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RESEARCH ARTICLE

Protective effect of vitamin E on biochemistry, oxidative stress and histopathological alterations induced by acrylamide in wistar rats (*Rattus norvegicus*)

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Abstract : Acrylamide (ACR) has recently been found in fried and backed foods, suggesting widespread public exposure. ACR is an industrial chemical material designated as a probable human carcinogen by IARC and USEPA. The aim of the present study was to evaluate the protective effects of vitamin E against acrylamide induced toxicity in rats. Forty Wistar male and female rats were divided in 4 different groups (each group have 5 male and 5 female). Group I served as control. Group II received ACR at dose of 15 mg/kg body weight. Group III received vitamin E at dose of 200 mg/kg body weight and Group IV administered vitamin E (200 mg/kg body weight) with acrylamide (15 mg/kg body weight). The biochemical results revealed that ACR (Group II) caused significant decrease in plasma albumin and plasma cholinesterase in male and female rats. In oxidative stress, the Group II male and female rats showed singnificant increase in LPO (lipid peroxidase) level and showed singnificant decrease SOD (superoxide dismutase) level. Histopathological alterations evidenced in brain, lung and spleen in male and female rats of Group II. Cotreatment of vitamin E with ACR (group IV) revealed improvement in biochemical and oxidative stress profile as well as in pathomorphology.

Key words : Acrylamide, Vitamin E, Albumin, Cholinesterase, Oxidative stress

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INTRODUCTION

Acrylamide (ACR) is an α, β-unsaturated carbonyl compound with a significantly high chemical activity. It is extensively used in many fields from industrial manufacturing to laboratory personnel work, so it is often absorbed during occupational exposure (Boettcher *et al*., 2005). ACR is used for the production of polymers used as flocculants for purification of drinking and waste water, thickeners for agricultural sprays, gel chromatography and electrophoresis, soil stabilizers, and in the paper and pulp industry.

The presence of acrylamide in starch rich foods and processed by roasting or baking was detected in 2002 by the Swedish researchers. Therefore, ACR becomes the main concern regarding the analytical and toxicological researches

(Zyzak *et al*., 2003). The World Health Organisation estimated total daily intakes of ACR from food to be in the range of 0.3-0.8 Ìg/kg of body weight (WHO, 2002). ACR may form in certain foods cooked at high temperatures (120-180°C). ACR is thought to be detected in food principally from the interaction of the amino acid asparagines with glucose or other carbohydrates. High levels of ACR were unexpectedly detected in widely consumed food items such as fried bread and potato chips.

Acrylamide is largely oxidized to glycidamide in mice, rats and humans by its oxidizing agent CYP450 2EI (Summer *et al.*, 1999). In humans, at relatively low doses of acrylamide, glycidamide is formed at higher extent than in rats, because of the higher levels of CYP450 2EI. Both compounds, acrylamide and glycidamide, are detoxified by glutathione conjugation and to some extent glycidamide is detoxified by hydrolysis.

Vitamin E is a family of lipid–soluble vitamins, and there are eight naturally occurring vitamin forms. The most abundant form found in nature is α-tocopherol with the highest rate of biological activity. Vitamin E can protect the critical cellular structures against damage from both free radicals such as peroxy radical, hydroxyl radical, and super oxide and from oxidation products such as malondialdehyde and hydroxynonenal (Erin *et al*., 1984). Vitamin E is an important antioxidant, plays a role in inhibition of mutagen formation, and repair of membranes and DNA. Therefore, it has been suggested that Vitamin E may be useful in cancer prevention (London *et al*., 1985). Vitamin E is believed to be the primary component of the antioxidant system of the spermatozoa and is one of the major membrane protectants against ROS and LPO attack (Yousef *et al*., 2003).

RESEARCH METHODOLOGY

The present research project was presented to the Institutional Animal Ethics Committee (IAEC) and experimental protocol number VET COLL-06-2012 was approved for conducting the work. Acrylamide and Vitamin E obtained from Himedia Laboratory Pvt. Ltd., Mumbai, India. Wistar rats were procured from Cadila Healthcare Limited, Gujarat, India and were maintained under standard management conditions. Animal care, housing, and environmental conditions (temperature, humidity and light dark cycle) were according to recommendation stated in the Guide for Care and Use of Laboratory Animals before and during study period.

