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DOI: 10.5603/FM.a2018.0105

Article type: ORIGINAL ARTICLES

Submitted: 2018-09-13

Accepted: 2018-10-10

Published online: 2018-10-29

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Acrylamide adverse cerebellar changes in rats: possible oligodendrogenic role of omega 3 and green tea

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Abstract

Background: Humans are widely exposed to Acrylamide (ACR) and its neurotoxicity is a significant public health issue attracting wide attention.

Aim of work: Investigating ACR-induced adverse cerebellar changes in rats and studying the possible Oligodendrogenic role of Omega 3 and Green Tea.

Material and Methods: Twenty four adult albino rats weighing 150 -200gm were randomly divided into four equal groups (6 rats each) as follows; Group I: (control), Group II: The rats received ACR 45mg/kg/day, Group III: The rats received ACR concomitant with Omega3 at a dosage of 200mg/kg/day, Group IV: The rats received ACR concomitant with Green Tea dissolved in drinking water at a dosage of 5gm/litre. The rats were euthanized 8 weeks from the experiment. Malondialdehyde (MDA) and Glutathione (GSH) were measured in cerebellar homogenates. Sections of 5 µm thickness from specimens from the cerebellum were stained with Hx & E, Silver stain and immunohistochemical stains; PDGFα (for Oligodendrocytes), GFAP (for Astrocytes) and BCL2 (Antiapoptotic).

Results: Omega3 and Green Tea had improved MDA & GSH as compared to ACR group. Histologically, ACR group showed variable degrees of cellular degeneration. Omega3 had induced Oligodendrogenesis in group III. The optical density of silver stain was significantly p<0.05 increased in group III & IV as compared to ACR group. Area % of positive PDGFα was significantly increased in ACR+omega3 group as compared to ACR group. Area % of positive GFAP was significantly decreased in...
group III & IV as compared to ACR group. Area % of positive BCL2 was significantly increased in omega3 received groups as compared to ACR group.

**Conclusions:** Concomitant administration of Omega 3 or green Tea with ACR might mitigate its adverse cerebellar changes with an Oligodendrogenic role of Omega 3.

**Key words:** acrylamide, cerebellum, omega 3, green tea, rats, oligodendrocytes

**INTRODUCTION**

Acrylamide (ACR) is an industrial neurotoxic chemical that has been found in carbohydrate-rich foods cooked at high temperatures such as potato chips [15]. Therefore, humans are widely exposed to ACR and its neurotoxicity in humans is a significant public health issue attracting wide attention [19]. It was also associated with carcinogenicity and reproductive toxicity [6]. The neurotoxicity of ACR has been known to affect nerve terminal and cysteine residues on the functionally important presynaptic proteins, resulting in inhibition of neurotransmitter release and eventual process degeneration [25, 26]. Cerebellum controls the maintenance of equilibrium (balance), influences posture and muscle tone as well as coordinating movement [8]. Cerebellar ataxia might be acquired from exposure to many toxic materials [39]. Myelin is an electrical insulator that increases conduction velocity of nerve fibers and is the physical basis for rapid saltatory conduction (in which impulses jump from one node of Ranvier to another) [29]. Inflammatory damage to the oligodendroglia and white matter is involved in the pathogenesis of Demyelinating diseases such as Multiple Sclerosis [5]. Oligodendrocyte is the cell responsible for producing CNS myelin by the concentric layers of its plasma membrane [34]. It is located in both the gray and the white matter of the CNS; Interfascicular oligodendrocytes (white matter) and Satellite oligodendrocytes (gray matter only) [14].

