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Role of Crocin on Acrylamide Induced Neurotoxicity in Adult Male Albino Rats

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Abstract

Acrylamide (ACR) is one of the most organic compounds which is present in many products in our life. It is one of the potential environmental health problems resulting from its increased accumulation in the process of cooking foods at high-temperature. Also, it is an industrial chemical with a wide range of uses in research laboratories, waste water treatment and cosmetic products.The aim of the study was to evaluate the toxic effect of acrylamide on the brain of adult male albino rats and the possible protective role of crocin.Forty adult male albino rats were included in the study. Rats were divided randomly into five groups (8 rats in each group);Group I: (negative control). Group II(positive control): rats received 1mldistilled water orally. Group III(crocin treated group): rats received crocin (50 mg/kg) five days per week for 12 weeks dissolved in 1ml distilled water. Group IV(ACR treated group): rats received ACR (15mg/kg) five days per week for 12 weeks dissolved in 1ml distilled water. Group V(crocin and ACRtreated group): rats received crocin in the same dose thenACRin the same dose five days per week for 12 weeks.At the end of the study, animals were subjected to estimation of acetylcholinestersae,creatine kinase BB, malondialdehyde and reduced glutathione. The cerebral cortex tissue was processed for estimation of brain derived neurotrophic factor plus histological and immunohistochemical examination.The results revealed a statistically significant decrease in acetylcholinesterase, brain derived neurotrophic factor and reduced glutathione levels in ACR group as compared to control group. Also, there was a significant increase in creatine kinase BB and malondialdehyde levels in ACR group when compared with the other groups.Histopathological examination revealed neuronal degeneration, astrogliosis, cytoplasmic vacuolations and thick meninges. Immunohistochemical examination revealed positive immunoreaction to the apoptotic marker caspase-3in the neuronal cells of ACR group when compared with the control group.Marked improvement in all these hazardous effects nearly to control values was recorded in (crocin and ACR) group.From the above mentioned results, it can be concluded thatACR induces oxidative stress, apoptosis and alterations in the neurotransmitters levels, leading to neurodegenerative disorders. Crocin can protect from these hazardous effects.

Keywords: *Acrylamide, neurotoxicity, Oxidative stress, Apoptosis, crocin.*

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I. Introduction

Thermal treatment may initiate the production of several detrimental compounds in foodstuffs during processing. Acrylamide (ACR) is one among such compounds that is formed as a result of Millard reaction between reducing sugars and free amino acids. ACR has gained much attention owing to its wide occurrence, dietary exposure and toxicity. **(Maan et al., 2020)**.ACR is known not only as a synthetic material used in industry but also a cytotoxic compound which is formed during heat-induced process mostly in food such as potatoes, bakery, and coffee. Environmental Protection Agency published that lifetime risk for cancer is 1.7 − 5.5/1,000 based on consumption of 1 μg ACR/kg BW/day for humans. Also, WHO guidelines set the acceptable ACR level in potable water at <0.5 μg/l **(Gedik et al., 2018)**

After metabolic process, ACR is distributed to all organs and tissues in human body. ACR was found to cause apoptosis by mitochondrial dysfunction. ACR was found to cause oxidative stress, neurotoxicity, and carcinogenicity. Methods of ACR inactivation by microorganisms and bioactive diet composites must be applied to ensure food safety **(Koszucka et al., 2019)**

Crocin is a carotenoid that reveals antioxidant effects due to its free oxygen radical scavenging properties. It has protective effects on ACR induced damage by inhibiting oxidative stress. Crocin may exert an inhibitory effect on CYP2E1, which causes the conversion of ACR to glycidamide (GA), which is more toxic than ACR **(Gedik et al., 2018)**

II. Materials and methods

2.1 Chemicals:

- **Acrylamide:** It is manufactured by Sigma -Aldrich chemical company, USA and purchased from Sigma, Cairo, Egypt. Its CAS No is A3553. Purity of ACR is 99%
- **Crocin:** It is manufactured by Sigma -Aldrich chemical company, USA and purchased from Sigma, Cairo, Egypt. Its CAS No is 17304. Purity of ACR is 99%
- **Distilled water:**It was obtained from SEDICO Company, Egypt. It is a vehicle for both ACR and crocin.

