



Study Neurotoxicity Effect of Acrylamide and Ameliorating Effect of Curcumin on Adult Male Rats

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Received: June 4, 2023 / Accepted: August 22, 2023/ Published: September 23, 2024

Abstract: Acrylamide (ACR) is also present in tobacco smoke and foods heavy in carbohydrates that have cooked at high temperatures. It was thought that ACR exposure exclusively occurred at work and was partially brought on by cigarette smoking, water, and cosmetics. The study was aimed to study the harmful and neurotoxic effect of the acrylamide exposure and the ameliorating effects of curcumin in neurotoxicity that induced by acrylamide. Thirty adult male rats were subjected in this study, animal were divided into three groups, ten rats per group, group control were intubated distilled water for 40 days, group ACR (Acrylamide), rat in this group were administered acrylamide 5mg/kg B.W. for 40 days and group ACR+CUR (Acrylamide+ curcumin), animals had acrylamide 5mg/kg B.W. and curcumin 100mg/kg B.W. for 40 days. The results showed a significant decrease in the dopamine in the brain and increase AchE in serum, moreover, acrylamide affected spatial memory and locomotor activity behavioral testing, however curcumin restored these parameters close to control values. Histopathological examination of brain sections of ACR rats revealed proliferation of microglial cells with Alzheimer type II astrocyte, whereas, section from ACR+CUR, animals showed mild congested blood vessels in the pia mater. In conclusion Acrylamide induced alteration in neurotransmitters, neurobehaviors and structure of brain and this can be protected by curcumin.

Keywords: curcumin, Acrylamide, neurotoxicity, dopamine, Ach-s, Neurobehaviors.

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Introduction

Acrylamide (ACR) is a significant chemical utilized in numerous industrial and scientific procedures, including the purification of water, the manufacture of cosmetics, and gel electrophoresis (1). Acrylamide (ACR) is also present in tobacco smoke and foods heavy in carbohydrates that have cooked at high temperatures. It was thought that ACR exposure exclusively occurred at work and was partially brought on by cigarette smoking, water, and cosmetics (2). Moreover, Acrylamide is a chemical building block that is used to create polyacrylamide and can be altered to have non-ionic, an-ionic, or cationic characteristics for certain applications

(3). Only the neurotoxicity of ACR has been demonstrated in both human occupational exposure and animal testing.

Repeated daily exposure to ACR causes a triad of manifestation effects, including ataxia, hindlimb foot splay, and skeletal muscular weakening, according to studies in a variety of laboratory animal species, including cats, rats, mice, guinea pigs, rabbits, and monkeys. In rat primary astrocytes, ACR-induced apoptosis led to mitochondrial malfunction and apoptosis in BV-2 microglial cells being neurotoxin, ACR may slow down nerve conduction, paralyze the hind limbs of rats, increase the landing foot spread, and cause the animals to drag their feet (4). On the other

hand, The life's secret ingredient is worldwide, tropical and subtropical environments support the growth of turmeric, whose scientific name is *Curcuma longa*. Numerous studies conducted over the past 50 years have established that curcumin is primarily responsible for the majority of the effects attributed to turmeric. The anti-inflammatory, antioxidant, anti-coagulant, anti-diabetic, anti-microbial, anti-ulcer, wound-healing, and anti-fertility effects of curcumin are among its medical benefits. Diabetes, numerous cancers, Alzheimer's disease, and other chronic diseases can all be treated successfully with it. It has been shown to help in the treatment of inflammatory illnesses, discomfort from metabolic syndrome, and degenerative and inflammatory eye problems (5). The curcumin therapeutic effects of on the nervous system, in particular the brain and the disorders associated with this important organ, have been the subject of much research. Been made some solid progress in understanding how curcumin protects against neurodegenerative conditions like disease (Alzheimer's and Parkinson's) in animal models. Expectations for the administration of curcumin in inflammatory brain disorders and brain malignancies have increased (6).

Materials and methods

Animals and housing

Thirty mature male Wistar Albino rats (aged 8–10) weeks, weighted between 160 and 190) were used in this study. They were acclimatized for two weeks prior the experiment. The animal housed in the house of the College of Veterinary Medicine at University of Baghdad over the 40-day period that spanned September and November 2022. Throughout the trial, they were kept in plastic cages (3rats/cage) in a well-ventilated room and were given unrestricted access to a regular pellet food as well as water. Animals were

maintained at 22 ± 2 °C. During the experiment, there was a light/dark cycle with a light on from 7:00 a.m. to 7:00 p.m.