The observation that Eskimos had a very low incidence of atherosclerosis and its complications, had led to the first indication of a protective effect of fish oil on atherosclerosis. For so, today fish oil is one of the most popular nutraceuticals available in health food stores [27]. Surprisingly, fish oil preparations are also anti-arrhythmic, particularly in patients who have already suffered a myocardial infarction [9]. In addition to the treatment of hypertriglyceridaemia, a preparation of omega 3-acid ethyl esters is licensed in the UK for the prevention of recurrent events after
myocardial infarction [32]. Recently, the combined treatment of fish oil dietary supplement and omega-3 polyunsaturated fatty acids injections had been proved to promote post-traumatic brain injury (TBI) restorative processes in the brain, including generation of immature neurons, microvessels, and oligodendrocytes, each of which was significantly correlated with the improved cognitive recovery [30].

Green Tea extract had been partially efficacious in preventing neurodegeneration in the brain of lead treated rats resulting from its inhibition of free radical chain reactions generated during oxidative stress caused by lead and from an increase in antioxidant enzyme capacity [23]. Over the last two decades polyphenols, one of Green Tea constituents, have drawn attention as promising natural dietary molecules for the prevention of aging and neurodegenerative diseases [22]. Recently, dietary polyphenols, endowed of antioxidant and anti-inflammatory properties, had been reported to extend brain health span as they were actively investigated as potential adjuvants to support proliferation and survival of neural progenitors, and counteract age-dependent neurogenic decline [36].

The present study deals with the influence of green tea extract and Omega-3 fatty acids in Acrylamide-induced adverse cerebellar changes in rats.

**MATERIAL AND METHODS**

**Animals**

The experiment was ethically approved by the Institutional Animal Care and Use Committee of Cairo University (CU-IACUC) under the number of CU/III/F/48/18. The present study was carried out on twenty-four male albino rats weighing 150-200. The animals were housed in cages, five rats/cage, under standard laboratory and environmental conditions with free access to food and water at a temperature of (20±2°C) with a natural 12-h light/dark cycle and free access to standard pellet chow and drinking water ad libitum. Animals were obtained from Animal House, Faculty of Medicine, Cairo University. The rats were set in the laboratory for a period of two weeks for acclimatization before carrying out the experiment.

**Chemicals**

1. **Acrylamide**: The dose of acrylamide used was 45mg/kg/day [31].

   Acrylamide (99% pure) was purchased from Sigma Chemical Company (St
Louis, Missouri, USA). It was dissolved in distilled water and offered every
day by oral gavage.

2. **Omega 3 plus**: Omega-3 fatty acids (Sedico, Egypt) was given in a dosage of
(200 mg/kg/day; DHA: 100 mg/kg/day + EPA: 100 mg/kg/day) which was
offered every day by oral gavage [38].

3. **Green Tea**: Green tea. Royal Regime Tea, packed in Egypt by Royal Herbs,
was dissolved in the drinking water at a concentration of 5 g/L. Tea was
prepared freshly three times per week and stored at 48°C until use. The content
of drinking vessels was renewed every day [23].

**Experimental design**

The rats were randomly divided into four equal groups (6 rats each) as
follows:

- **Group I (control group)**: The rats received no medications.
- **Group II**: The rats received Acrylamide.
- **Group III**: The rats received Acrylamide concomitant with Omega 3.
- **Group IV**: The rats received Acrylamide concomitant with Green Tea.

All animals were sacrificed after eight weeks (the end of the experiment) by
decapitation using Guillotines to avoid brain injury [10]. Just before sacrifice, the
body weight of all animals was measured. After euthanasia of animals, the cerebellum
of all animals was immediately removed and underwent the following:

**a- Biochemical analysis:**

The cerebellum of all animals was immediately washed in ice-cold glass
slides, homogenized separately in 10 volumes (w/v) of 0.1M phosphate buffer, pH 7.4
using a Polytron homogenizer for one minute. The homogenates were centrifuged at
4000 r.p.m. for 20 minutes and refrigerated at 4 °C. The supernatant was used for
estimation of the quantitative activities of both Malondialdehyde (MDA) and the total
glutathione (GSH).