2.2 Animals and experimental design

The study was carried out on forty adult healthy male albino rats, each weighing 150-200 gram with an average age of 50-60 days. They were obtained from the Animal House, Faculty of Medicine, ZagazigUniversity. Rats received balanced food rich in all stuffs necessary to maintain their health before and during drug administration. It consisted of bread, barley and milk. Water was offered in separate clean containers. All animals received humane care in compliance with the animal care guidelines of the National Institutes of Health (NIH), and the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC) approved the experimental design with the approval number: ZU-IACUC/3/F/46/2018

2.3 Grouping of animals:

The rats were divided into five groups:

Group I: (Negative control group): 8 rats received regular diet and tap water to measure the basic parameters.

Group II: (Positive control group): 8 rats received (1 ml/kg BW) of distilled water orally (a vehicle for both ACR and crocin) five days per week orally.

Group III: (Crocin treated group): 8 rats received crocin (50 mg/kg BW) dissolved in distilled water (1ml/kg BW), orally, five days per week for 12 weeks. This dose at which crocin has antioxidant properties, according to **Erdemli et al. (2017)** studies.

Group IV:(Acrylamide (ACR) treated group): 8 rats received ACR (15mg/kg BW) (1/20 of LD50) dissolved in distilled water (1ml/kg BW), orally, five days per week for 12 weeks.

Oral LD50 of ACR in rats =300 mg/kg body weight **(Environmental Protection Agency (EPA))**.

Group V:(Crocin and ACR treated group): 8 rats will receive crocin (50 mg/kg BW) dissolved in distilled water (1ml/kg BW), one hour later, ACR was given (15 mg/kg BW) dissolved in distilled water (1ml/kg BW), orally, five days per week for 12 weeks.

At the end of the 12^{th} week,blood samples were collected to estimate acetylcholine esterase (AChE) activity, creatine kinase- BB,reduced glutathione (GSH) and malondialdehyde (MDA).Then rats were sacrificed, parts of the cerebrum were homogenized for estimation of brain derived neurotrophic factor (BDNF) while the other parts were processed for histological and immunohistochemical examination.Immunohistochemical staining of the cerebrum for detection of caspase-3 as a marker of apoptosis.

2.4 Methods

Assessment of serum acetylcholinesterase(U/L)

Acetylcholine esterase activity was measured by using spectrophotometer based on Ellman's method **(Ellman et al., 1961)**.

Assessment of serum creatine kinase (CK-BB) (U/L)

Creatine kinase-BB was measured by spectrophotometer according to the method described by **Oliver (1995)**.

Assessment of serum malondialdehyde (MDA) level (nmol/ml)

It was measured by spectrophotometer according to the method described by**(Yoshioka et al., 1979**)

Assessment of serum reduced glutathione (GSH) (ng/ml)

It was measured by spectrophotometer according to the method described by**Moron et al.(1979).**

Assessment of Brain derived neurotrophic factor (BDNF) (pg/ml)

This assay employs the quantitative sandwich enzyme immunoassay technique ELISA according to **Elfving et al. (2010)**

Methods used for histopathological examination of the cerebrum

At the time of sacrifice, animals were anaesthetized by ether inhalation, then were sacrificed.Brain tissues were fixed in 10% formalin saline. After fixation, they were embedded in paraffin blocks and processed for the preparation of 5 u. thickness sections. These sections were subjected for hematoxylin and eosin stainingaccording to the method described by **Mustafa et al. (2015)**

Methods used for Immunohistochemical examination of the cerebrum

Immunohistochemical reactions were carried out on brain tissues of the experimental rats using caspase-3. The caspases are a family of cytosolic aspartate-specific cysteine proteases involved in the initiation and execution of apoptosis.It was processedaccording to the method described by **Mustafa et al. (2015)**

Statistical analysis

Analysis of data was done using SPSS program (Statistical Package for Social Science) version 25.0 . For normally distributed data, comparison between the five studied groups was analyzed using one way analysis of variance (ANOVA or F-test) and least significant Difference (LSD) test. All data were expressed as mean ± SD (Standard deviation) **(SPSS, I. 2017).**

III. Results

3.1 Biochemical results

There was no statistically significant difference between the control groups in the neurological parameters or oxidative stress parameters. So, we considered the -ve control group as the control one for comparison with other groups **(Table 1).**

There was a high significant difference in AChE level between negative control group, crocin group, ACR treated group and (crocin+ACR) treated group (P<0.001) as shown in **(Table 2)**. There was a high significant decrease in serum AChE mean values (1748 VS 13726 U/L) when compared with negative control group (p<0.001). Co-administration of crocin in (crocin+ACR) treated group showed a high significant increase in AChE mean values (15081 VS 1748 U/L) when compared with those of ACR treated group (p<0.001) **(Table 3)**

Table (1): Statistical comparison between negative and positive control groups as regard mean values of serum acetylcholine esterase (AChE), creatine kinase- BB (Ck-BB), brain derived neurotrophic factor (BDNF), malondialdehyde (MDA) and reduced glutathione (GSH) using student t- test.

