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Protective Role of 5-Aminosalicylic acid and Vitamin-E against the Acrylamide Induced Neurotoxicity in Rats

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ABSTRACT

The aim of this study was to characterize the toxic effect of acrylamide (ACR) on sciatic nerve and brain of rats; and examine the protective effect of 5-aminosalicylic acid (5-ASA) and vitamin-E on sciatic nerve and cerebrum injury induced by acrylamide. This study was performed at King Fahad Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. A total of 49 adult wistar rats (250 ± 20g) of 60 days age were divided into seven groups (control, acrylamide alone, acrylamide + 5-ASA, acrylamide + vitamin -E, acrylamide + 5-ASA + vitamin-E, vitamin-E alone, 5-ASA alone). After 5 days of acrylamide treatment, rats were observed for 24 hours and sacrificed. Histopathology for the brain and sciatic nerve were performed. Administration of acrylamide produced neuronal damage in rats. No significant changes were observed in lactate dehydrogenase serum level and rats' body weight. Injection of acrylamide treated rats with vitamin-E and 5-ASA concomitantly showed strong improvement in general histology of neurons. However, good improvement in morphology of sciatic nerve was observed after injection of 5-ASA to ACR-treated rats. Compared to this improvement by 5-ASA, treatment of vitamin-E to ACR-treated rats also exhibited marked improvement in morphology of sciatic nerve (myelin and vacuolar-like degeneration). We concluded that ACR induced neuronal damage in nervous tissue of rats mainly by the induction of lipid peroxidation. 5-ASA and vitamin E as powerful antioxidants, played a protective role against acrylamide neurotoxicity. On histological level, Vitamin-E showed more protection in comparison to 5-ASA.

Keywords: Acrylamide, 5-Aminosalicylic acid, Antioxidant, Neurotoxicity, Vitamin-E.

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INTRODUCTION

Acrylamide (ACR) is a colorless, odorless, crystalline, highly water soluble vinyl monomer formed from the hydration of acrylonitrile. It is used in the production of polyacrylamides mainly in the sewage and water treatment industry, pulp and paper industry, textile treatment industry and is used as a laboratory reagent for electrophoresis, chromatography and electron microscopy. ACR is also a food contaminant formed in carbohydrate rich food when cooked at high temperatures.[1] The main chemical process that results in the production of ACR is known as the Maillard Reaction; it is the same reaction that 'browns' food and affects its taste. Acrylamide forms from sugars and amino acids (mainly one called asparagine) that are naturally present in many foods.[1] ACR was shown to be a neurotoxicant, reproductive toxicant, and carcinogen in animal species.[2] Extensive studies in rodents and other laboratory animals have reported that exposure to monomeric ACR causes cellular damage in both nervous and reproductive systems, and causes tumours in certain hormonally responsive tissues.[2,3] A significantly elevated incidence of neurotoxicity induced by ACR in occupationally exposed workers was reported previously.[3] In 1994 the International Agency for Research on Cancer (IARC) categorized acrylamide as "potentially carcinogenic to humans".[4] While in 2001, the Scientific Committee on Toxicity, Ecotoxicity and the Environment (SCTEE) reported its inherent toxicity properties: neurotoxicity, genotoxicity to both somatic and germ cells, carcinogenicity, mutagenicity and reproductive toxicity.[5]

Biological activity of ACR is mainly due to adduction with protein, while its primary activity with DNA is alkylation, mainly due to its active metabolite glycidamide.[6] The two main pathways elaborated for ACR metabolism are: glutathione conjugation and epoxidation to glycidamide (GA).[7] Cytochrome P450 E1 (CYP2E1) is believed to be the main enzyme responsible to mediate the formation of glycidamide from ACR.[8,9] During this metabolic process by CYP2E1, ACR has been found to cause the release of free radicals-reactive oxygen species (ROS), which initiate the oxidative stress resulting the imbalance between production and destruction of ROS, hence leading to lipid peroxidation along with DNA and protein alterations.[7,10]

