RESEARCH ARTICLE

Crocin's Protective Effect Against Valproate-Exposed Model of Autism Spectrum Disorder: A Histological and Oxido-Inflammatory Investigation

Sagarika Majhi[1*, 2,] Lubhan Singh[2]
[1]I. T. S College of Pharmacy, Ghaziabad, Uttar Pradesh, India
[2]Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India
Corresponding Author Email ID: sagarika.majhi@gmail.com

Received: 19/02/2025 **Accepted:** 08/03/2025 **Published:** 15/04/2025

Abstract

Background: Autism spectrum disorder (ASD), is shown to be associated with oxidative stress and damage to neurons induced by valproic acid (VPA) in a postnatal animal model. Crocin (CRO) was considered to have potential advantages in ASD due to its putative anti-inflammatory, Nrf2 (nuclear factor erythroid 2–related factor 2) stimulation, and gut microbiota alteration capabilities.

Objective: The study aimed to investigate the histological and biochemical alterations associated with crocin treatment in an ASD model induced by valproic acid (VPA). By examining both the structural and molecular changes in affected brain tissues, the therapeutic potential of crocin in mitigating the adverse effects of VPA exposure was studied.

Methods: Wistar albino rat, aged thirteen days, were randomly assigned to five groups of six each. VPA was effectively used to cause autism on PND 14 with a single 400 mg/kg s.c. dosage. Crocin was given out every day between PND 14 and 40. After the study concluded, the animals were euthanized for oxido-inflammatory estimations and histopathological investigation.

Results: Following administration of VPA, a rise in Ca^{2+} and myeloperoxidase level was observed. Also, the inflammatory markers *viz.* IL-6, TNF-α level increased and BDNF, CREB and IL-10 level was decreased along with mitochondrial complexes. Intervention with crocin considerably improved the histological and inflammatory alterations in rodents contrasted with those in the VPA-exposed category. Increasing the dosage of crocin had more noticeable effects, suggesting that the autistic deficiencies induced by VPA may be reversed.

Conclusion: Crocin may be helpful in the treatment of ASD because it has neuronal cytoprotective properties, possibly as a result of its anti-inflammatory properties. Future studies are required to completely comprehend the biological mechanism of action of crocin.

Keywords: Autism spectrum disorder (ASD), Valproic acid (VPA), Crocin (CRO), Oxido-inflammatory stress, Neuroprotective activity, Postnatal Day (PND).

Journal of Applied Pharmaceutical Sciences and Research, (2025); DOI: 10.31069/japsr.v8i1.04

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by challenges in social interaction, communication, and repetitive behaviours. Its aetiology is believed to involve a multifaceted interplay of environmental factors and genetic predispositions. The intricate interaction between environmental and genetic factors contributes to the heterogeneous nature of ASD, making it challenging to pinpoint specific causative factors [1]. Ongoing research efforts aim to further elucidate the complex aetiology of ASD, identify biomarkers for early detection, and develop personalized interventions tailored to the unique needs of individuals with ASD [2]. By gaining insights into the underlying causes of ASD, researchers hope to improve diagnostic accuracy, enhance treatment options, and ultimately improve outcomes for individuals affected by the disorder [3].

Studies have also indicated a higher prevalence of autism among children exposed to valproic acid (VPA), with approximately 1 in 36 children affected by the disorder. The association between prenatal VPA exposure and autism highlights the potential teratogenic effects of the drug and underscores the importance of cautious use and thorough risk assessment when prescribing VPA to pregnant individuals. It emphasizes the need for further research to elucidate the underlying mechanisms and to develop strategies for mitigating these risks in clinical practice [4,5]. Pregnant rodents exposed to VPA experience neurological effects and aberrant behaviours resembling the symptoms of autism [6]. Additionally, giving VPA to mice on PND14 causes autism-like symptoms, including impaired motor and cognitive functions, the advent of intrusive behaviours, and ongoing deficiencies in the social connection development [7]. Adults did not exhibit this impact, indicating that PND14

is within the critical developmental window for brain damage caused by VPA [8].