The Rats were randomly divided into three groups each group consisted of 10 rats:

1. Group Control: Ten animals were received distilled water by gavage needle for 40 days.
2. Group ACR: Ten animals in this group were received acrylamide 5mg/kg B.W. orally by gavage needle daily for 40 days.
3. Group ACR+ CUR: Ten animals in this group received acrylamide 5mg/kg B.W. every day and curcumin 100mg/kg B.W, orally by gavage needle for 40 days.

Serum preparation

After administering general anesthesia using intramuscular injections of ketamine (60 mg/kg B.W.) and xylazine (40 mg/kg B.W.) blood samples were obtained using the heart puncture technique after the experiment. After centrifuging blood samples for 15 minutes at 4000 rpm to extract the serum, the serum was stored in tightly sealed tubes and kept frozen for analysis at -20°C . Blood samples were put in non-heparinized gel tubes for 10 minutes.

Tissue collection and homogenization

All rats from all groups were sedated with intramuscular injections of ketamine (60 mg/kg) and xylazine at the conclusion of the 40-day trial, and their heads were removed by cervical dislocation to extract the whole brain. The left and right halves of the brain were separated during dissection. To conduct the ELISA test, a 0.5 grams of the right part of the brain was wash with phosphate buffer solution (PBS) (0.02mol/L, pH 7.0-7.2) solution and placed in container then frozen at -20°C . The left part of the brain was kept in plastic contain-

ers with formalin (10%) for routine histological examination.

Determination of serum acetylcholine esterase (AChE) activity

Quantitative determination of serum Acetylcholine esterase ($\mu\text{mol/L}$) was conducted by an Enzyme Linked Immunosorbent Assay through using rat Acetylcholine Esterase Competitive ELISA kit. The Catalog Number (MBS725468) according to company instruction (MyBioSource), Country(USA).

Determination of dopamine level in brain

Right brain tissues were weighed at 500 mg and washed in ice-cold PBS (0.02 mol/L, pH 7.0-7.2) to fully eliminate any extra blood. Using a glass homogenizer set on ice, tissue was chopped into minute pieces and homogenized in 500ul of PBS. The cell membranes in the resultant solution were further damaged by ultrasonic or two freeze-thaw cycles. The homogenates were then centrifuged for 15 minutes at 5000 rpm to obtain an aliquot of supernatant for dopamine analysis (7).

Y- Maze spatial memory

The test was performed at the beginning of the study and (40) day. Animal was permitted to move through apparatus for 5 minutes after being positioned just within arm A distant from the center. The succession of arm entries were graphically documented. Each arm entry was determined when all four paws enter the arm. The floor of three arms was wiped with alcohol between trails before the rats were placed back in their cages. The number of entries should not include the first recorded arm, which is always A. Any trail that had all three letters was recorded as an alteration (8).

Open field test

Open field apparatus consists of a wooden open box at measurement (100 x 100 cm²) the arena divided into 16 equal squares each small squares (20x20 cm²). This test evaluates the general emotional activity in the other words measure the time that animal spent close to the walls or in the center of the apparatus during 5 minutes for each animal (9).

Histopathology

The dissected left brain samples from the scarified animals were promptly preserved in a 10% formalin solution, and then they were transferred to the next processes after being rinsed with saline for 1–2 hours: dehydration, clearing, Embedding with paraffin wax, blocking, and sectioning, followed by staining by Hematoxylin and Eosin (H and E) (10).

Statistical analysis

Graph Pad Prism software version 9.1.0 (San Diego, CA, United States) was used to statistically analyze the obtained data by Two-way ANOVA and One-way ANOVA. When ANOVA was significant, the data were post *hoc tested* Tukey's test. Number of animals represented by (*n*) Data are presented as mean \pm standard error (SE). Statistically significant was accepted when ($P < 0.05$)(11).

Results and discussion

Dopamine level (ng/L)

In this study after 40 days of treatment there was significant ($P < 0.05$) decline in the dopamine level of ACR group (14.41 ± 3.10) compared with control group (51.80 ± 5.34) and ACR+CUR groups (29.65 ± 5.58). However, there were significantly differences ($P < 0.05$) in Dopamine level in Control, and ACR+CUR groups when compared with each other as shown in (Figure1).