Experimental design :

Forty colony bred Wistar male and female animals were divided in 4 different groups (each group have 5 male and 5 female) *viz.*, I, II, III and IV. Group I served as control while, Group II rats were orally administered ACR at dose rate of 15 mg/kg b.wt., Group III rats were orally administered with vitamin E at dose rate of 200 mg/kg b.wt. and Group IV rats were orally administered with ACR and vitamin E @ dose rate of 15 and 200 mg/kg b.wt., respectively for 28 days. The group IV rats administered vitamin E one hour prior to acrylamide administration.

Parameters evaluated :

Blood was collected from all experimental groups on 29th day of study from retro-orbital plexus with the help of capillary tube in heparinised vial for clinical biochemistry analysis included, albumin (g/dl) and acetylcholinesterase (AchE-IU/L). In oxidative stress, the LPO (nmol/ml of RBC) was measured by method of Shafiq-Ur-Rehman (1984) and SOD (U) was estimated by method of Madesh and Balasubramanian (1998). Finally, all rats were sacrificed by decapitation method after the completion of experiment on 29th day and a complete gross and microscopic examinations were performed from various organs. Further, these tissues were processed and stained with H and E stain.

Statistical analysis :

The statistical analysis of data generated on various parameters (Body weight, clinical biochemistry and oxidative stress) was subjected to statistical analysis using 2-way analysis of variance (ANOVA). Pairwise comparisons with control, for each sex separately, was made using Dunnett's test. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

All the animals of group I and III did not show any behavioural changes during to the 28 days of the experiment. The group II rats revealed weakness, ataxia, tremors, hair loss and neurological signs of paralysis, splaying and dragging especially of the hind limbs (Fig. 1) while the rats of group IV revealed the same signs but, intensity was in milder extent as compare to the rats of group II.

The body weight of rats were significantly decreased ($P < 0.05$) in male and female of group II on 28th day (week 4) as compared to control while, male and female rats of group IV showed less decrease in the body weight as compared to group II. In group III, male and female rats showed no significant changes in body weight as compared to control.

Biochemical parameters of male and female rats are summerized in Table 1. The male and female rats revealed significant decrease ($p < 0.05$) in albumin concentration and AchE activity in group II as compared to group I rats. The group IV male and female rats revealed increase in albumin concentration and AchE activity as compared to group II male and female rats which indicated the ameliorative effect of vitamin E against acrylamide toxicity.

Oxidative stress was evaluated in all the treatment group of rats by measuring the LPO and SOD levels in RBC

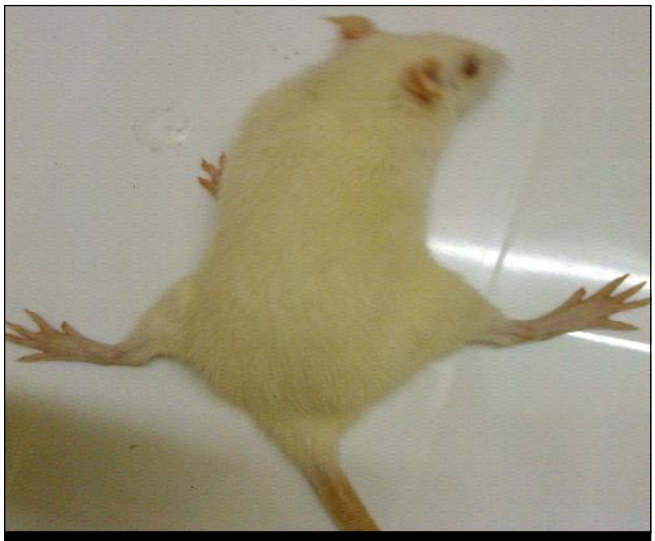

Fig. 1 : Group II rat showing splaying of hind limb

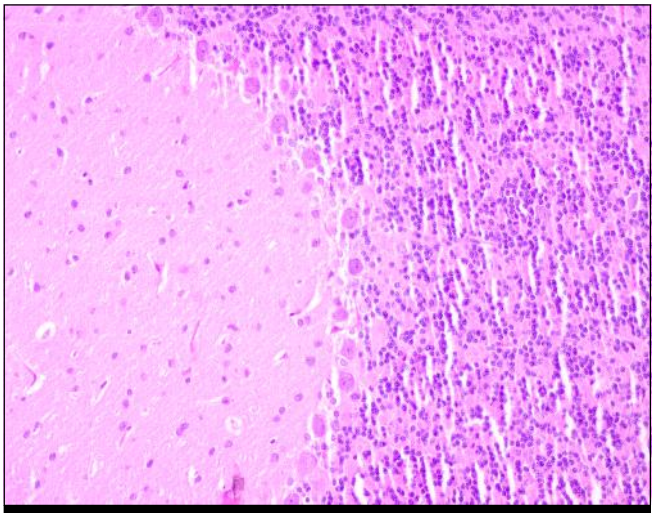

Fig. 2 : Brain from group I rat showing normal architecture (H and E × 200)