Although the mechanism involved in ACR neurotoxicity is not well understood, however recent studies suggested that acrylamide neurotoxicity is due to cerebellar purkinje cell injury and axonal degeneration in peripheral and central nervous system.[3] Moreover, recent studies have shown that acrylamide neurotoxicity is associated mainly with the increase in lipid peroxidation

(LPO) and the reduction of the antioxidative power in nervous tissue mostly caused by a primary depletion of reduced glutathione (GSH).[11,12] Recent studies have unfolded the incapability of ACR to activate the nuclear factor erythroid 2-related factor 2-antioxidant-responsive element (Nrf2-ARE) pathway in the nerve terminal which makes them susceptible to oxidative damage.[13] Moreover, it has been found that ACR increases lipid peroxidation, declines antioxidant capacity in nervous tissue and sciatic nerve and induces apoptosis in cerebral cortex.[12,14]

In general, antioxidants, such as Vitamin-E and 5-aminosalicylic acid (5-ASA) play a major defensive role against oxidative stress caused by ROS generation induced by ACR .[15] Vitamin-E is lipid soluble compound; its most active biological form is α-tocopherol which obstructs lipid peroxidation by supplying hydrogen atom to ROS in lieu of polyunsaturated fatty acids present in lipid membrane .[15] It has been observed that 5-ASA has antioxidant and anti-inflammatory role, which strongly protects liver from ROS mediated damage.[7] Even though the mechanism of ACR-induced neurotoxicity and the protective role of various natural antioxidants against ACR-induced neurotoxicity have been studied. Still, less research has been done on the sciatic nerve injury induced by ACR and the protective role of antioxidants. Furthermore, no research has been done to compare the protective role of 5-ASA & vitamin -E on the sciatic nerve & brain injury induced by ACR.

The theoretical account of this study is that 5-ASA may have protective effect against ACR induced sciatic nerve injury, which will be compared to the antioxidant effect of vitamin-E. This would be potentially due to their anti-inflammatory and anti- antioxidative effect, which empowers 5-ASA and Vitamin-E to prevent the increase in lipid peroxidation; decline in antioxidant capacity in nervous tissue and sciatic nerve; and apoptosis in cerebral cortex. Therefore, in the current study we will characterize the toxic effect of acrylamide on sciatic nerve and brain of rat; and examine the protective effect of 5-ASA and vitamin-E on sciatic nerve and cerebrum injury induced by acrylamide. This study can be carried out in two parts:

(a) Inducing toxic neuronal changes in the neural tissue by exposing rats to high toxic dose of ACR.

(b)Investigation of antioxidant effect of 5-ASA and Vitamin-E on ACR-induced neurotoxicity in rats.

MATERIALS AND METHOD

Materials

Plus one acrylamide (PAGE) grade with purity >99.95 was purchased from Pharmacia Biotech (Upsala, Swedan). 5-ASA 95%, Vitamin-E (DL-α-tocopherol Acetate) and > 98% HPLC (High Performance Liquid Chromatography) was purchased from Sigma-Aldrich (Steinheim-Germany). Unless otherwise mentioned, other chemicals and materials of molecular biology grade purchased from BHD laboratory supplies (Analar®, England) were used in carrying out the study.

Animals and Treatment

Forty-nine adult male Wister rats (250 ±20 g), 60 days old on arrival, were purchased from King Fahad Medical Research Centre (KFMRC), Jeddah, Kingdom of Saudi Arabia (KSA) and were housed four per polypropylene cage with wood shavings as bedding. Animals were maintained in controlled environment of temperature 22 ± 2°C, relative humidity of 40 - 65 % and 12 hours / 12 hours light / dark cycles throughout the experiment. The rats were fed laboratory chow and an adlibitum supply of tap water.

