited 2023 Jan 5];247:39–48. Available from: <https://pubmed.ncbi.nlm.nih.gov/26807765/>
 18. Hossainzadeh H, Sadeghnia HR, Ghaeni FA, Motamedshariaty VS, Mohajeri SA. Effects of saffron (*Crocus sativus* L.) and its active constituent, crocin, on recognition and spatial memory after chronic cerebral hypoperfusion in rats. *Phytother Res* [Internet]. 2012 Mar [cited 2023 Jan 5];26(3):381–6. Available from: <https://pubmed.ncbi.nlm.nih.gov/21774008/>
 19. Vahdati Hassani F, Naseri V, Razavi BM, Mehri S, Abnous K, Hossainzadeh H. Antidepressant effects of crocin and its effects on transcript and protein levels of CREB, BDNF, and VGF in rat hippocampus. *Daru* [Internet]. 2014 Jan 8 [cited 2023 Jan 5];22(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/24401376/>
 20. Xiao Q, Shu R, Wu C, Tong Y, Xiong Z, Zhou J, et al. Crocin-I alleviates the depression-like behaviors probably via modulating “microbiota-gut-brain” axis in mice exposed to chronic restraint stress. *J Affect Disord* [Internet]. 2020 Nov 1 [cited 2023 Jan 5];276:476–86. Available from: <https://pubmed.ncbi.nlm.nih.gov/32871679/>
 21. Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology (Berl)* [Internet]. 2009 Jun [cited 2023 Jan 5];204(2):361–73. Available from: <https://pubmed.ncbi.nlm.nih.gov/19189082/>
 22. Banji D, Banji OJF, Abbagoni S, Hayath MS, Kambam S, Chiluka VL. Amelioration of behavioral aberrations and oxidative markers by green tea extract in valproate induced autism in animals. *Brain Res* [Internet]. 2011 Sep 2 [cited 2023 Jan 5];1410:141–51. Available from: <https://pubmed.ncbi.nlm.nih.gov/21820650/>
 23. Bakshi V, Sunand K, Begum N, Kakalij RM, Tekula MR. Neuroprotective Effect of Resveratrol on Valproic Acid induced Oxidative Stress Autism in Swiss Albino Mice. *International Journal of Pharmaceutical Sciences and Drug Research* [Internet]. 2018 May 1 [cited 2023 Jan 5];10(03):103–10. Available from: <https://www.ijpsdr.com/index.php/ijpsdr/article/view/615>
 24. Schneider T, Przewłocki R. Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology* [Internet]. 2005 Jan [cited 2023 Jan 5];30(1):80–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/15238991/>
 25. Barendse EM, Hendriks MP, Jansen JF, Backes WH, Hofman PA, Thoonen G, et al. Working memory deficits in high-functioning adolescents with autism spectrum disorders: neuropsychological and neuroimaging correlates. *J Neurodev Disord* [Internet]. 2013 Dec [cited 2023 Jan 5];5(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/23731472/>
 26. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* [Internet]. 1984 [cited 2023 Jan 5];11(1):47–60. Available from: <https://pubmed.ncbi.nlm.nih.gov/6471907/>
 27. Restall G, Magill-Evans J. Play and preschool children with autism. *Am J Occup Ther* [Internet]. 1994 [cited 2023 Jan 5];48(2):113–20. Available from: <https://pubmed.ncbi.nlm.nih.gov/8017504/>
 28. Byrge L, Dubois J, Tyszka JM, Adolphs R, Kennedy DP. Idiosyncratic brain activation patterns are associated with poor social comprehension in autism. *J Neurosci* [Internet]. 2015 Apr 8 [cited 2023 Jan 5];35(14):5837–50. Available from: <https://pubmed.ncbi.nlm.nih.gov/25855192/>
 29. Kumar H, Sharma BM, Sharma B. Benefits of agomelatine in behavioral, neurochemical and blood brain barrier alterations in prenatal valproic acid induced autism spectrum disorder. *Neurochem Int* [Internet]. 2015 Dec 1 [cited 2023 Jan 5];91:34–45. Available from: <https://pubmed.ncbi.nlm.nih.gov/26498253/>