**b- Histological and Immunohistochemical study:**

Specimens from the cerebellum were fixed in 10% neutral-buffered formalin.
Sections of 5 µm thickness were prepared from each specimen. The sections were
stained with Haematoxylin& Eosin (H &E) as well as the silver stain for histological
assessment. For silver staining [2], sections were deparaffinized, treated with 1%
potassium permanganate, bleached in 1% oxalic acid, treated with 2.5% iron alum,
placed in a Coplin jar of silver solution, reduced in 10% aqueous formalin, toned in 0.2% gold chloride solution, treated with 5% sodium thiosulfate, counterstained with eosin, dehydrated through ascending grades of alcohol then cleared in xylene. Deparaffinized sections were prepared for immunohistochemical study [24, 35]. They were mounted on positively charged slides for staining with PDGFα (as a marker for Oligodendrocytes), GFAP (to assess for astrocytic activity) and BCL 2 (as a measure for antiapoptosis). The deparaffinized Sections underwent rehydration, heat-induced epitope retrieval (HIER). The slides were placed on Dako autostainer instrument, EnVision Flex peroxidase blocked. Immune reactions were evaluated with various primary antibodies PDGFα (Genetex, USA), BCL2 (Dako, Denmark), GFAP (DAKO, Denmark). Secondary antibodies used were Dako EnVision Flex/HRP. Visualization had been done with EnVision FLEX DAB+ Chromogen. The prepared sections were examined and photographed using a Canon digital camera (Canon, Japan) attached to the IBM computer system.

**Histomorphometric study**

Image analysis was performed using the software Leica Quin 500, Germany. It was used to measure the areas percent of positive PDGF, GFAP and BCL2 immunostaining reaction in a standard measuring frame using a magnification x 400 by light microscopy transferred to the monitor's screen. These areas were masked by a green color using the computer system. Area percent values for each group were obtained from 6 different fields from different slides. Values were presented as a mean and standard deviation and statistically analyzed. For Optical Density of Silver staining, the video signals from the video camera were transmitted to the image capture board which digitally converted light intensity to 256 possible grey levels. The image was broken up into discrete picture elements or pixels, with each pixel assigned a specific grey color. These grey levels ranging from pure black to pure white were automatically converted to optical density values and stored. The number of Purkinje cells for each group was counted by the computer system using a magnification x 400 from 6 different fields from different slides. Values were presented as a mean and standard deviation and statistically analyzed.

**Statistical analysis**
Statistical analysis was performed using statistical package for the social sciences (SPSS) version 21.0 (IBM Corporation, Somers, NY, USA) statistical software. The data were expressed as means ± Standard Deviation (SD). Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by posthoc Tukey test. Significance was considered when p-value was less than 0.05.

RESULTS

Clinical observations and rats body weight

ACR group of rats became progressively less active and showed general weakness with a decrease of their appetite from the first time of treatment with the drug. Although, no mortality recorded, this group showed a decline in their body sizes. ACR+ Omega3 and ACR+ Green Tea groups were more active with slight decrease of their appetite with no mortality recorded. The body weight of ACR and ACR+ Gr. Tea groups was significantly decreased as compared to the control group. It was significantly increased in ACR+ Omega3 group as compared to the ACR and ACR+ Gr. Tea group (Table. I).

Biochemical results

The Mean GSH of ACR group was significantly decreased as compared to the control group. It was significantly increased in ACR+ Omega3 and ACR+ Gr. Tea group as compared to the ACR group (Table. I). The Mean MDA of ACR group was significantly increased as compared to the control group. It was significantly decreased in ACR+ Omega3 and ACR+ Gr. Tea group as compared to the ACR group (Table. I).

Histological results

On Hx & E (Fig.1) staining, the control group showed regularly arranged Purkinkje cells and granular cells. ACR group exhibited regular Purkinje cells alternating with swollen degenerated ones with pale chromatin materials in their nuclei. Some Purkinje cells were seen displaced in the granular layer. ACR group also exhibited degenerated Purkinje cells surrounded with empty spaces. The granular cells were markedly diminished in number with clumps of pyknotic cells with intercellular eosinophilic areas (necrosis). ACR+ Omega3 group showed largely
preserved Purkinje cells with apparently intact all layers of the cortex. Many specimens of this group showed multilayers of Purkinje cells surrounded by many vacuolated cells (clear cells) mostly Oligodendrocytes. ACR+ Gr. Tea group showed largely preserved Purkinje cells with apparently intact all layers of the cortex.