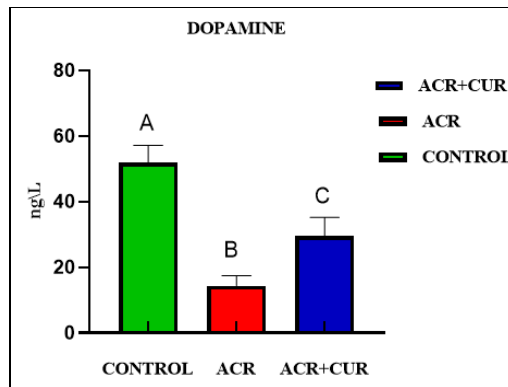


Figure (1): Effect of Acrylamide and Curcumin on dopamine level in brain tissue of adult male rats

* Different letters indicate to the differences between groups

*Data represented as mean \pm SEM

* N=9 per group

It has recently been demonstrated that ACR shows a toxic to neurons activity by binding to cysteine-rich receptor proteins that then participate in membrane reuptake (membranous dopamine transporter), vesicular neuroconduction (vesicular dopamine transporter), and presynaptic release of a neurotransmitter (a component sensitive to N-ethylmaleimide) (12). Additionally, it has been shown that dopamine levels decrease significantly in response to ACR's intoxication in rat. It causes a disturbance of neuroconductions and nerve function which ultimately leads to morphological changes and biochemical changes disturbance or visible neurotoxicity (13). This study shows that curcumin taken orally maintains the health of the dopaminergic system. Numerous studies have demonstrated that the ad-

ministration of curcumin dramatically lowers the toxin-induced death of dopaminergic neurons in animal models. In fact, it has been demonstrated that curcumin lessens dopaminergic cell line caused neurotoxicity. This research demonstrated curcumin's neuroprotective effect and ability to improve brain functioning in diverse animal models by highlighting its antioxidant, anti-apoptotic qualities, and anti-inflammatory (14).

AchE concentration

The current findings was significant ($P < 0.05$) elevation in serum Acetylcholine esterase after oral received of ACR (249.91 ± 10.97) group in 40-day trial when compared with the control (144.41 ± 10.22) group and the ACR+CUR (158.63 ± 14.79) group at the 40-day trial as shown in (Figure 2).

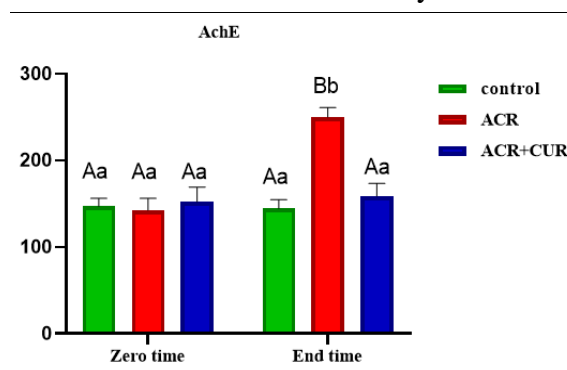


Figure (2): Effect of Acrylamide and Curcumin on AchE level in adult male rats

*Upper-case letters indicate the differences between groups in the same period of time

*Lower-case letters indicate the differences between time periods, 40 days vs. zero time for the same group.

* Data represented as mean \pm SEM, N=8 per group.

Another Neurotransmitters was subjected this study, Acetylcholine is hydrolyzed into acetic acid by acetylcholine esterase, it does not cause excessive ACh to accumulate in synaptic gaps as a result of an instant ACh's breakdown because this might impair an organism's ability to operate properly. Due to the cleavage of Ach, the cholinergic anti-inflammatory pathway's activity can be stopped by acetylcholinesterase. It's interesting to note that ACR increases acetylcholinesterase activity levels (15). Additionally, of counteract the reduction in dopamine levels caused by ACR in some brain areas. The results were consistent with (16) and showed that ACR dramatically increased the activity levels of AChE. AChE activity is increased in Alzheimer's disease patients, which accelerates acetylcholine breakdown and lowers cognitive performance moreover, AChE activity, is a sign of cholinergic dysfunction, which has been demonstrated to be correlated with elevated oxidative stress. According to (17) ACR toxicity is caused by

pro-inflammatory cytokines, oxidative damage, and decreased antioxidant activity in the kidneys, liver, and brain (3). (18) Suggested that, curcumin therapy led to substantial increases in AChE activity. Other findings revealed that rotenone-induced deficiencies in a variety of areas might be reduced by curcumin-induced restoration of cerebellar AChE activity and increased expression of AChE (19). Curcumin therapy decreases mRNA expression and has anti-AChE action in the rat cerebral cortex (20).