Parameter	-ve control (regular diet)	+ve control (1ml/kg distilled water)	T	p-value
	Mean±SD			
AChE(U/L)	13726.1±1483.3	14221.1±1306.8	0.708	0.490 NS

Ck-BB (U/L)	4.01±0.97	4.26±1.51	0.375	0.713 NS
BDNF(pg/ml)	133.63±14.94	136.38±13.22	0.390	0.702 NS
GSH (U/L)	44±3.12	42.13±1.96	1.441	0.172 NS
MDA(nmol/L)	0.6±0.30	0.68±0.19	-0.619	0.546NS

All values are expressed as mean±SD

SD: standard deviation

+ve: positive

N=Number of rats in each group. NS: non-significant (P >0.05).

-ve: negative

Table (2): Statistical comparison amongnegative control, crocin, acrylamide (ACR) and (crocin+ACR) treated groups as regard mean values of serum acetylcholine esterase (AChE), creatine kinase- BB (Ck-BB) and brain derived neurotrophic factor (BDNF)using ANOVA test.

Group N=8 / **Parameter**	**-ve Control (Regular diet)**	**Crocin (50mg/kg)**	**ACR (15mg/kg)**	**Crocin (50mg/kg) + ACR (15mg/kg)**	**F**	**P-value**
	Mean ± SD					
AChE(U/L)	13726.1±1483.3	13075±645.3	1748±439.39	15081.13±761.62	358.610	<0.001**
Ck-BB (U/L)	4.03±0.97	4.03±1.03	75.63±8.91	4.23±0.96	497.129	<0.001**
BDNF (pg/ml)	133.63±14.94	131.63±10.76	18.19±4.81	140.5±11.92	218.453	<0.001**

All values are expressed as mean±SD. (SD: standard deviation)

N=Rats number in each group.

**: highly significant (P<0.001).

-ve: negative

Table (3): Least significance difference (LSD) for comparison between mean values of serum acetylcholine esterase (AChE) of negative control, crocin, acrylamide (ACR) and (crocin+ACR) treated groups.

Groups N=8	Crocin (50mg/kg)	ACR (15mg/kg)	Crocin (50mg/kg) +ACR (15mg/kg)
-ve control	0.168 NS	<0.001**	0.006**
Crocin		<0.001**	<0.001**
ACR			<0.001**

NS: statistically non significant (p>0.05).

**: statistically highly significant (P<0.001)

-ve: negative

N= Number of rats in each group

Table (4): Least significant difference (LSD) for comparison between mean values of serum creatine kinase- BB (Ck-BB) of negative control, crocin, acrylamide (ACR) and (crocin+ACR) treated groups.

Groups N=8	Crocin (50mg/kg)	ACR (15mg/kg)	Crocin (50mg/kg) + ACR(15mg/kg)
-ve control	1.000 NS	<0.001**	0.930 NS
Crocin		<0.001**	0.930 NS
ACR			<0.001**

NS: statistically non significant (p>0.05)

-ve: negative

**: statistically highly significant (P<0.001)

N=Number of rats in each group

Table (5): Least significant difference (LSD) for comparison between mean values of brain derived neurotrophic factor (BDNF) of negative control, crocin, acrylamide (ACR) and (crocin+ACR) treated groups.

Groups N=8	Crocin (50mg/kg)	ACR (15mg/kg)	Crocin (50mg/kg) + ACR (15mg/kg)
-ve control	0.724 NS	<0.001**	0.231NS
Crocin		<0.001**	0.125 NS
ACR			<0.001**

NS: statistically non significant (p>0.05)

-ve: negative

**: statistically highly significant (P<0.001)

N=Number of rats in each group

Table (6): Statistical comparison amongnegative control, crocin, acrylamide (ACR) and (crocin+ACR) treated groups as regard mean values of serum reduced glutathione (GSH) and malondialdehyde (MDA) using ANOVA test.