Crocus sativus L., commonly known as saffron or crocus, belongs to the family Iridaceae and is recognized for its medicinal properties. Among its bioactive constituents is crocin (CRO), a compound with therapeutic potential. Studies have shown that dietary polyphenols and plant-based medications, including those derived from saffron, possess neuroprotective effects and may offer treatment options for neurodevelopmental disorders like autism [9]. These compounds have been found to inhibit inflammatory responses and neuronal cell death while promoting neurogenesis and neuroplasticity in the brain. Numerous pharmacological activities of crocin have been demonstrated through recent research, such as its anti-inflammatory [10] and neuroprotective properties [11]. Furthermore, oral crocin therapy improved the function of the intestinal barrier, altered the composition of gut microbiota, and decreased neuroinflammation. These actions effectively decreased animal behaviour suggestive of depression [12].

There is growing recognition that early dietary modification may have an impact on the onset of autism spectrum disorder (ASD). One area of research is the possible health advantages of carotenoids, namely their neuroprotective properties. This led to the notion that the carotenoid component crocin, which is present in saffron, would be able to lessen behavioural regressions in rodents that are caused by postnatal exposure to valproic acid (VPA). Given its established qualities in these domains, the evaluation of crocin's neuroprotective effectiveness focused on its anti-inflammatory processes. Crocin was investigated for its ability to reverse the neurodevelopmental defects linked to ASD-like behaviours brought on by VPA administration by focusing on oxidative stress and inflammation.

Materials and Methods

Experimental protocol

Swami Vivekanand Subharti University's Institutional Animal Ethical Committee accepted the experimentation techniques, which complied with CCSEA. (Approved Protocol No. 1204/PO/Re/S/08/CPCSEA/22-01). The study used thirty healthy male wistar albino rats (weighing between thirty and forty grammes) kept in vented animal rooms with a 12-hour light/dark cycle, 24 ± 2°C temperature, and 60 ±5% humidity. The required number of animals were selected as per power analysis and previous literature survey. As previously reported [7], the rat was given a single subcutaneous injection of 400 mg/kg of valproic acid (VPA) (Sigma-Aldrich) in saline (vehicle) on PND14. The rats were split into various groups *viz.* I (Control), II (VPA-exposed), III (Crocin *per se*), IV (VPA + Crocin I 50mg/kg), V (VPA + Crocin II 100mg/kg). From PND 14 to 40, CRO administration once a day was performed. Further on specific date, anti-inflammatory and histological investigation was carried out.

Dissection and homogenization

Animals were euthanized and each brain was removed and weighed separately. Subsequently, the homogenizer was used to produce a 10% w/v homogenization of brain in 0.1 M phosphate buffer (pH 7.4). The resulting mixture was centrifuged for 15 minutes at 4°C and 3000 rpm/10,000g. After being separated, the supernatant was employed as oxido-inflammatory stress indicators in further research.

Determination of calcium (Ca^{+2}) levels

An auto analyzer (RMSBCA 201) was used to determine the calcium (Ca^{+2}) levels throughout the entire brain. By reacting Ca^{+2} with O-Cresolphthalein Complexone in an alkaline media, the absorbance was measured, which is directly proportional to Ca^{+2} content. The purple colour complex is formed in this approach (OCPC) for the measurement of calcium. The test was conducted in accordance with scientific recommendations [13].

Determination of myeloperoxidase (MPO)

Using a modified spectroscopic technique, the MPO activity at 460 nm was evaluated. Brain tissue was homogenised using a 50 mM potassium phosphate buffer (pH = 6) containing 0.5% of hexadecyl trimethyl ammonium bromide. The homogenate was centrifuged at 11,000 g for 20 minutes at 4°C after freezing. The supernatant (34 µl) received the same volume of phosphate buffer (0.0005% hydrogen peroxide, 0.167 mg/ml (986 µl) ortho-dianisidine dihydrochloride). One unit of MPO activity was defined as 1 nM of peroxide used per minute at 22°C. The protein values were expressed in units/mg [14].

Determination of mitochondrial complex I (NADH dehydrogenase) activity

Complex I, or NADH dehydrogenase activity, was evaluated by spectrophotometric analysis (UV-1800). Cytochrome-C is reduced throughout the process when NADH is catalytically oxidised to NAD+. The reaction starts when the required amount of solubilized mitochondrial substance is added to the reaction mixture. The alteration in absorbance at 550 nm was noted for two minutes [15].

Determination of mitochondrial complex II (succinate dehydrogenase—SDH) activity

SDH was subjected to spectrophotometric examination with a UV-1800. A synthetic acceptor of electrons called potassium ferricyanide is employed in the procedure to oxidise succinate. The procedure was initiated by adding the brain mitochondrial extract to the reaction mixture, and the shift in absorbance at 420 nm was recorded for two minutes [15].