All animal care procedure and treatments were carried out at KFMRC. Experimental design and animals were approved by the Unit of Biomedical Ethics, King Abdulaziz University (KAU), Medical College, Jeddah, KSA in accordance with the guidelines of the KAU. These guidelines follow the national and international laws and policies (National Institutes of Health Guiding Principles on the Care and Use of Laboratory Animals, USA).

Rats were allowed to acclimatize at the experimental environment for 3 days before dosing initiation. The rats were grouped into seven groups (n=7). Group I normal control rats were fed with laboratory chow and tapped water throughout the experimental period. Group II rats were treated with ACR (45 mg/kg bw/day) by oral gavage, using orogasteric needle.[7] Group III rats were administered with ACR+5-ASA (25mg/kg bw/day).[7] Group IV rats were treated with ACR +Vitamin-E (200mg/kg bw/day).[16] Group V rats were treated with ACR +5-ASA +Vitamin-E. Group VI and VII rats served as drug control groups and were given Vitamin-E alone (200 mg/kg bw/day) and 5-ASA alone (25mg/kg bw /day) respectively. 5-ASA was given by intraperitoneal injection and Vitamin-E was gavaged orally throughout the experiment. The experiment continued for 5 consecutive days. All rats were weighed and checked daily for sudden death or any abnormal behaviour during the experiment and recovery period.

After 5 days of treatment, blood was collected from retro-orbital sinus of the left eye and centrifuged for further use of the produced serum. After a recovery period of one day of ACR cessation, animals were sacrificed by cervical dislocation and the left sciatic nerve together with

section of frontal lobe of left cerebral hemisphere of all rats were isolated for further experimental evaluation.

Histopathology

Tissue Preparation and Histopathological Examination

Sciatic nerve and frontal lobe of left cerebral hemisphere of all rats were isolated and fixed immediately by 10% natural buffered formalin for 24 hrs. Following overnight fixation, the brain (left cerebral hemisphere) was cut coronally and sliced. Tissues were then processed using automatic tissue processor (Shandon, England) by using standard laboratory procedures for histology. Tissues were briefly embedded in paraffin blocks, sectioned and then stained with Periodic acid-Schiff (PAS). Tissues were examined for histological changes using light microscopy (Olympus BX51TF) at 10X, 20X, 40X magnification and representative images were captured with Olympus DP 72 camera. Brain sections were evaluated in terms of cerebral cortex layers and any abnormal neuronal cells.

Biochemical Analysis

Lactate Dehydrogenase (LDH) Assay

Lactate dehydrogenase (LDH) is commonly found in the cytoplasm within different mammalian bodies and can be easily determined by using quantitative data measurements obtained by Dimension Vista® System and Flex® reagent cartridge. The reaction took place within 96 micro-well plate where all reagents are ready to use liquid solutions and the available wells that numbered from one to eight contained different concentration of N-Methyl-D-glucamine and L-(+)-lactate and sodium chloride (NaCl) while the last four wells involved with β-nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide lithium salt, added with preservatives and stabilizers. The enzyme linked immunoassay began with alkaline buffer solution of pH (9.4) that included a substrate. In fact, the presence of NAD^+ would help the enzyme (LDH) to oxidize the substrate which resulted in formation of pyruvate and NADH that absorbs light at 340 nm and can be measured. These readings can be measured at 340/ 700 nm and are directly proportional to the lactate dehydrogenase levels in the serum.

Statistical Analysis

All statistical analysis was done using SPSS (Statistical package for the Social Sciences) 16.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± 2SD. Differences among the groups were analysed by one-way analysis of variance (ANOVA) followed by the Tukey's test as a post hoc for multiple comparisons. A P -value of less than 0.05 was considered as criterion for a statistically significant difference.

RESULTS AND DISCUSSION

General Observation

Rats gavaged with ACR (45 mg/kg bw/day) showed signs of aggression and rough coat, with reduction in food and water intake. Improvement in water and food intake was detected in group treated with ACR and 5-ASA. Further, rats in the control group showed no symptoms of illness or mortality during the experimental period. No mortality or hind limb disability was reported among ACR treated or other groups.