Group N=8 / Parameter	-ve Control (regular diet)	Crocin (50mg/kg)	ACR (15mg/kg)	Crocin (50mg/kg)+ ACR (15mg/kg)	F	P-value
	Mean ± SD					
GSH	44±3.12	41.5±2.07	8.91±0.93	37.63±5.04	210.483	<0.001**

MDA	0.6±0.30	0.8±0.21	12.93±1.35	1.4±0.41	535.888	<0.001**

SD: standard deviation

-ve: negative

N: Number of rats in each group

**: highly significant (P<0.001)

ANOVA: analysis of variance

Table (7): Least significance difference (LSD) for comparison between mean values of serum reduced glutathione of negative control, crocin, acrylamide (ACR) and (crocin+ACR) treated groups:

Groups N=8	**Crocin (50mg/kg)**	**ACR (15mg/kg)**	**Crocin (50mg/kg) + ACR (15mg/kg)**
-ve control	0.126 NS	<0.001**	<0.001**
Crocin		<0.001**	0.021*
ACR			<0.001**

NS: statistically non significant (p>0.05)

-ve: negative

*: statistically significant (p<0.05)

**: statistically highly significant (P<0.01)

N=Number of rats in each group

Table (8): Least significance difference (LSD) for comparison between mean values of serum malondialdehyde of negative control, crocin, acrylamide (ACR) and (crocin+ACR) treated group:

Groups N=8	**Crocin (50mg/kg)**	**ACR (15mg/kg)**	**Crocin (50mg/kg) + ACR (15mg/kg)**
-ve control	0.481 NS	<0.001**	0.026 *
Crocin		<0.001**	0.114 NS

ACR			<0.001**

NS: statistically non significant (p>0.05)

-ve: negative

*: statistically significant (p<0.05)

**: statistically highly significant (P<0.01)

N=Number of rats in each group

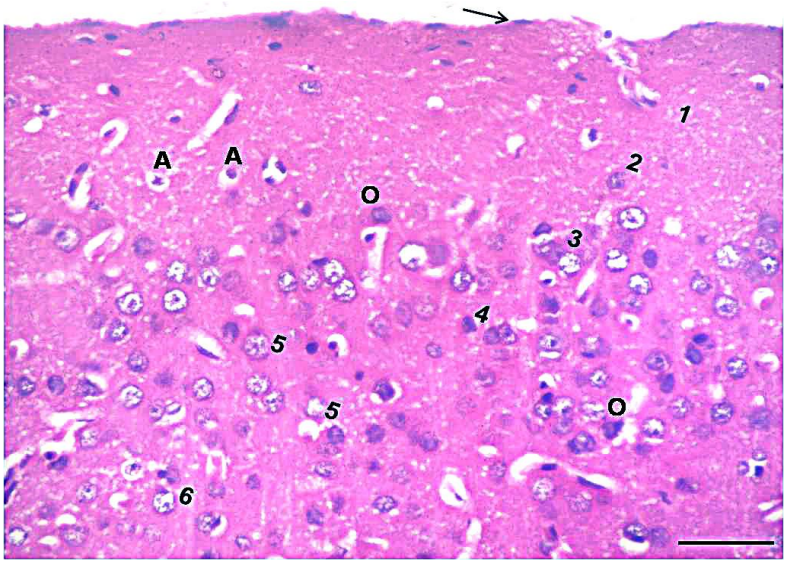

Figure (1):A photomicrograph of a section from cerebral cortex of both control and crocin treated groups showingpia matter **(arrow)** and distinct six layers; molecular layer **(1)**, outer granular layer **(2)**, outer pyramidal layer **(3)**, inner granular layer **(4)**, inner pyramidal layer **(5)** and multiform layer **(6)**. The pink stained neuropil is packed with neuronal and glial cell processes **(H&E× 400)**

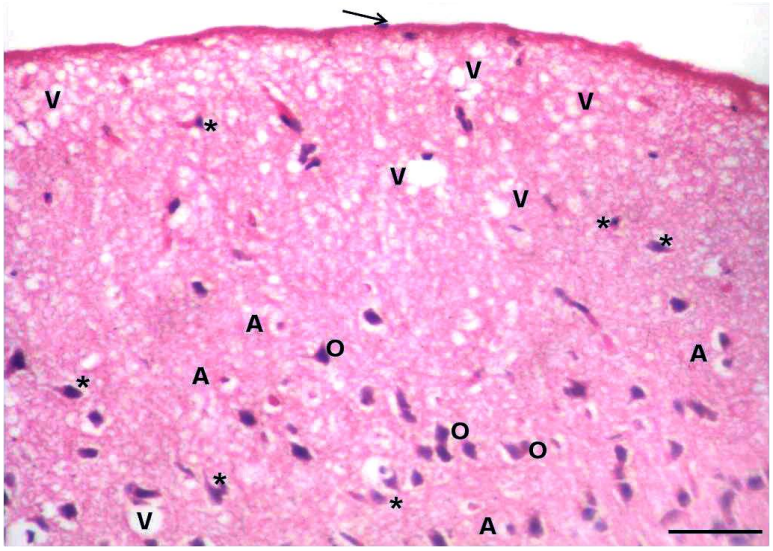

Figure (2):A photomicrograph of a section from cerebral cortex of acrylamide group, showing multiple sized vacuoles (**V**) in the neuropil. Most of the neuronal cells (mainly the pyramidal) and neuroglial cells had dark stained nuclei. Oligodendrocyte**(O)**, Astrocytes **(A)**. Scale bar 50 µm **(H and E× 400)**