Y maze test (spatial memory)

In this study there were significant reduction ($p < 0.05$) in the succession of arm entries in spatial memory Y maze test, it was observed in ACR group (36.85 ± 1.34) at 40 days of the experiment compared with ACR+CUR group (50.87 ± 1.17) and control group (55.58 ± 4.20) at the same period. Moreover, there is significant reduction on the succession of arm entries ($p < 0.05$) in ACR+CUR group (50.87 ± 1.17) compared with control group (55.58 ± 4.20) as shown in (Figure 3).

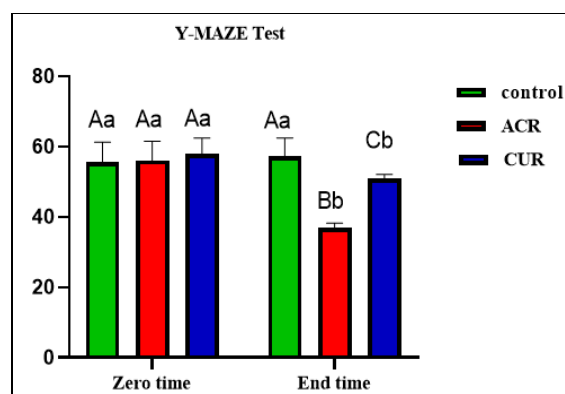


Figure (3): Effect of Acrylamide and Curcumin on spatial memory tested by Y maze test in adult male rats

*Upper-case letters indicate the differences between groups in the same period of time

*Lower-case letters indicate the differences between time periods, 40 days vs. zero time for the same group

* Data represented as mean \pm SEM. N=8 per group

The present results show spatial memory impairments and increase the anxiety in rats administered ACR, however Cur repair partially of the affected

parameters. ACR severely reduced spatial working memory ability. additionally, it hindered learning (21), According to a number of studies, ACR in-

creases oxidative stress and free radical generation, which in turn promotes lipid peroxidation and changes the blood-brain barrier's structural elements (22). ACR reduced inhibitory neurotransmitter activity, which negatively impacted hippocampus neuronal function. In addition, cognitive decline and hippocampus cell death were seen in the rats. This finding can be connected to the striking changes in dopamine and Ach-s, which serve as indicators of neuronal injury. According to a significant amount of experimental evidence from animal research, free radical overproduction has been connected to functional changes in the brain tissue (23). However, researches showed that curcumin has neuroprotective effects on spatial memory. memory deficit was averted by

curcumin, most likely by halting changes in oxidative stress in the hippocampus. This study showed that these substances could be potential treatment options for neurodegenerative diseases. Even though, the synthetic curcumin greatly improved memory, protected against neuronal degeneration, and restored memory deficiencies, which is consistent with published findings (24).

Open field test

In the current experiment there was significant elevation ($p < 0.05$) in the time spent in the center of the platform in 5 minutes at the end of experiment in the animals exposed to ACR (0.58 ± 0.11) compared with animals treated with curcumin (0.33 ± 0.22) and control group (0.24 ± 0.12) as illustrated in (Figure 4).

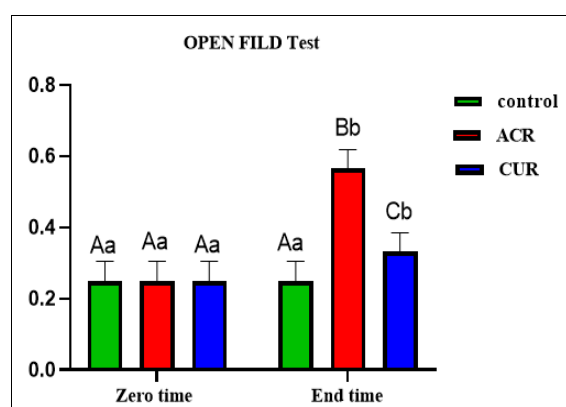


Figure (4): Effect of Acrylamide and Curcumin on the locomotor activity tested by Open Field test

*Upper-case letters indicate the differences between groups in the same period of time

*Lower-case letters indicate the differences between time periods, 40 days vs. zero time for the same group