Determination of mitochondrial complex IV (cytochrome oxidase) activity

It was determined how much cytochrome oxidase was active in the brain's mitochondria. The chemical reaction was initiated by adding the solubilized brain mitochondrial

material to the reaction mixture, and the resulting shift in absorbance at 550 nm was observed spectrophotometrically for a duration of two minutes [15].

Determination of inflammatory parameters

With RayBio® Rat ELISA kits, estimations of CERB, BDNF, IL-10, TNF-α, and IL-6 levels will be computed. They were assessed utilising the brain›s enzyme-linked immunosorbent assay sandwich method. The extracts were processed in triplets for optical density metrics, according to the precise instructions provided in the product information booklet. The final estimate of the concentration took the mean optical density into account. Supernatant at 450 nm was used to quantify IL-6, IL-10, TNF-α, BDNF, and CREB on 96-well percolated to specific antibody plates. The findings were given in ng/ml and pg/ml [16].

Histopathological studies

To conduct histopathological analyses, five-micron-thick sagittal brain slices will be prepared and will be stained using hematoxylin and eosin (H&E). Following staining, the slices will be photographed under a light microscope, allowing for detailed examination of the tissue morphology. Specifically, the Purkinje cell layer, a key region implicated in various neurological conditions, will be scrutinized for any observable alterations or abnormalities. This method enables researchers to visually assess the impact of experimental interventions.

Statistical Analyses

To illustrate the data, the Mean ± standard deviation (SD) will be the statistic used employing Graph-pad prism 5. The tukey post-test will be implemented after the statistical significance has been ascertained using analysis of variance (ANOVA).

Results

Assessment of calcium (Ca^{+2}) level

When comparing the VPA exposed group to the naïve control group, a substantial increase in Ca^{+2} levels was seen as a result of oxidative and nitrosative stress ($^{###}P < 0.001$) (Table 1). Comparing the naïve control group to the administration of CRO alone revealed no discernible difference in outcome. In comparison to the VPA exposed group, the CRO treated group showed a significant dose-dependent outcome (*P < 0.05, **P < 0.01) by lowering the levels of Ca^{+2}, suggesting reduced oxidative damage.

Table 1: Effect of crocin on Calcium levels in VPA and CRO treated groups

Groups (PND 41)	*Calcium (µg/g of tissue)*
Naive control	54.72 ± 11.87
VPA group	90.27 ± 14.83$^{###}$
Crocin (per se)	54.32 ± 9.35
VPA + Crocin I	65.37 ± 12.05*
VPA + Crocin II	60.02 ± 11.00**

Values are expressed as Mean ± SD (One-way ANOVA + Tukey's Multiple Comparison Test) $^{###}P < 0.001$ comparison of naïve control vs. VPA group; *P < 0.05, **P < 0.01 comparison of VPA group vs. CRO I and CRO II groups on PND 41 (n = 6).

Assessment of brain mitochondrial complex I, II and IV Activities

Table 2 shows that the VPA exposed group had lower mitochondrial complex activity (Complexes I, II, and IV) than the naïve controls ($^{###}P < 0.001$). Treatment of CRO *per se* produced non-significant results when compared to the naïve control group. In a dose-dependent way, the administration of CRO considerably reduced the amount of dysfunction of mitochondria (*P < 0.05, **P < 0.01, ***P < 0.001).

Assessment of Myeloperoxidase (MPO)

Comparing the VPA exposed group to the naïve control group, Table 3a shows a substantial increase in MPO levels ($^{###}P < 0.001$). Comparing the naïve control group to the administration of CRO alone revealed no discernible disparity in outcome. In comparison to the VPA-treated group, the CRO-treated group showed a significant dose-dependent outcome (*P < 0.05, **P < 0.01) by lowering the levels of MPO, suggesting reduced oxidative damage.

Estimation of various inflammation parameters

BDNF, CREB, and IL-10 concentrations in the brains of the VPA-exposed group were significantly lower than those of the naïve controls ($^{###}P < 0.001$). In comparison to the naïve control, the administration of CRO *per se* had a non-significant outcome. The positive impact of CRO on the neuronal activity markers BDNF, CREB (*P < 0.05, **P < 0.01), and IL-10 (**P < 0.01, ***P < 0.001) were dose-dependent and significantly favourable (Table 3b).