On silver staining (Fig. 2) of the cerebellar cortex, ACR group revealed marked loss of Purkinje cells with nuclear degeneration in the remaining ones. ACR+ Omega3 group revealed a multilayer deposition of regular Purkinje cells with oligodendrocytes inbetween. Granular cells appeared regular despite their decreased staining. ACR+ Gr. Tea group revealed largely preserved Purkinje and granular cell layers. On silver staining of the cerebellar medulla, the control group exhibited normal silver staining of the axons while ACR group exhibited markedly decreased axonal staining. ACR+ Omega3 group exhibited mildly decreased axonal staining while ACR+ Gr. Tea group exhibited normal axonal staining.

**Immunohistochemical results**

On PDGF immunostaining (Fig. 3), Control group showed many Oligodendrocytes with a positive PDGF reaction while the ACR group showed few Oligodendrocytes with decreased reaction. ACR+ Omega3 group exhibited an increased number of Oligodendrocytes with a positive PDGF reaction esp. at the junction between molecular and granular cell layers while the ACR+ Gr. Tea group exhibited a considerable amount of Oligodendrocytes with a positive reaction.

On GFAP immunostaining (Fig. 4), Control group showed a considerable number of astrocytes with a positive GFAP reaction while ACR group showed a huge number of astrocytes with a positive reaction. ACR+ Omega3 group exhibited a considerable number of astrocytes with a positive GFAP reaction while ACR+ Gr. Tea group exhibited mild increase in the number of astrocytes with positive GFAP reaction.

On BCL2 immunostaining (Fig. 5), Control group showed mild positive BCL2 reaction while ACR group showed a scanty BCL2 reaction. ACR+ Omega3 showed mild increase in BCL2 reaction while ACR+ Gr. Tea groups exhibited mild positive BCL2 reaction.

**Histomorphometric results**

a) The Mean Number of Purkinje cell in ACR group was significantly decreased
as compared to the control group. It was significantly increased in ACR+ Omega3 and ACR+ Gr. Tea group as compared to the ACR group (Table. 1).
b) The Optical density of silver stain was significantly decreased in ACR group as compared to control group. It was significantly increased in ACR+ Omega3 and ACR+ Gr. Tea group as compared to ACR group (Fig. 6).
c) The area% of BCL2 positive reaction was significantly decreased in ACR group as compared to control group. There was a significant difference between ACR+ Omega3 as compared to group IV. There was a significant difference between ACR+ Omega3 as compared to ACR group and no significance between ACR+ Gr. Tea group as compared to ACR group (Fig. 6).
d) The area% of PDGF positive reaction was significantly decreased in ACR group as compared to control group. It was significantly increased in ACR+ Omega3 as compared to all groups. ACR+ Gr. Tea group showed significant increase as compared to ACR group (Fig. 6).
e) The area% of GFAP positive reaction was significantly increased in ACR group as compared to control group. It was significantly decreased in ACR+ Omega3 and group IV as compared to ACR group (Fig. 6).

DISCUSSION

Human beings and animals are exposed every day simultaneously and concurrently to environmental contaminants. Acrylamide is known to exert its toxicity through oxidative stress by generating reactive oxygen species [21]. Body weight is an important marker in toxicology experiments. In our study, acrylamide administered to adult rats reduced the body weight. This might be due to a decrease in food and water intake by adult rats by decreasing appetite [1]. Moreover, ACR toxicity could affect absorption or metabolism of the food, resulting in a decrease in body weight [37].