* Data represented as mean \pm SEM. N=8 per group

In the open field test, the amount of locomotor activity and exploratory behavior reduced after the administration of ACR, ACR promotes hypolocomotion, which drastically shortens the walk in open field test's distance and duration in order to measure emotional activity. Dopamine and GABA levels were significantly decreased as a result of ACR. The Curcumin's ability to protect against neurotoxicity and the result-

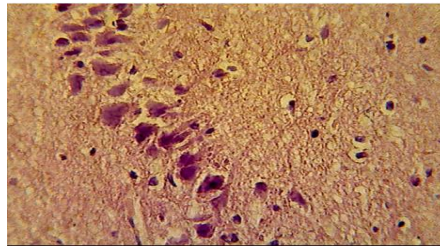
ing locomotor hypoactivity. By functioning as an antioxidant and an anti-lipid peroxidative against a variety of oxidative stimuli, Cur is known to be able to reduce oxidative damage. This antioxidant action of cur has been linked to the presence of chain-breaking or hydrogen-donating phenolic groups in its molecular structure, in addition to being demonstrated to scavenge NO-based radicals. Cur is a potential medi-

cine for both the prevention and therapy of neurodegenerative illnesses because its polyphenolic nature allows it to pass the blood-brain barrier and bind redox metal ions.

Histopathological study of the brain

The histological examination results demonstrated the detrimental impact of an Acrylamide on the tissue of rats' brains as well as Curcumin anti-inflammatory and antioxidant properties, as well as its involvement in minimizing that impact. Brain sections apparent in the cerebrum Figure (5) of control group show no lesions. In contrast, the sections of brain that obtained from group of ACR show marked

perivascular microglial cell proliferation and oligodendrocytes attached to necrotized neurons as well as vacuolation and congested blood vessels in the ependymal layer of the cerebrum, moreover, there is a proliferation of microglial cells and Alzheimer type II astrocyte (Figures 6,7,8, and 9). Whereas, brain section of CUR+ACR animals show inflammatory proliferation of astrocytes and microglia, as well as congested blood vessels with proliferation of microglial cells of the cerebrum, in addition, mild congested blood vessels in the pia mater can be seen as well (Figures 10,11, and 12).



Figure(5): Section in the cerebrum of control group shows no clear lesions 40X (H and E).

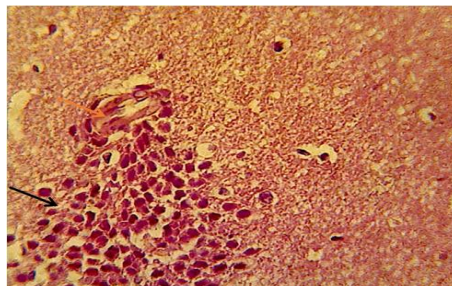
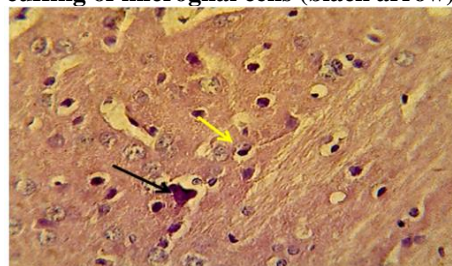


Figure (6): Section in the cerebrum of rat treatment with ACR group shows marked perivascular (orang arrow) cuffing of microglial cells (black arrow) 40X (H and E)



Figurer (7): Section in the cerebrum of rat treated with ACR shows proliferation of oligodendrocytes (yellow arrow) with neuronal swelling (black arrow) 40X (H and E)

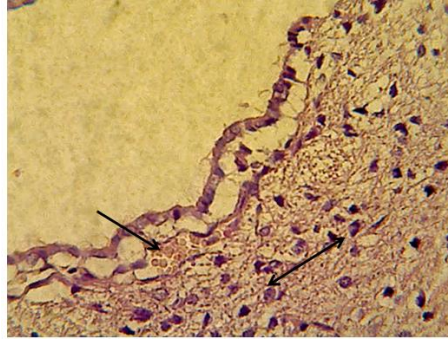


Figure (8): Section in cerebrum of animal treatment with ACR group shows vacillation (black double-headed arrow) and congested of meningeal vessels (black arrow) 40X (H and E)