Table 2: Effect of crocin on mitochondrial enzyme activities in VPA and CRO treated groups

Groups (PND 41)	*Mitochondrial enzyme complexes (% of control)*		
	Complex I	*Complex II*	*Complex IV*
Naive control	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
VPA group	48.67 ± 15.43$^{###}$	41.97 ± 13.77$^{###}$	50.40 ± 16.87$^{###}$
Crocin (per se)	98.87 ± 16.11	98.96 ± 20.61	98.58 ± 19.62
VPA + Crocin I	76.06 ± 9.26*	71.50 ± 8.87*	83.06 ± 11.38*
VPA + Crocin II	83.02 ± 18.69**	82.97 ± 16.02***	89.40 ± 15.69**

Values are expressed as Mean ± SD (One-way ANOVA + Tukey's Multiple Comparison Test) $^{###}P < 0.001$ comparison of naïve control vs. VPA group; *P < 0.05, **P < 0.01, ***P < 0.001 comparison of VPA group vs. CRO I and CRO II groups on PND 41 (n = 6).

Table 3a: Effect of crocin on MPO, IL-6 and TNF-α levels in VPA and CRO treated groups

Groups (PND 41)	*MPO (nM/min/mL)*	*IL-6 (pg/ml)*	*TNF-α (pg/ml)*
Naive control	2.79 ± 1.61	91.04 ± 12.34	42.60 ± 16.49
VPA group	11.79 ± 2.77$^{###}$	209.86 ± 24.41$^{###}$	140.94 ± 22.50$^{###}$
Crocin (per se)	2.78 ± 1.70	90.31 ± 12.01	43.96 ± 17.66
VPA + Crocin I	6.61 ± 2.22*	167.34 ± 18.67**	92.60 ± 23.16**
VPA + Crocin II	5.48 ± 2.32**	120.23 ± 19.23***	55.42 ± 17.49***

Values are expressed as Mean ± SD (One-way ANOVA + Tukey's Multiple Comparison Test) $^{###}$P < 0.001 comparison of naïve control vs. VPA group; *P < 0.05, **P < 0.01, ***P < 0.001 comparison of VPA group vs. CRO I and CRO II groups on PND 41 (n = 6).

Table 3b: Effect of crocin on BDNF, CREB and IL-10 levels in VPA and CRO treated groups

Groups (PND 41)	*Anti-inflammatory Parameters (Mean ± SD)*		
	BDNF (pg /mL)	*CREB (ng/mL)*	*IL-10 (pg/ml)*
Naive control	42.77 ± 14.02	1.62 ± 0.30	86.45 ± 15.37
VPA group	10.44 ± 10.68$^{##}$	0.61 ± 0.23$^{###}$	34.75 ± 12.88$^{###}$
Crocin (per se)	42.51 ± 13.70	1.54 ± 0.28	86.12 ± 14.14
VPA + Crocin I	33.44 ± 8.03*	1.18 ± 0.25*	71.52 ± 17.91**
VPA + Crocin II	39.97 ± 8.09**	1.33 ± 0.35**	78.33 ± 13.96***

Values are expressed as Mean ± SD (One-way ANOVA + Tukey's Multiple Comparison Test) $^{###}$P < 0.001 comparison of naïve control vs. VPA group; *P < 0.05, **P < 0.01, ***P < 0.001 comparison of VPA group vs. CRO I and CRO II groups on PND 41 (n = 6).

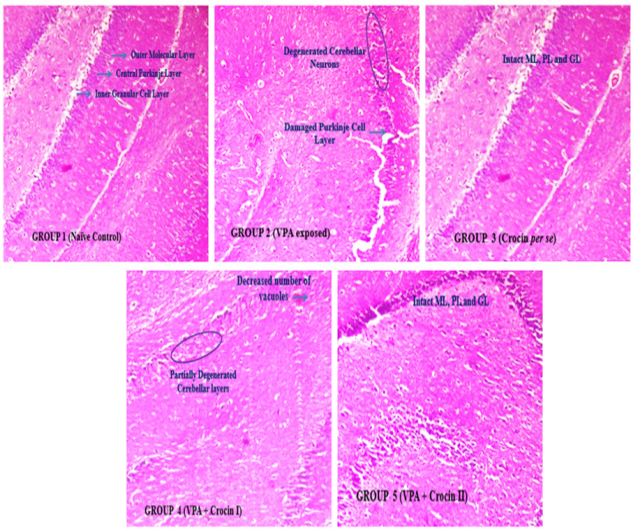

Figure 1: Effect of crocin on histopathology of brain in VPA-exposed and CRO treated groups.