Effects of Acrylamide and Antioxidants on Body Weight Changes of Rats

Treatment of ACR to rats at a dose of 45 mg/kg bw/day for 5 consecutive days did not show any significant difference in body weight change between groups, one day after cessation of ACR treatment (Figure 1). At the end of observation body weight changes were determined. Oral administration of vitamin-E to rats at a dose of 200mg/kg bw/day, and intraperitoneal (IP) injection of 5-ASA at a dose of 25mg/kg bw/day with gum acacia as a solvent, concomitantly with oral ACR treatment, did not show change in body weight of rats. Data were expressed as mean ± 2SD (twice the value of standard deviation), n = 7. No statistically significant difference was detected between groups.

Effect of ACR and Antioxidants on Serum Lactate Dehydrogenase (LDH) Concentration

In this study, no significant difference ($P > 0.05$) of serum lactate dehydrogenase concentration was detected among all groups (Figure 2).

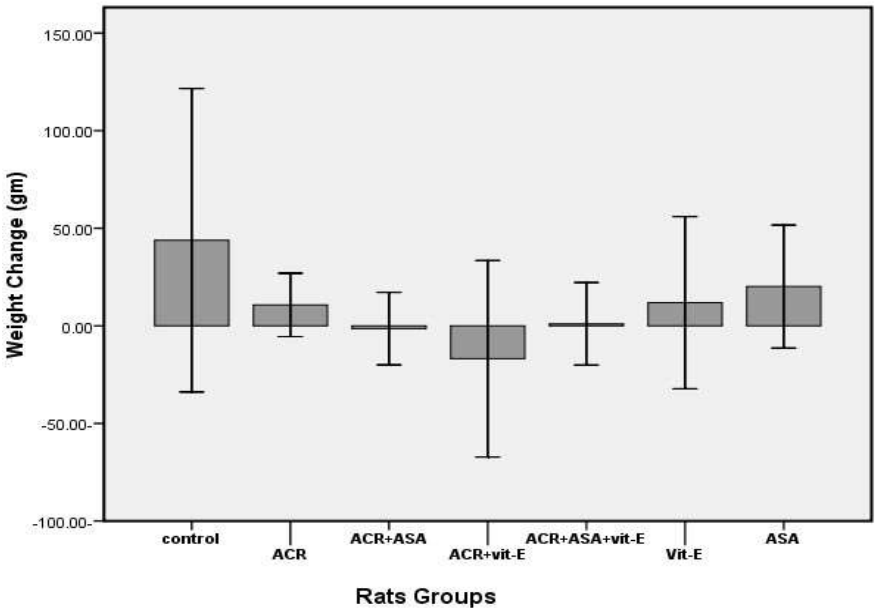

Figure 1: Effect of acrylamide (ACR) and antioxidants on body weight changes of rats at the end of observation period.