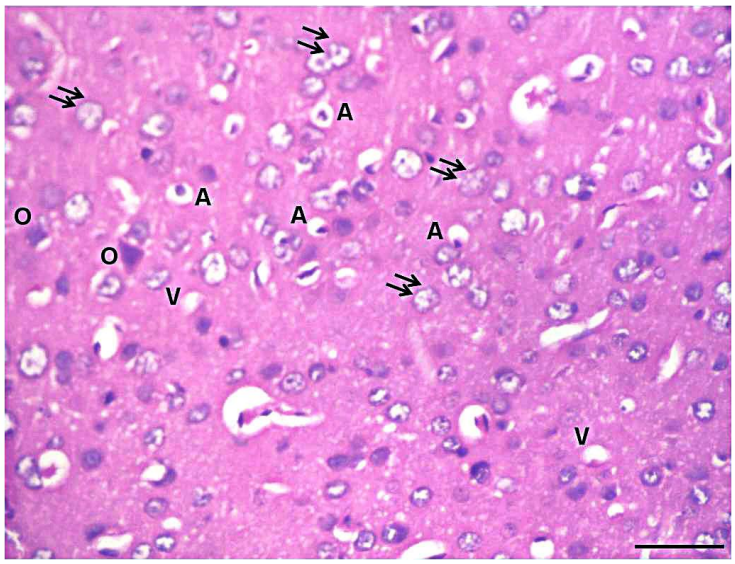

Figure (3): A photomicrograph of a section of the cerebral cortex of (crocin+acrylamide) group, showing improvement in the histological architecture. The nerve and glial cells had bright nuclei **(double arrows)** in many areas. Vacuoles **(V),**oligodendrocyte**(O)**, astrocytes **(A)**. Scale bar 50 µm **(H and E× 400)**

Figure (4): A section from the cerebral cortex of both negative control and crocin treated group, showing negative caspase-3 immunoreactivity in the cytoplasm of neurons **(Immunohistochemical × 400)**

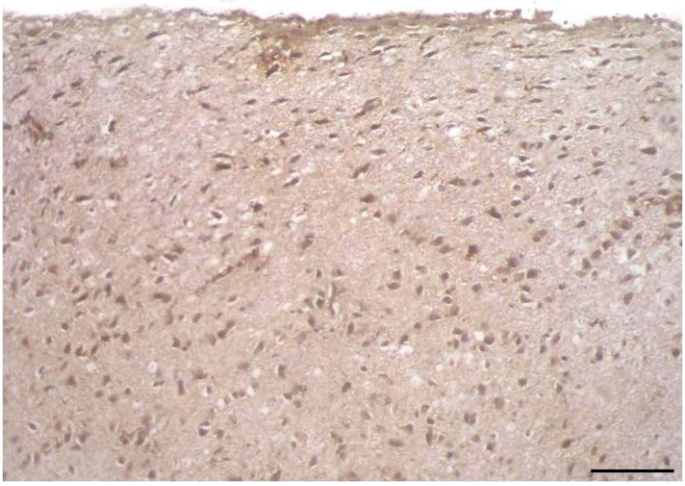

Figure (5): A section from the cerebral cortex of acrlamide group, showing strong positive caspase-3 immunoreactivity in the cytoplasm of neurons **(Immunohistochemical × 400)**

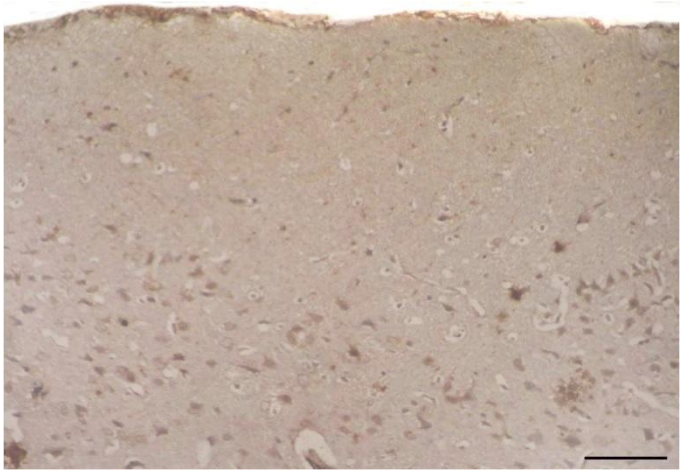

Figure (6): A section from the cerebral cortex of (crocin+acrylamide) group, showing decreasing in positive caspase-3 immunoreactivity in the cytoplasm of neurons **(Immunohistochemical × 400)**