When juxtaposed to the naïve group of controls, the inflammatory markers in the brains of rats exposed to VPA showed a substantial rise ($^{###}$P < 0.001) in the levels of IL-6 and TNF-α (Table 3a). The use of CRO in isolation did not significantly differ from the naïve control group. Regarding the aforementioned findings, it was found that, in comparison to the VPA group, the concentrations of IL-6 and TNF-α were dramatically lowering in a dose-dependent way (**P < 0.01, ***P < 0.001), suggesting that the use of CRO had decreased inflammation.

Histopathological evaluation

Examining the cerebellar areas of rats exposed to VPA revealed pronounced degeneration in key regions, including the middle Purkinje cell layer (PL), inner granular cell layer (GL), and molecular layer (ML). This degeneration suggests a significant impediment to the normal development of neurons within these regions. Interestingly, in rat neonates exposed to VPA and subsequently treated with crocin (CRO), the integrity of the PL appeared to be preserved, as depicted in Figure 1. Further analysis showed that CRO treatment led to a reduction in both the number and size of vacuoles compared to the VPA-exposed group. This finding suggests that CRO possesses the ability to protect against oxidative damage induced by VPA through robust antioxidant mechanisms. Moreover, the protective effects of CRO on Purkinje cell loss seemed to be dependent on its dosage, indicating a dose-dependent attribute of this phytoconstituent. In summary, the study highlights the potential neuroprotective effects of CRO in mitigating VPA-induced neuronal damage in the cerebellum, offering insights into its therapeutic potential for conditions associated with neurodevelopmental abnormalities such as ASD.

Discussion

The study verified a robust defence against VPA-exposed symptoms in the CRO-treated animals. An increase in the level of intracellular calcium causes aberrant neural circuit functioning in autism [17]. A rise in calcium levels may be caused by an overexpression of the NMDA receptor and surface transient receptor potential [18]. Rats treated with CRO showed a relative decrease in intracellular calcium levels as compared to rats given VPA. As a result, CRO inhibits neurological inflammation, which has been verified by earlier research [19,20].

Neuroinflammation is often characterised by a surge in the death of neuronal cells, glial cell activation [21], excessive inflammatory cytokine production, and an elevation in myeloperoxidase (MPO) values [22,23]. The findings of our investigation corroborated this, since MPO levels were exclusively increased in the VPA group. In CRO groups, there was a discernible decrease in MPO, which is consistent with other studies that showed their involvement in downregulating MPO and suppressing inflammation and oxidative stress [24].

The neuronal mitochondria are shielded by the effective antioxidant activity of crocin against VPA-induced autism. Our findings showed that CRO's antioxidant activity reduced the lipid peroxidation of mitochondrial membranes in the brain. It has been demonstrated in earlier investigations that crocin has a protective effect against lipid peroxidation and preserves the lipid membrane of cells. Additionally, the addition of CRO dramatically reduced the swelling of the mitochondria, indicating that oxidative stress plays a direct role in MMP disintegration [25,26].

It has been established that BDNF has a role in synaptic plasticity, neuronal rebuilding, and brain development [27]. Numerous studies have confirmed the role of BDNF in learning, memory, emotion recognition, perception, and regulation. Additionally, it has been researched how BDNF alteration might contribute to a variety of diseases [28]. One of the target genes of CREB is BDNF, which is responsible for regulating its gene expression [29]. CREB deactivation or reduction might lower BDNF gene expression. We demonstrated in the current study that crocin may greatly boost BDNF and CREB gene expression in the brain of VPA-treated rats, and this finding has been supported by earlier research [30,31]. Furthermore, according to earlier research, oral treatment of crocin enhanced the composition of the gut microbiota to restore SCFA ratios and the functioning of the intestinal barrier, thus, reducing neuroinflammation and elevating BDNF protein [12]. The blood-brain barrier is penetrated by proinflammatory cytokines, which then triggers microglia. Synaptic pruning is negatively impacted by dysfunctional microglia, which affects how neurons transmit signals and aids in the emergence of ASD [32]. Also, crocin appears to block TNF-α and IL-6 to suppress up-regulation of inflammatory mediators [33]. Crocin's protective properties may be linked to a decreased inflammatory response. Our findings, particularly show that crocin may hinder the progression of autism, would thus be highly significant due to its anti-inflammatory activities.