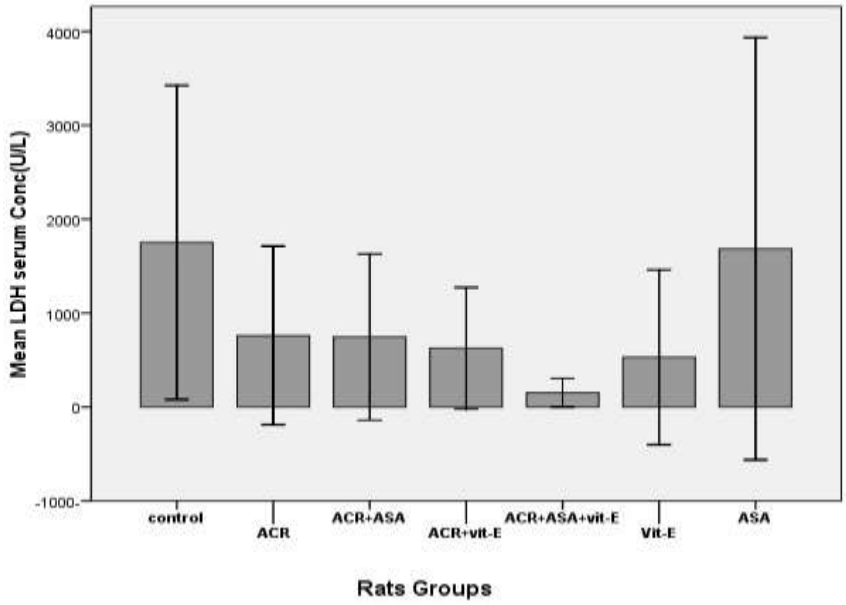

Figure 2: Effect of acrylamide (ACR) and antioxidants on serum lactate dehydrogenase (LDH) concentration of rats.

Effect of ACR and Antioxidants on Histology of Sciatic Nerve and Cerebral Cortex

In histopathological examination, control frontal lobe cerebral cortex showed six normal layers starting with molecular layer and ending with fusiform layer. It also showed apparent normal neurons in the second layer of external granular lamina with abundant pyramidal cells (Figure 3A, 3B). The normal pyramidal cells showed their general characteristic shape. The nuclei of these cells were rounded, large and centrally located and the neurons were arranged in a regular pattern (Figure 3C).

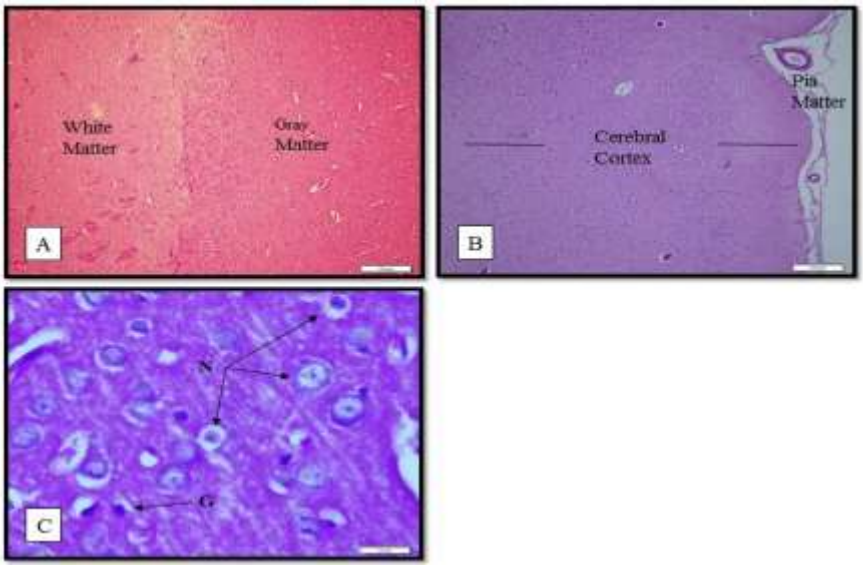

Figure 3: Photomicrograph of a section of cerebral cortex taken from control rat. (A) and **(B)** Shows normal arrangement of layers and normal cells; **(C)** Shows magnified neurons of

cerebral cortex (100X magnification, N-neuron, G-glial cells). Sections were stained with PAS stain and viewed with light microscopy (A and B scale bar =100 μm).

After acrylamide treatment, cerebral cortex showed increase in number of dark abnormal neurons with pyknosis, increased basophilic chromatin and absence of nuclei with small cell bodies which are scattered throughout all layers of cerebral cortex (Figure 4A). These pathological changes indicate neuronal degeneration. Improvement in cerebral cortex morphology after the treatment of 5-ASA and Vitamin-E was also observed (Figure 4B, 4C).