There was a high significant difference in Ck-BB level between negative control group, crocin group, ACR treated group and (crocin+ACR) treated group (P<0.001) as shown in **(Table 2)**. There was a high significant increase in serum CK-BB mean values (75 VS 4 U/L) when compared with negative control group (p<0.001). Co-administration of crocin in (crocin+ACR) treated group showed a high significant decrease in CK-BB mean values (4.2 VS 75 U/L) when compared with those of ACR treated group (p<0.001) **(Table 4)**

There was a high significant difference in BDNF level between negative control group, crocin group, ACR treated group and (crocin+ACR) treated group (P<0.001) as shown in **(Table 2)**. There was a high significant decrease in BDNF mean values (18VS 133 pg/ml) when compared with negative control group (p<0.001). Co-administration of crocin in (crocin+ACR) treated group showed a high significant increase in BDNF mean values (140 VS 18 pg/ml) when compared with those of ACR treated group (p<0.05) **(Table 5)**

There was a high significant difference in GSH level between negative control group, crocin group, ACR treated group and (crocin+ACR) treated group (P<0.001) as shown in **(Table 6)**. There was a high significant decrease in GSH mean values (9 VS 44ng/ml) when compared with negative control group (p<0.001). Co-administration of crocin in (crocin+ACR)-treated group showed a high significant increase in GSH mean values (37.5 VS 9 ng/ml) when compared with those of ACR treated group (p<0.001) **(Table 7)**

There was a high significant difference in MDA level between negative control group, crocin group, ACR treated group and (crocin+ACR) treated group (P<0.001) as shown in **(Table 6)**. There was a high significant increase in serum MDA mean values (13 VS 0.6 nmol/L) when compared with negative control group (p<0.001). Co-administration of crocin in (crocin+ACR)- treated group showed a high significant decrease in MDA mean values (1.4 VS 13nmol/L) when compared with ACR treated group (p<0.001) **(Table 8)**

3.2 Histological results:

The light microscopic examination of hematoxylin and eosin (H&E) stained sections from the cerebral cortex of the control groups showed normal histological architecture of the cerebral cortex. The normal cerebral cortex contained six layers from outwards to inwards. These are molecular layer, outer granular layer, outer pyramidal layer, inner granular layer, inner pyramidal layer and the multiform layer. The most obvious cells in these layers are granular, pyramidal and supporting cells. The pink stained eosinophilicneuropil is packed with neuronal and glial cell processes (oligodendrocytes and astrocytes) with blood vessels **(Figure: 1)**.Sections obtained from ACR treated group at the end of the 12^{th} week, revealed obvious histological changes in all of the crotical layers compared to the control group. Lots of vacuolations were seen between cell layers. Most of nerve cells (pyramidal and granular cells) were shrunken with loss of their processes and had pkynotic dark nuclei. The neuropil around the neurons and glial cells showed extensive vacuoles of different sizes **(Figure: 2)**. While sections from (crocin and ACR) treated group exhibited enhancement in neuronal and glial cell architecture in different cortical areas. The neuronal cells, pyramidal and granular, were near to normal control group concerning the histological structure. The majority of nerve in this group showed bright vesicular nuclei, but a small number of pyramidal neuronal cells were shrunken inbetween the normal granular cells. Their nuclei were dark stained with vacuolated neuropil. Some supporting cells were almost near to control littermates, while only some cells were presented with dark stained nuclei with vacuolated nearby neuropil**(Figure: 3)**

3.3 Immunohistochemical results

Immunohistochemical examination of the cerebral cortex of control groups showed no immunoreactivity for caspase–3 along the duration of the study **(Figure: 4).** While in ACR treated group, there was many neurons with strong positive reactions to the caspase-3 antibody in their cytoplasm **(Figure: 5).**In (crocin and ACR)treated group, there was few numbers of positively reacted neurons**(Figure: 6)**