The histological analyses demonstrate that CRO has neuroprotective benefits by shielding Purkinje cells from valproate-induced impairment. In several motor activities, the animals who received VPA exhibited considerably worse performance. These findings imply that VPA exposure causes substantial cerebellar impairment and that employing CRO in animals to explore the changes in the cerebellum associated with ASD is a useful approach. The anxious conduct and performance deficits in VPA rats may be influenced by purkinje cell degeneration i.e., smaller Purkinje cells, shorter and less complex dendritic arbors. Purkinje cell degeneration might result from this, which would disrupt the corticocerebellar circuit. The result concludes with the acceptance of the hypothesis i.e., the probable neuroprotective role of crocin in VPA induced ASD in wistar albino rats.

Authors' previous research emphasized on the assessment of Berberine's potential in addressing autism spectrum disorder, and suggests a focus on exploring its therapeutic application [34]. In case of berberines' study, no prior pre-natal or any other studies on animal model have been performed. Thus, no affirmation and more studies are required to confirm the results of post-natal model of ASD. Previous studies on crocin shows its usage on pre-natal ASD rat model [35] with productive results. The behavioural effect of crocin on valproate induced ASD was also studied by the authors [36] and the current manuscript affirms the study with an additional study on antioxidant and anti-inflammatory effects of crocin. Also, with usage of different post-natal model of ASD, the study gives an advantage and doesn't compare the results between berberine and crocin, rather it affirms the usage of bioactive phytoconstituent for the treatment of autism spectrum disorder. Both Berberine and Crocin studies share similarities in their experimental design, particularly in the utilization of control and VPA-exposed groups, indicating similarities in methodology between the two investigations with different results.

Conclusion

Because crocin has high antioxidant and anti-inflammatory properties, all the studies discussed above suggest that it has considerable ability for safeguarding towards oxidative stress, behavioural impairments, and cerebellar degeneration induced by VPA. By diminishing the consequences of anxiety and the motor coordination deficits caused by VPA, it shielded the neurons. Since this treatment reestablished the oxidative balance, decreased mediators of inflammation, strengthened Purkinje cells in the cerebellum, and boosted cerebellar-related activities, it may have favourable benefits and be more helpful in treating autism. The results of the present research provide information that is positive enough to justify more investigation into the bioactive component's mode of action along with the genesis of ASD and potential therapeutic approaches employing crocin.

Acknowledgment

None

Sources of Support

The research was self-funded.

Author Agreement

The corresponding author ensures that all appropriate co-authors and no inappropriate co-authors are included on the paper, and that all co-authors have seen and approved the paper and have agreed to its submission for publication.

Credit Authorship Contribution Statement

Dr. Sagarika Majhi - Writing original draft, review & editing, Data curation, Formal analysis, Investigation. **Dr. Lubhan Singh -** Project administration, Supervision. All authors have read and approved the published version of the manuscript.

Author Declarations

Authors declare no conflicts of interest, including financial and non-financial.