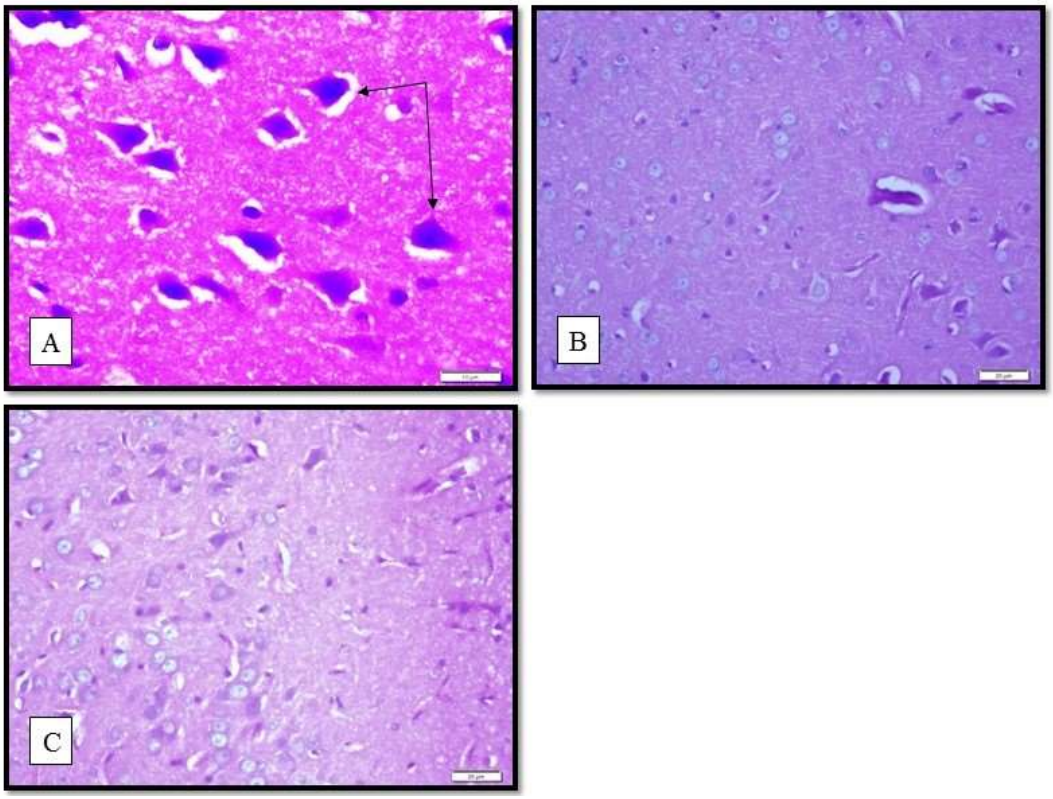

Figure 4: Photomicrograph of a section of cerebral cortex taken from acrylamide treated rats. (A) Shows abnormal pyknotic dark cells (arrows) with absent nucleoli and small cell bodies; **(B)** Shows improvement in cerebral cortex morphology after 5-aminosalicylic acid treatment; **(C)** Shows improvement in cerebral cortex morphology after vitamin-E treatment; Sections were stained with PAS stain and viewed with light microscopy at 100X magnification (scale bar = 10 μm).

Cross section of sciatic nerve of normal control rat showed densely appeared myelin, round and uniform with ordered lamellar structure presenting neither axonal shrinkage nor its swelling (Figure 5A, 5B). On the other hand, sciatic nerve from acrylamide treated rats showed reduction in the number of axons with axonal atrophy and swelling with myelin vacuolization and shrinkage indicating demyelination (Figure 5C, 5D). However good improvement in morphology

of sciatic nerve was observed after injection of 5-ASA to ACR-treated rats (Figure 5E, 5F). Compared to this improvement by 5-ASA, treatment of vitamin-E to ACR-treated rats also exhibited marked improvement in morphology of sciatic nerve (myelin & vacuolar-like degeneration) (Figure 5G, 5H). However, concomitant treatment of acrylamide treated rats with both antioxidants did not show improvement in cerebral cortex or sciatic nerve morphology.