The significant increase of malondialdehyde (MDA) levels in ACR group might be due to the high content of lipids in the cerebellum which makes lipid peroxidation is the main landmark of brain oxidative stress [42]. The decline in glutathione (GSH) levels in ACR group might reflect its consumption through oxidative stress [7, 41, 12]. Supporting our results, it was reported that acrylamide had
enhanced oxidative stress through increased formation of reactive oxygen species and lipid peroxidation while decreased superoxide dismutase activity and glutathione levels [19].

Histologically, ACR group exhibited variable degrees of cellular degeneration esp. Purkinji cells, confirmed by the significant decrease in their number as compared to control group. In agreement to these results, it was observed undifferentiated Purkinje cells with frequent pyknotic ones, a loss of their number and also marked oedema in the upper part of the internal granular layer in the cerebellum of drug-received rats [15, 18]. The observed migration of Purkinje cells into the granular layer in this study might be due to ACR delaying of the cells proliferation in the granular layer as well as migration and differentiation [1]. These changes might be explained by the possible action of acrylamide to induce alterations in the cytoskeleton, membrane necrosis, free radicals, oxidative stress and mitochondrial dysfunction [41]. Some specimens in group II of this work showed clumping of the granular layer with areas of necrosis inbetween the cells. Partially supporting these findings, it was revealed that chronic acrylamide administration had led to thinning of the external granular layer which in turn delays the proliferation of the cells of this layer [1].

On GFAP immunostaining in the current work, group II showed strong positive GFAP reaction. GFAP is an important skeleton protein of astrocytes [37]. Upregulation of GFAP is associated with proliferation and activation of astrocytes. Almost all types of brain injury may stimulate the upregulation of GFAP in reactive astrocytes, so GFAP could be used as a marker of CNS injuries. ACR had been reported to upregulate the expression of GFAP in the cerebellum in response to the Purkinje cells damage [40]. On BCL2 immunostaining of this work, although mild reaction was observed in all groups, the area % of positive reaction in ACR group was significantly decreased as compared to the control group. Supporting this result, it was reported that bcl-2 mRNA expression had significantly decreased in PC12 cells treated with ACR implicating increased apoptosis [19].

Omega3 received group showed significantly improved results in comparison with those in the ACR group. Histochemically, there was significant decrease in cerebellar MDA level of Omega 3 received rats as compared to ACR received ones. Also, there was significant increase in cerebellar glutathione level of Omega 3 received rats as compared to the ACR group. Concordantly, Omega-3 fatty acid supplementation elevated superoxide dismutase and catalase activity in various organs
such as the kidney, liver, and intestine [4]. In addition, Omeg3 had significantly increase GSH and neuroprotectin D1 (NPD1) in the brain of posttraumatic brain injury in rats [37].

Oligodendrocytes precursor cells (OPCs) are present in the adult CNS and have NG2 and PDGFα receptors [13]. Omega 3 had promoted oligodendrogenesis in the cerebellar cortex in group III of this work esp. at the junctional area between molecular and granular cell layers, confirmed by their positive PDGFα immunostain and negative GFAP immunostain to exclude ACR-induced Bergmann astrocytosis. Confirming these findings, the area% of PDGF positive reaction in this work was significantly increased in group III as compared to all groups. Supporting our results, the combined treatment of fish oil dietary supplement and omega-3 polyunsaturated fatty acids injections had been proved to promote post-traumatic brain injury (TBI) restorative processes in the brain, including generation of immature neurons, microvessels, and oligodendrocytes, each of which was significantly correlated with the improved cognitive recovery [30]. Oligodendrocytes were abundant in Hx & E sections of Omega 3 plus received rats in this work. Oligodendrocytes are identified histologically by having condensed, rounded nuclei and unstained cytoplasm due to very abundant Golgi complexes, which stain poorly [28]. The observed Oligodendrocytes in this work were in the grey matter of cerebellum in its Molecular layer i.e (Satellite oligodendrocytes). It was suggested that the function of this type of Oligodendrocytes as to monitor the extracellular fluid around neuronal cell bodies, act in a reserve capacity, and, if the need arises, they may migrate into the white matter to replenish interfascicular oligodendrocytes [14].