 30. Singh P, Gupta S, Sharma B. Melatonin receptor and KATP

- channel modulation in experimental vascular dementia. *Physiol Behav* [Internet]. 2015 Apr 1 [cited 2023 Jan 5];142:66–78. Available from: <https://pubmed.ncbi.nlm.nih.gov/25659733/>
31. Georgiadou G, Tarantilis PA, Pitsikas N. Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of obsessive-compulsive disorder. *Neurosci Lett* [Internet]. 2012 Oct 18 [cited 2023 May 2];528(1):27–30. Available from: <https://pubmed.ncbi.nlm.nih.gov/22985509/>
 32. Lopresti AL, Drummond PD. Saffron (*Crocus sativus*) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. *Hum Psychopharmacol* [Internet]. 2014 Nov 1 [cited 2023 May 2];29(6):517–27. Available from: <https://pubmed.ncbi.nlm.nih.gov/25384672/>
 33. Taheri-Amlashi M, Hassanpour S, Vazir B. Effects of the prenatal exposure to crocin in the expression of withdrawal syndrome on reflexive motor behaviors in mice offspring's. *Neurosci Lett* [Internet]. 2022 Jan 1 [cited 2023 May 2];766. Available from: <https://pubmed.ncbi.nlm.nih.gov/34788677/>
 34. Altinoz E, Oner Z, Elbe H, Vardi N. Neuro-protective effects of crocin on brain and cerebellum tissues in diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines* [Internet]. 2015 Apr 9 [cited 2023 May 2];11(6):33–9. Available from: <https://www.ajol.info/index.php/ajtcam/article/view/115422>
 35. Tashakori A, Hassanpour S, Vazir B. Protective effect of crocin on cuprizone-induced model of multiple sclerosis in mice. *Naunyn-Schmiedeberg's Arch Pharmacol* [Internet]. 2023 [cited 2023 May 2]; Available from: <https://pubmed.ncbi.nlm.nih.gov/36805765/>
 36. Markram K, Rinaldi T, Mendola D La, Sandi C, Markram H. Abnormal fear conditioning and amygdala processing in an animal model of autism. *Neuropsychopharmacology* [Internet]. 2008 Mar [cited 2023 Apr 21];33(4):901–12. Available from: <https://pubmed.ncbi.nlm.nih.gov/17507914/>
 37. Yuan Y, Shan X, Men W, Zhai H, Qiao X, Geng L, et al. The effect of crocin on memory, hippocampal acetylcholine level, and apoptosis in a rat model of cerebral ischemia. *Biomed Pharmacother* [Internet]. 2020 Oct 1 [cited 2023 May 2];130. Available from: <https://pubmed.ncbi.nlm.nih.gov/32738637/>
 38. Fisher BE, Boyd L, Winstein CJ. Contralateral cerebellar damage impairs imperative planning but not updating of aimed arm movements in humans. *Exp Brain Res* [Internet]. 2006 Oct [cited 2023 May 3];174(3):453–66. Available from: <https://pubmed.ncbi.nlm.nih.gov/16741716/>
 39. Fine EJ, Ionita CC, Lohr L. The history of the development of the cerebellar examination. *Semin Neurol* [Internet]. 2002 Dec [cited 2023 May 3];22(4):375–84. Available from: <https://pubmed.ncbi.nlm.nih.gov/12539058/>
 40. Hosseinzadeh H, Noraei NB. Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract and its constituents, crocin and safranal, in mice. *Phytother Res* [Internet]. 2009 Jun [cited 2023 May 3];23(6):768–74. Available from: <https://pubmed.ncbi.nlm.nih.gov/19142981/>
 41. Georgiadou G, Grivas V, Tarantilis PA, Pitsikas N. Crocins, the active constituents of *Crocus sativus* L., counteracted ketamine-induced behavioural deficits in rats. *Psychopharmacology (Berl)* [Internet]. 2014 Feb [cited 2023 May 3];231(4):717–26. Available from: <https://pubmed.ncbi.nlm.nih.gov/24096536/>