IV. Discussion

In the present study, there was a high significant decrease in AChE mean values in ACR treated group as compared to their corresponding values in the control groups. Co-administration of crocin caused a high significant increase in AChE activity when compared with ACR group, reflecting that crocin supplementation may have a protective role against ACR neurotoxic effects.This declined AChE activity may occur due to the direct binding of ACR with the active -SH sites of the AChE enzyme may result in oxidation of the cysteine group in the enzyme by free radicals as ACR enhance the production of ROS (**Venkataswamyet al., 2013)**. ROS are toxic to neurons due to their action on polyunsaturated fatty acids, decrease of membrane fluidity, and oxidation of biological molecules, nuclear and mitochondrial damage. Moreover, free radicals significantly inhibit AChE activity in the brain **(Kopa̓nska et al ., 2015)**.The results of this study are in accordance with **Kopa´nska et al., (2015)** who recorded that ACR induced a significant decline in AChE activity in the brain structures of Swiss mice after intraperitoneal ACR administration in a dose dependent manner. In the same way,**Elblehi et al. (2019)** studied the effectsof repeated ACR administration on the brain tissueof the rats and concluded that ACR toxicity decreases the activity of AChE in the brain, which disturbs metabolic and nervous activity leading to increased membrane permeability and cholinergic dysfunction.

In the present study, there was a significant increase in CK-BB mean values in ACR treated group as compared to the control groups. Co-administration of crocin caused a significant decrease in CK-BB when compared with ACR treated group; reflecting that crocin supplementation may have a protective role against ACR neurotoxic effects. This can be explained by the theory of**Xintong et al. (2019)** who reported that inflammatory neuropathology is a crucial factor in neuronal damage after ACR exposure which might occur via the gut-brain axis, as ACRdiet causes a defect in the intestinal barrier of mice leading to elevated plasma levels of bacteria-derived lipopolysaccharide which passes through the gut epithelium and enter the systemic circulation inducing inflammatory response and destabilize the tight junctions of the blood brain barrier, causing neurogenic inflammation. The results of this study are in accordance with **El-Shamy et al. (2013)** who studied the neurotoxic effects of ACR when applied orally in a dose 5mg/kg/day, and showed significant increase in CK-BB level indicating brain injury and neuronal loss. Also, in a study by **AbdlGawad et al. (2017)**, ACR induced neurotoxic effects when administered to albino rats in dose 25mg/kg/day for 3 weeks.

In the present study, there was a high significant decrease in BDNF level in ACR treated group as compared to the control groups. Co-administration of crocin caused a high significant increase in BDNF level compared with ACR group; reflecting that crocin supplementation may have a protective role against ACR neurotoxic effects.This declined BDNF level may be due to ACR induced overproduction of free radicals and increasing the oxidative stress in the brain tissue, which finally results in neuronal dysfunction. Several studies

indicated that oxidative stress is a key mechanism in ACR induced neurodegenerative diseases **(Ahmed et al., 2016)**.These results are in accordance with**Erdemli et al. (2016)** who studied the effect of ACR on fetal brain tissues when administered to pregnant female rats for 20 days, which showed thedramatically decreased BDNF levels. Also, **AbouZaid et al. (2017)** investigated the neurotoxic effects of ACRwhenadministrated in a dose 50 mg/kg BW per day for 21 days in male Wister albino rats, which showedthe significant decrease in BDNF levels in brain structures. Moreover,**Xintong et al. (2019)** reported that ACR diet induced a significant decrease of BDNF expression at night, which indicated that ACR treatment exhibited more neurotoxicity at night disturbing the circadian clock besides impairing the memory performance.

Crocin administration has significant neuroprotective effect as it significantly decreased CK-BB level and significantly increased AChE and BDNF. It modulate the neurotoxicity and brain damage induced by ACR. These results are in agreement with **Farkhondeh et al. (2018)** who studied the protective effects of crocin in the management of neurodegenerative diseases via its antioxidant and antinflammatory effects. In the same way, **Ahmadi et al. (2017)**investigated the effect of crocin on improving memory deficits and cerebral oxidative damage in streptozotocin-induced diabetic rats. In another study by **AbdlGawad et al. (2017)** crocin showed a significant increase in BDNF level when administered orally in a dose 50mg/kg for 3 weeks. Also, **Sadough, (2019)** studied the neuroprotective role of crocin against trimethyltin chloride induced brain damage.

The studied oxidative stress markers revealed that ACR administration caused a significant elevation of serum MDA and a significant reduction of serum GSH level. Co-administration of crocin showed a significant decline in MDA with a significant increase in GSH level reflecting that crocin supplementation improved the ACR toxic effects. This can be explained by **Swamy et al. (2013)** theory in which ACR is oxidized to glycidamide, a reactive epoxide that undergoes conjugation with GSH; explaining the observed reduction in GSH level. Preservation of GSH level is dependent upon availability of glutathione reductase enzyme (GR). The reduction of this enzyme's activity is due to the interaction of free radicals formed by ACR with the sulfhydryl group present at the active site of GR enzyme; preventing this enzyme from participating in the formation of GSH, leading to reduction in GSH level.