References

1. Rossignol DA, Bradstreet JJ. Evidence of Mitochondrial Dysfunction in Autism and Implications for Treatment. Am J Biochem Biotechnol 2008;4:208–17. https://doi.org/10.3844/AJBBSP.2008.208.217.
2. Gerlai R, Gerlai J. Autism: A target of pharmacotherapies? Drug Discov Today 2004;9:366–74. https://doi.org/10.1016/S1359-6446(04)03039-9.
3. Llaneza DC, DeLuke S V., Batista M, Crawley JN, Christodulu K V., Frye CA. Communication, interventions, and scientific advances in autism: a commentary. Physiol Behav 2010;100:268–76. https://doi.org/10.1016/J.PHYSBEH.2010.01.003.
4. Data & Statistics on Autism Spectrum Disorder | CDC n.d. https://www.cdc.gov/ncbddd/autism/data.html (accessed January 10, 2024).
5. 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 2000;37:489–97. https://doi.org/10.1136/JMG.37.7.489.
6. 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 2001;43:202–6. https://doi.org/10.1111/J.1469-8749.2001.TB00188.X.
7. Wagner GC, Reuhl KR, Cheh M, McRae P, Halladay AK. A new neurobehavioral model of autism in mice: pre- and postnatal exposure to sodium valproate. J Autism Dev Disord 2006;36:779–93. https://doi.org/10.1007/S10803-006-0117-Y.
8. Yochum CL, Dowling P, Reuhl KR, Wagner GC, Ming X. VPA-induced apoptosis and behavioral deficits in neonatal mice. Brain Res 2008;1203:126–32. https://doi.org/10.1016/J.BRAINRES.2008.01.055.
9. Majhi S, Kumar S, Singh L. A Review on Autism Spectrum Disorder: Pathogenesis, Biomarkers, Pharmacological and Non-Pharmacological Interventions. CNS Neurol Disord Drug Targets 2023;22:659–77. https://doi.org/10.2174/1871527321666220428134802.
10. 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 2016;247:39–48. https://doi.org/10.1016/J.CBI.2016.01.014.
11. Hosseinzadeh 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 2012;26:381–6. https://doi.org/10.1002/PTR.3566.
12. 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 2020;276:476–86. https://doi.org/10.1016/J.JAD.2020.07.041.
13. Lopez J. Carl A. Burtis, Edward R. Ashwood and David E. Bruns (eds): Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (5th edition): Elsevier, St. Louis, USA, 2012, 2238 pp, 909 illustrations. ISBN: 978-1-4160-6164-9. Indian Journal of Clinical Biochemistry 2013;28:104. https://doi.org/10.1007/S12291-012-0287-7.
14. Gupta S, Sharma B. Protective effects of phosphodiesterase-1 (PDE1) and ATP sensitive potassium (KATP) channel modulators against 3-nitropropionic acid induced behavioral and biochemical toxicities in experimental Huntington's disease. Eur J Pharmacol 2014;732:111–22. https://doi.org/10.1016/J.EJPHAR.2014.03.032.
15. Aguiar CCT, Almeida AB, Araújo PVP, Vasconcelos GS, Chaves EMC, Do Vale OC, et al. Effects of agomelatine on oxidative stress in the brain of mice after chemically induced seizures. Cell Mol Neurobiol 2013;33:825–35. https://doi.org/10.1007/S10571-013-9949-0.
16. Luhach K, Kulkarni GT, Singh VP, Sharma B. Vinpocetine amended prenatal valproic acid induced features of ASD possibly by altering markers of neuronal function, inflammation, and oxidative stress. Autism Res 2021;14:2270–86. https://doi.org/10.1002/AUR.2597.
17. Rinaldi T, Silberberg G, Markram H. Hyperconnectivity of local neocortical microcircuitry induced by prenatal exposure to valproic acid. Cereb Cortex 2008;18:763–70. https://doi.org/10.1093/CERCOR/BHM117.
18. Mizoguchi Y, Kato TA, Seki Y, Ohgidani M, Sagata N, Horikawa H, et al. Brain-derived Neurotrophic Factor (BDNF) Induces Sustained Intracellular Ca2+ Elevation through the Up-regulation of Surface Transient Receptor Potential 3 (TRPC3) Channels in Rodent Microglia. J Biol Chem 2014;289:18549. https://doi.org/10.1074/JBC.M114.555334.
19. Krishnaswamy VKD, Alugoju P, Periyasamy L. Effect of short-term oral supplementation of crocin on age-related oxidative stress, cholinergic, and mitochondrial dysfunction in rat cerebral cortex. Life Sci 2020;263. https://doi.org/10.1016/J.LFS.2020.118545.
20. Sama DM, Norris CM. Calcium dysregulation and neuroinflammation: discrete and integrated mechanisms for age-related synaptic dysfunction. Ageing Res Rev 2013;12:982–95. https://doi.org/10.1016/J.ARR.2013.05.008.
21. Mortezaee K, Khanlarkhani N, Beyer C, Zendedel A. Inflammasome: Its role in traumatic brain and spinal cord injury. J Cell Physiol 2018;233:5160–9. https://doi.org/10.1002/JCP.26287.
22. Yatsiv I, Grigoriadis N, Simeonidou C, Stahel PF, Schmidt OI, Alexandrovich AG, et al. Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal