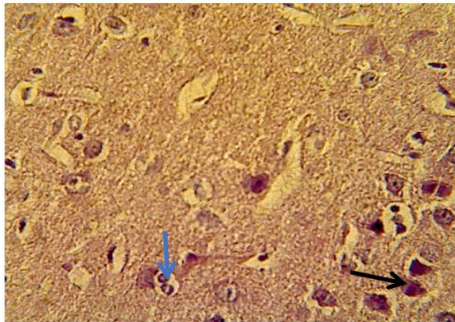


Figure (9): Section in cerebrum of animal treatment with ACR group shows marked neuronal swelling (black arrow) with evidence Alzheimer type II astrocyte (blue arrow) 40X (H and E)

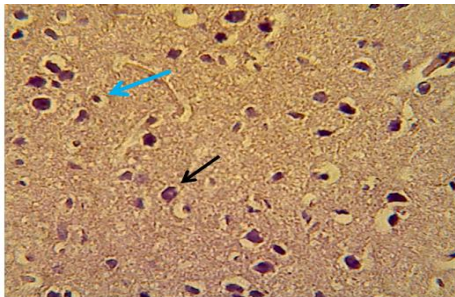


Figure (10): Section in the cerebrum of rat treated with ACR+ CUR group shows proliferation of astrocytes (black arrow) and oligodendrocytes (blue arrow) 40X (H and E)

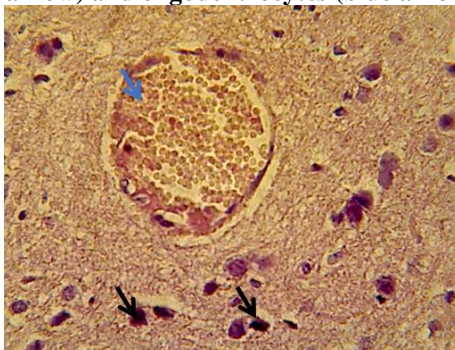


Figure (11): Section in the cerebrum of rat treated with ACR+CUR group shows congested blood vessels (blue) with proliferation of microglial cells (black) 40X (H and E).

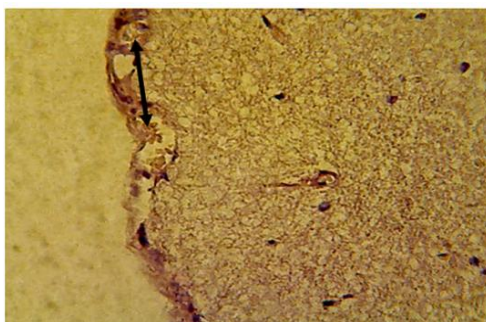


Figure (12):Section in cerebrium of one rat treatment with ACR+ CUR group shows mild congested of meningeal vessels (black double-headed arrow) 40X (H and E).

The behavioral and biochemical findings concur with the histology findings. Our histology findings showed that ACR animals had structural changes in their cerebrum. It has been shown that exposure to ACR disturbs the equilibrium between the production and removal of free radicals, the latter of which is mediated by antioxidant mechanisms. Additionally (14) revealed that, Pro-inflammatory cytokines, oxidative damage, and a decline in antioxidant function in the brain are what lead to ACR toxicity. The elevation in AChE activity in the brain may be a sign of damage to neurons as it elevates brain neurotransmitters, stimulates inflammatory cells, and increases the levels of inflammation in the brain as well. This study agrees with (25) who revealed that the examination of the histological alterations in the rat brain's cerebral cortex treated with ACR showed neuronal necrosis, shrinkage, and pyknosis in addition to neuronophagia the examination of the histological alterations in the rat brain's cerebral cortex treated with ACR revealed neuronal necrosis, shrinkage, and pyknosis in addition to neuronophagia (25). The current study's findings suggested that curcumin may be utilized to prevent or delay the neurological damage caused by ACR exposure by having anti-apoptotic, anti-

inflammatory, and antioxidant effects on ACR-induced neurotoxicity in rats. Importantly, the results of this study showed that curcumin treatment may effectively repair brain damage caused by ACR. As a crucial occasion in the regulation of neuronal cell quantity. The number of cells can be maintained, the severity and progression of brain illnesses may be decreased, and Alzheimer's disease can be slowed down. Additionally, anti-apoptotic capability of curcumin's impact against exposure to ACR was demonstrated by the significantly reduced ACR-impaired brains apoptotic nerve cells in the hippocampus and cortex, and the dual functions in the elimination of ROS amount within cells, which appeared to be extremely cell type dependent (14).

Conclusion

Acrylamide induced neurobehavioral impairments in adult rats by inducing neurotransmitters deficits and pathological changes in brain, however these changes can be avoided by Curcumin administration.

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