 42. Anand A, Shekhar A. Brain imaging studies in mood and anxiety disorders: special emphasis on the amygdala. *Ann N Y Acad Sci* [Internet]. 2003 [cited 2023 Apr 21];985:370–88. Available from: <https://pubmed.ncbi.nlm.nih.gov/12724172/>
 43. Pitsikas N, Zisopoulou S, Tarantilis PA, Kanakis CD, Polissiou MG, Sakellaridis N. Effects of the active constituents of *Crocus sativus* L., crocins on recognition and spatial rats' memory. *Behavioural brain research* [Internet]. 2007 Nov 2 [cited 2023 May 3];183(2):141–6. Available from: <https://pubmed.ncbi.nlm.nih.gov/17628713/>
 44. Pitsikas N, Boultsadakis A, Georgiadou G, Tarantilis PA, Sakellaridis N. Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of anxiety. *Phytomedicine* [Internet]. 2008 Dec [cited 2023 May 3];15(12):1135–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/18693098/>
 45. Natori T, Kodaira F, Hirasawa T, Gao YY, Nagai K. Augmentation of polysialic acid by valproic acid in early post-natal mouse hippocampus and primary cultured hippocampal neurons. *J Biosci Bioeng* [Internet]. 2008 Feb [cited 2023 May 3];105(2):164–7. Available from: <https://pubmed.ncbi.nlm.nih.gov/18343346/>
 46. Wang C, Luan Z, Yang Y, Wang Z, Cui Y, Gu G. Valproic acid induces apoptosis in differentiating hippocampal neurons by the release of tumor necrosis factor- α from activated astrocytes. *Neurosci Lett* [Internet]. 2011 Jun 22 [cited 2023 May 3];497(2):122–7. Available from: <https://pubmed.ncbi.nlm.nih.gov/21543053/>
 47. Berger F, Hensel A, Nieber K. Saffron extract and trans-crocetin inhibit glutamatergic synaptic transmission in rat cortical brain slices. *Neuroscience* [Internet]. 2011 Apr 28 [cited 2023 May 3];180:238–47. Available from: <https://pubmed.ncbi.nlm.nih.gov/21352900/>
 48. Chauhan A, Chauhan V. Oxidative stress in autism. *Pathophysiology* [Internet]. 2006 Aug [cited 2023 Apr 21];13(3):171–81. Available from: <https://pubmed.ncbi.nlm.nih.gov/16766163/>
 49. Sandhya T, Sowjanya J, Veeresh B. *Bacopa monniera* (L.) Wettst ameliorates behavioral alterations and oxidative markers in sodium valproate induced autism in rats. *Neurochem Res* [Internet]. 2012 May [cited 2023 Mar 28];37(5):1121–31. Available from: <https://pubmed.ncbi.nlm.nih.gov/22322665/>
 50. Kermanshahi S, Ghanavati G, Abbasi-Mesrabadi M, Gholami M, Ulloa L, Motaghinejad M, et al. Novel Neuroprotective Potential of Crocin in Neurodegenerative Disorders: An Illustrated Mechanistic Review. *Neurochem Res* [Internet]. 2020 Nov 1 [cited 2023 May 4];45(11):2573–85. Available from: <https://pubmed.ncbi.nlm.nih.gov/32940861/>
 51. Rajaei Z, Hadjzadeh MAR, Nemati H, Hosseini M, Ahmadi M, Shafiee S. Antihyperglycemic and antioxidant activity of crocin in streptozotocin-induced diabetic rats. *J Med Food* [Internet]. 2013 Mar 1 [cited 2023 May 4];16(3):206–10. Available from: <https://pubmed.ncbi.nlm.nih.gov/23437790/>
 52. Chen Y, Zhang H, Tian X, Zhao C, Cai L, Liu Y, et al. Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents. *Food Chem*. 2008 Aug 1;109(3):484–92.
 53. Knott C, Stern G, Wilkin GP. Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. *Mol Cell Neurosci* [Internet]. 2000 [cited 2023 May 4];16(6):724–39. Available from: <https://pubmed.ncbi.nlm.nih.gov/11124893/>
 54. Nam KN, Park YM, Jung HJ, Lee JY, Min BD, Park SU, et al. Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. *Eur J Pharmacol* [Internet]. 2010 Dec 1 [cited 2023 May 4];648(1–3):110–6. Available from: <https://pubmed.ncbi.nlm.nih.gov/20854811/>