apoptosis and inflammation in a rodent model of experimental closed head injury. FASEB J 2005;19:1701–3. https://doi.org/10.1096/FJ.05-3907FJE.
23. Davies MJ, Hawkins CL. The Role of Myeloperoxidase in Biomolecule Modification, Chronic Inflammation, and Disease. Antioxid Redox Signal 2020;32:957–81. https://doi.org/10.1089/ARS.2020.8030.
24. Soeda S, Ochiai T, Paopong L, Tanaka H, Shoyama Y, Shimeno H. Crocin suppresses tumor necrosis factor-alpha-induced cell death of neuronally differentiated PC-12 cells. Life Sci 2001;69:2887–98. https://doi.org/10.1016/S0024-3205(01)01357-1.
25. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. Cell Death & Differentiation 2006 13:9 2006;13:1423–33. https://doi.org/10.1038/sj.cdd.4401950.
26. Razavi BM, Hosseinzadeh H, Movassaghi AR, Imenshahidi M, Abnous K. Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure. Chem Biol Interact 2013;203:547–55. https://doi.org/10.1016/J.CBI.2013.03.010.
27. Lee BH, Park TY, Lin E, Li H, Yang CH, Choi KH. Altered Acoustic Startle Reflex, Prepulse Inhibition, and Peripheral Brain-Derived Neurotrophic Factor in Morphine Self-Administered Rats. Int J Neuropsychopharmacol 2017;20:383–91. https://doi.org/10.1093/IJNP/PYW107.
28. Sasi M, Vignoli B, Canossa M, Blum R. Neurobiology of local and intercellular BDNF signaling. Pflugers Arch 2017;469:593–610. https://doi.org/10.1007/S00424-017-1964-4.
29. Nair A, Vaidya VA. Cyclic AMP response element binding protein and brain-derived neurotrophic factor: molecules that modulate our mood? J Biosci 2006;31:423–34. https://doi.org/10.1007/BF02704114.
30. Ghodrati-Jaldbakhan S, Ahmadalipour A, Rashidy-Pour A, Vafaei AA, Miladi-Gorji H, Alizadeh M. Low- and high-intensity treadmill exercise attenuates chronic morphine-induced anxiogenesis and memory impairment but not reductions in hippocampal BDNF in female rats. Brain Res 2017;1663:20–8. https://doi.org/10.1016/J.BRAINRES.2017.02.024.
31. Ghasemi T, Abnous K, Vahdati F, Mehri S, Razavi BM, Hosseinzadeh H. Antidepressant Effect of Crocus sativus Aqueous Extract and its Effect on CREB, BDNF, and VGF Transcript and Protein Levels in Rat Hippocampus. Drug Res 2015;65:337–43. https://doi.org/10.1055/S-0034-1371876.
32. Nelson SB, Valakh V. Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism Spectrum Disorders. Neuron 2015;87:684–98. https://doi.org/10.1016/J.NEURON.2015.07.033.
33. Csiszar A, Smith K, Labinskyy N, Orosz Z, Rivera A, Ungvari Z. Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: role of NF-kappaB inhibition. Am J Physiol Heart Circ Physiol 2006;291. https://doi.org/10.1152/AJPHEART.00340.2006.
34. Majhi S, Kumar S. Neuroprotective effect of berberine by amelioration of behavioural, biochemical and histological anomalies on valproate induced autistic features in experimental animals. Pharmacological Research - Modern Chinese Medicine 2024;10:100352. https://doi.org/10.1016/J.PRMCM.2023.100352.
35. Seyedinia SA, Tarahomi P, Abbarin D, Sedaghat K, Rashidy-Pour A, Yaribeygi H, et al. Saffron and crocin ameliorate prenatal valproic acid-induced autistic-like behaviors and brain oxidative stress in the male offspring rats. Metab Brain Dis 2023;38:2231–41. https://doi.org/10.1007/S11011-023-01275-7.
36. 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;14:14–22. https://doi.org/10.25258/IJDDT.14.1.03.

How to cite this article: Majhi S, Singh, L. Crocin's Protective Effect Against Valproate-Exposed Model of Autism Spectrum Disorder: A Histological and Oxido-Inflammatory Investigation. Journal of Applied Pharmaceutical Sciences and Research. 2025; 8(1):27-33 Doi : 10.31069/japsr.v8i1.04