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) exist abundantly in the brain and play a crucial role in essential neuronal functions, such as axonal guidance, synapse and dendrite formation, neurotransmission, etc. [17, 20]. Other mechanisms by which, omega-3 polyunsaturated fatty acids exert potent protective effects following experimental traumatic brain injury (TBI) through had been reported, for example, amelioration of oxidative stress [33], mitigation of endoplasmic reticulum stress [3] and modulation of microglial activation [16].

The present work showed improvement of the histopathological findings with the co-administration of green tea extract with the acrylamide. Interestingly, the protective effects of Green Tea extract, against neurotoxicity caused by acrylamide exposure both in vivo and in vitro, was attributed to antioxidant effects of this extract
Both Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) which are green tea catechins showed inhibitory effects on ACR neurotoxicity. They increased GSH level and decreased lipid peroxidation in rat cerebral cortex [12]. A study on the PC12 cell line of pheochromocytoma revealed that EGCG attenuates ACR-induced neurotoxicity in PC12 cells by maintaining mitochondrial function and regulating the expression of bax and bcl-2 mRNA and redox state [19].

Optical Density of silver staining in this work was significantly lower in ACR received group as compared to the control. This might indicate the demyelinating effect of ACR. Having the major role of myelinating central axons, each oligodendrocyte can myelinate individual intermodal segments of an average of 30 separate axons (as high as 60 axons); adjacent internodal segments are myelinated by different oligodendrocytes [13]. This pattern of central myelination leaves periodic nodes of Ranvier bare, separating between adjacent segments, with sodium channels, at which action potentials (APs) are reinitiated as they travel down the myelinated axon and its branches (called saltatory conduction) [8, 13]. Both Omega3 and Green Tea significantly increased the optical density of silver staining of the cerebellar axons as compared to ACR received rats in the current work; this might suggest their myelinating role and their potential Oligodendrocytes recruitment. Oligodendrocytes can be attacked by antibodies directed at specific oligodendrocyte proteins in multiple sclerosis, leading to oligodendrocyte death and axonal dysfunction [13]. Also, they could be attacked by a polyoma virus (JC virus) causing demyelinization of axons especially in the occipital and parietal lobes of the brain leading to Progressive multifocal leukoencephalopathy [14].

Clinically implicated from the current work, Omega 3 might have an Oligodendrogenic role and might be used in CNS injury to help myelin sheath regeneration. However, the exact mechanism by which Omega 3 induces Oligodendrogenesis is not clear and still needs further investigations. Also, this Oligodendrogenic role after already established CNS injury should be thoroughly investigated.

CONCLUSIONS

In conclusion, Acrylamide has adverse cerebellar changes. Concomitant administration of Omega 3 or green Tea with ACR might mitigate these adverse changes with an Oligodendrogenic role of Omega 3.
Conflict of interest
The authors declare no conflict of interests.

Acknowledgement:
This research was not supported by any type of funds.

References


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**Table 1.** Comparison of the body weight, number of Purkinje cells and oxidative/antioxidative markers among the different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean body weight ± SD</th>
<th>Mean Number of Purkinje cells ± SD</th>
<th>Mean GSH mmol/mg ptn ±SD</th>
<th>Mean MDA nmol/mg ptn±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>217.3±23.9</td>
<td>10.8±1.8</td>
<td>55.0±6.3</td>
<td>2.4±0.6</td>
</tr>
<tr>
<td>ACR</td>
<td>157.0±13.0*</td>
<td>4.74±0.7*</td>
<td>30.8±4.2*</td>
<td>12.9±1.9*</td>
</tr>
<tr>
<td>ACR+ Omega3</td>
<td>204.4±20.0**</td>
<td>11.26±1.8**</td>
<td>41.3±4.2*,**</td>
<td>5.4±1.2*,**</td>
</tr>
<tr>
<td>ACR+Gr. Tea</td>
<td>169.9±24.7*,<strong>,</strong>*</td>
<td>9.8±2.6**</td>
<td>46.6±5.6*,**</td>
<td>4.0±0.61.11*,**</td>
</tr>
</tbody>
</table>