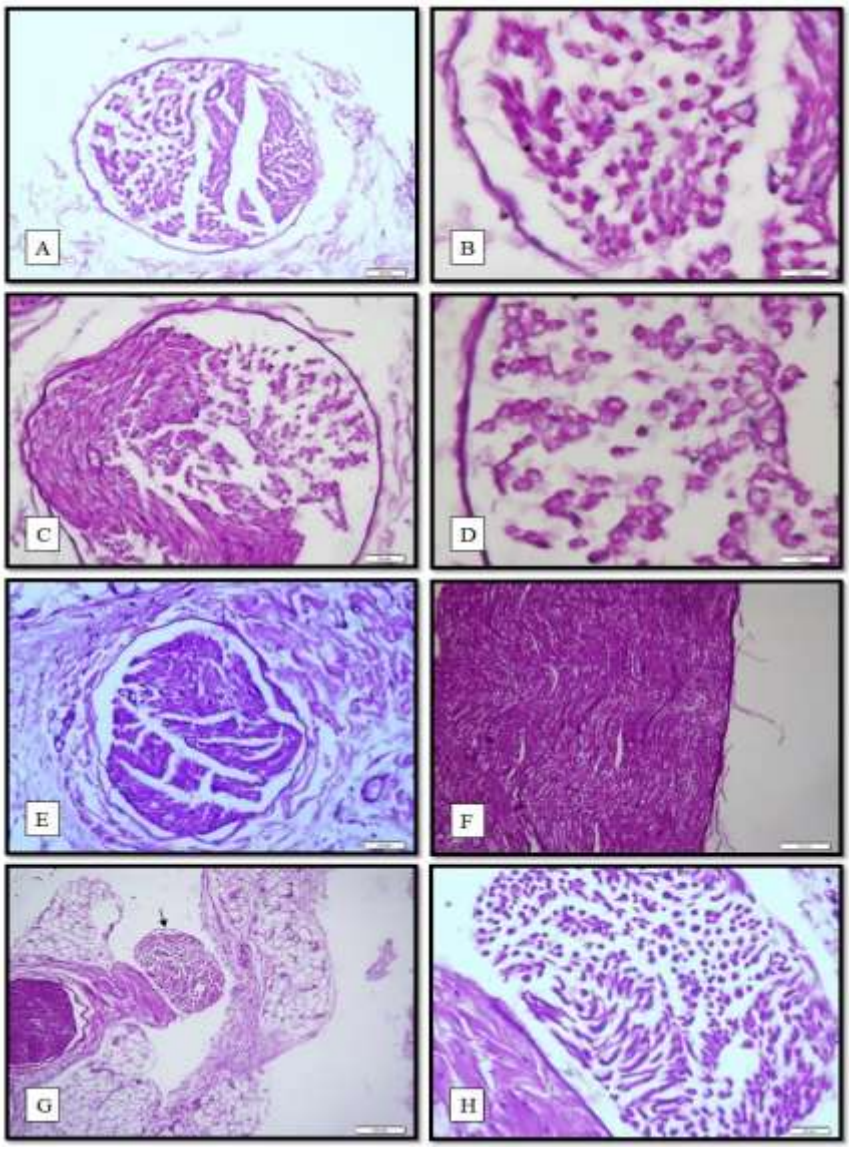

Figure 5: Light microscopy of cross section of sciatic nerve from control, acrylamide treated, acrylamide + 5- aminosalicylic acid, and acrylamide + vitamin-E treated rats. (5A and 5B) Control shows densely apprered myelin, round and uniform with ordered lamellar structure presenting neither axonal shrinkage nor its swelling; **(5C and 5D)** Shows axonal atrophy and swelling with myelin vacuolization and shrinkage from acrylamide treated rats; **(5E and 5F)** Shows improvement in the morphology of sciatic nerve after administration of 5-

amonisalicylic acid to acrylamide treated rats; **(5G and 5H)** Shows marked improvement in the morphology of sciatic nerve in the form of decrease in myelin (arrow) & vacuolar-like degeneration after the administration of vitamin-E treatment to acrylamide treated rats.