These results are in accordance with **Zhao et al. (2015**) study in which ACR was administered to albino rats in a dose of (50 mg/kg) for five days intraperitoneally. Also, the results of the present study are in accordance with **Al-Agele and Khudiar, (2016)** who reported the same results after ACR oral administration in a dose 0.5 and 1mg/kg B.W in adult albino rats. Furthermore,**Elhelaly et al. (2019)** demonstrated that flavonoid antioxidants have a great benefit in ameliorating the toxic effects of ACR when they utilized hesperidin and diosmin which are flavonoid glycosides presented mainly in the citrus family plants. Hesperidin and diosmin supplementation prevented lipid peroxidation, normalized the serum parameters altered by ACR, decreased MDA level, increased GSH level in rat tissues, and enhanced the tissue concentrations and activities of antioxidant biomarkers. Moreover,**Gedik et al. (2018)** investigated the protective effects of crocin on ACR induced toxic damage in rats, proving that crocin inhibit ACR-induced oxidative stress when administered in a dose (50 mg/kg), presented by decreased MDA level and increased GSH level. This could be explained by the study of **Paolini et al. (2001)** who reported the capability of crocin to exert an inhibitory effect on CYP2E1, which causes the conversion of ACR to glycidamide (GA), which is more toxic than ACR. Therefore, inhibition of the CYP2E1 enzyme prevented oxidative damage by inhibiting the formation of the toxic metabolite GA.

(B) Histopathological results:

In the present study, the cerebral cortex showed neuronal disorganization, hypercellularity, thick meninges, dilated blood vessels and areas of severe hemorrhage. Increased cytoplasmic vacuolations and red neurons were noticed. Few nerve cells with vesicular nuclei were observed, glial cells with small nuclei were present.Regarding (crocin+ACR)-treated group, the light microscopic examination of the cerebral cortex showed enhancement in neuronal and glial cell architecture in different cortical areas. The neuronal cells, pyramidal and granular were near to normal, the majority of nerves in this group showed bright vesicular nuclei. A small number of pyramidal neuronal cells were shrunken inbetween the normal granular cells, their nuclei were dark stained with vacuolated neuropil. Some supporting cells were near to normal control group, reflecting that crocin supplementation had a protective effect of on the neuronal damage induced by ACR. These results are confirmed by results of **AbouZaid et al. (2017)** who demonstrated that ACR administration in a dose 50 mg/kg BW per day for 3 weeks.

In the present study, immunohistochemical examination of sections of the cerebral cortex of ACR treated group showed many neurons with strong positive reactions to the caspase-3 antibody in their cytoplasm compared with control group. On the other hand, examination of sections of the cerebral cortex of (crocin +ACR) treated group showed few neurons with mild positive reactions to the caspase-3 antibody in their cytoplasm in comparison to ACR treated group. This can be explained by the ability of ACR to penetrate the neuronal membrane, which provokes ROS production mediated mitochondrial damage and disruption of mitochondrial permeability resulting in the release of key apoptotic proteins, including cytochrome-c and caspase-3 **(Pan et al., 2017)**. These results are in agreement with those reported by **Oda, (2017)** who reported that ACR activated the mitochondrial apoptotic pathway via downregulation of Bax, activation of Bcl2, caspases cascade, releasing of cytochrome c and mitochondrial dysfunction. Moreover, our results are in accordance with **Elblehi et al. (2019)** who revealed that a strong positive expression of the apoptosis marker caspase-3 in the immunohistochemical examination of ACR-treated rats.

The studied histopathological and immunohistochemical findings were parallel to the biochemical results. This can be confirmed by the study of**Kunnel et al. (2019)**who observed various histopathological alterations in the ACR-intoxicated group i.e. neuronal degeneration and necrosis with glial cell activation together with oxidative stress, ROS generation, mitochondrial dysfunction and cytoskeleton changes resulted in cell death necrosis or apoptosis.

V. Conclusion

Acrylamide should be taken into consideration while evaluating the increasing trend of cognitive impairment. It induces oxidative stress, apoptosis, alteration in the neurotransmitters levels, leading to neuronal degeneration. Crocin can protect from these hazardous effects via its antioxidant and antiapoptotic properties.

Conflict of interest

The authors report no conflicts of interest.

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