*Statistically significant as compared to Control group, **statistically significant as compared to ACR group. *** Statistically significant as compared to ACR+ Omega3 group

**Figure 1.** A. Control group showing regularly arranged Purkinje cells (black arrow) and granular cells (arrow head). B. ACR group showing regular Purkinje cells (black arrow) alternating with swollen degenerated ones with pale chromatin materials in their nuclei (incomplete arrow). Purkinje cells (green arrow) are seen displaced in the granular layer and surrounded with dark granular cells (arrow head). C. ACR group showing degenerated Purkinje cells surrounded with empty spaces (*). Purkinje and granular cells are markedly diminished in number. D. ACR group showing degenerated Purkinje cells and clumps of pyknotic granular cells (arrow heads) with intercellular eosinophilic areas (necrosis). E. Omega3 received group showing largely preserved all layers of the cortex. F. Omega3 received group showing multilayers of Purkinje cells surrounded by many cells with clear cytoplasm mostly Oligodendrocytes (red arrows). The granular cells appear preserved. G, H. Gr. Tea received group showing largely preserved all layers of the cortex. (Hx & E x 400)

**Figure 2.** A. Control group showing regularly arranged granular cells (arrow head) and flask shaped Purkinje cells (black arrows); B. ACR group showing loss of Purkinje cells with nuclear degeneration in the remaining ones (black arrow). The
Granular cells (arrow heads) appear with decreased staining and wide intercellular spaces. C. Omega3 received group showing multilayers of regular Purkinje cells with oligodendrocytes (red arrows) inbetween. Granular cells appear regular despite their decreased staining. D. Gr. Tea received group showing largely preseved Purkinje cells and granular layer. E. Control group cerebellar medulla showing normal silver staining of axons (blue arrows). F. ACR group cerebellar medulla showing markedly decreased silver staining of axons. G. Omega3 received group cerebellar medulla showing mildly decreased silver staining of axons in the cerebellum. H. Gr. Tea received group cerebellar medulla showing normal silver staining of axons. (Silver x 400)

**Figure 3.** A, E. Control group (cerebellar cortex & medulla respectively) showing many Oligodendrocytes (arrows) with positive PDGF reaction. B, F. ACR group cerebellar (cerebellar cortex & medulla respectively) showing few Oligodendrocytes (arrows) with positive reaction. C, G. Omega3 received group (cerebellar cortex & medulla respectively) showing many Oligodendrocytes with positive reaction esp. at the junction between molecular and granular cell layers. D, H. Gr. Tea received group (cerebellar cortex & medulla respectively) showing a considerable amount of Oligodendrocytes with positive reaction. (PDGF x 400)

**Figure 4.** A. Control group showing considerable number of astrocytes (arrow heads) with positive GFAP reaction. B. ACR group showing huge number of astrocytes with positive reaction. C. ACR+ Omega 3 received group showing considerable number of astrocytes with GFAP reaction. Note the cells (red arrow) with negative GFAP reaction at the junction of molecular layer and Purkinje cell (black arrow) layer are mostly Oligodendrocytes. D. ACR+ Gr. Tea received group showing mild increase in the number of astrocytes with positive GFAP reaction. (GFAP x 400)

**Figure 5.** A. Control group showing mild positive BCL2 reaction (arrow). B. ACR Group showing scanty BCL2 reaction. C. Omega3 received group showing mild increase in BCL2 reaction. D. Gr. Tea received group showing mild positive BCL2 reaction. (BCL2 x 400)
Figure 6. Bar charts of the mean; A. Optical density of silver stain. B. Area % of positive BCl2 reaction. C. Area % of PDGF positive reaction. D. Area % of GFAP positive reaction. * = statistically significant as compared to group I, ** = statistically significant as compared to group II, *** = statistically significant as compared to group III.