DISCUSSION

Acrylamide is a known neurotoxicant to humans, which on longterm exposure might lead to toxic accumulation and results in neurotoxicity.[3,17] The current study aimed to characterize acrylamide induced neurotoxicity on brain and sciatic nerve of male rat . We also examined and compared the possible antioxidant effects of 5-ASA and Vitamin-E on the induced central and peripheral neurotoxicity in rats.

The current study showed that administration of ACR to rats at a dose of 45 mg/kg/day for a period of 5 continuous days had no effect on body weight change of rats. This result was similarly to the study carried out by Lafferty J. et al.[18] where ACR treatment did not show any significant difference in rats' body weights. Contradictory to our result, other researchers revealed a significant reduction in body weight and food intake in experimental rats, after administrated of ACR.[7]

In the current study, the histological examination showed that acrylamide caused neurotoxicity in brain cerebral cortex in the form of neuronal degeneration and peripheral axonal demylination of sciatic nerve. This finding is in consistency with several other previous studies which also exhibited the brain toxicity upon the exposure to acrylamide.[19,20,21] Moreover, peripheral demylination of peripheral spinal nerves after acrylamide treatment was reported in earlier studies.[22,23,24] However, one of the studies was not able to reveal this peripheral neuropathy in sciatic nerve after acrylamide treatment.[25]

While the mechanism behind the acrylamide neurotoxicity is unclear but several theories have been reported to explain the acrylamide neuronal damage.[21] Earlier studies had made it evident that nerve end degeneration is the initial trigerring sequelae of acrylamide toxicity in peripheral and central nervous system.[26,27] Recent studies indicated that acrylamide induces neurotoxicity because of its great ability to produce reactive oxygen species (ROS) that causes cellular damage and initiates series of cell membrane damage with induction of lipid peroxidation in cerbral cortex and cerebellum causing oxidative stess.[11,12,19] Further, acrylamide causes marked depletion of glutathione (GSH) in cerebral cortex and cerebellum, which makes the brain tissue more sensitive to oxidative stess.[19] Researches on the acrylamide neurotoxicity suggest that antioxidants might be considered as powerfull antagonists to acrylamide induced oxidative stress

and ACR-induced neurotoxicity.[7,14,19] In the current study treatment of 5-ASA and vitamin-E to ACR treated rats showed good protection of cerebral cortex and sciatic nerve by reducing abnormal dark neurons scattered throughout cerebral cortex and restoration of mylein sheath demylination seen in cross section of stained sciatic nerve. However, vitamin-E showed more protection against acrylamide neurotoxicity than 5-ASA. Vitamin-E has been considered as a neuroprotective compound in many studies and its antagonistic action is believed to be due to its antioxidant activity.[20,28] So, the result of our study is consistent with the studies determining the neuroprotective role of vitamin-E.[20,28] Recent study also revealed antiapoptotic effect of vitamin-E against acrylamide produced apoptosis in neural tissue.[28] It has been studied that 5-ASA as antioxidant has protective role against reproductive toxicity induced by acrylamide.[7] In the current work 5-ASA proved effective against ACR induced neurotoxicity.

CONCLUSION

We concluded that ACR induced neuronal damage in nervous tissue of rats mainly by the induction of lipid peroxidation. 5-ASA and vitamin E as powerful antioxidants, played a protective role against acrylamide neurotoxicity. On histological level, vitamin-E showed more protection in comparison to 5-ASA. Further investigations like immunohistochemistry of brain and sciatic nerve, and staining of nervous tissue with osmic acid stain to assess myelin sheath accurately, are required to understand the molecular basis behind ACR-induced neurotoxicity. Biochemical assay to determine lipid peroxidation by the assessment of thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) of brain tissue should be carried out. Therefore, we advocate restraint of ACR exposure either occupationally or in food products.

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