Table 1 : Effect of acrylamide and vitamin E on biochemical parameters (mean ± S.D.) of male and female rats of different experimental groups (n = 5 rats)

Parameters		Group-I control	Group-II acrylamide	Group-III vitamin E	Group-IV ACR + Vit. E
Albumin (g/dl)	Male	4.18±0.126	3.58±0.241*	4.10±0.461	3.94±0.312
	Female	4.43±0.194	3.75±0.343*	4.39±0.209	4.15±0.110
Cholinesterase (IU/L)	Male	2946.20±829.200	1950.00±118.954*	2858.60±357.872	2151.60±172.947*
	Female	8002.40±804.332	5560.20±1048.757*	6847.40±766.465	6504.60±871.084*

* indicates significance of values at P = 0.05; ± Standard deviation

Table 2 : Effect of acrylamide and vitamin E on oxidative stress (mean ± S.D.) of male and female rats of different experimental groups (n = 5 rats)

Parameters		Group-I control	Group-II acrylamide	Group-III vitamin E	Group-IV ACR+Vit. E
LPO	Male	36.42±2.984	68.15±5.649*	32.59±2.932	52.70±2.021*
	Female	44.20±2.784	86.99±3.830*	41.12±3.166	57.13±2.701*
SOD	Male	49.11±1.739	9.09±1.440*	51.60±0.648	44.76±2.482*
	Female	47.98±1.296	10.84±0.411*	50.44±2.880	42.15±2.122*

* indicates significance of values at P = 0.05; ± Standard deviation

lysate, showed in Table 2. The male and female rats of group II and IV showed significant ($p < 0.05$) increase in LPO as compared to group I, but group IV revealed less increase in LPO as compared to group II. No significant changes were observed in LPO of group III in male and female rats as compared to control group I (Fig. 2). While, male and female rats of group II and IV showed significant ($p < 0.05$) decrease in SOD as compared to group I, but group IV revealed less decrease in SOD as compared to group II. No significant changes were observed in SOD of group III in male and female rats as compared to control group I.

Grossly, the group II rats revealed distended urinary bladder. Histopathological changes in brain of group II rats revealed mild eosinophilic purkinje cells (Fig. 3), meningeal congestion. Brain of group IV rats revealed similar lesions but of minimal extent. Pathomorphological alterations in lung of group II rats manifested by fibrosis, oedema and infiltration of neutrophils and haemorrhage (Fig. 4) as compared to control group I, whereas, the rats of group IV revealed same lesions with minimal extent (Fig. 5). Furthermore, microscopic changes in spleen of group II rats characterized by severe diffuse hemosiderosis with atrophy of white pulp (Fig. 6). The spleen of group IV rats revealed same lesions with minimal extent.

The present study was conducted to evaluate the repeated dose toxicity of acrylamide and its amelioration by well known natural antioxidant vitamin E in wistar rats. Grivas *et al*. (2002) reported that the levels of acrylamide

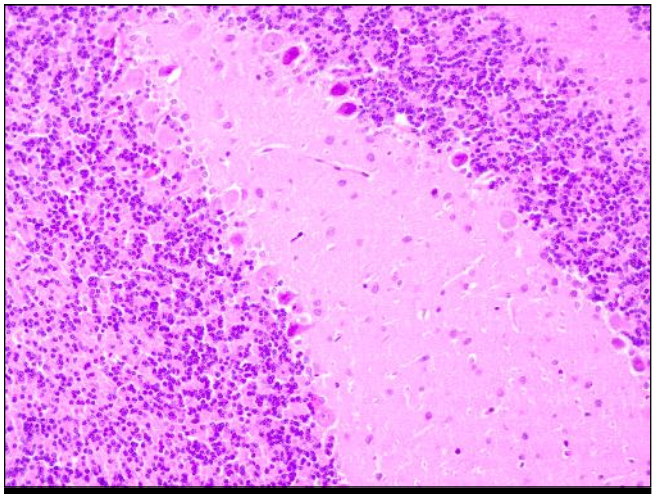

Fig. 3 : Brain from group II rat showing eosinophilic purkinje cells (H and E × 200)

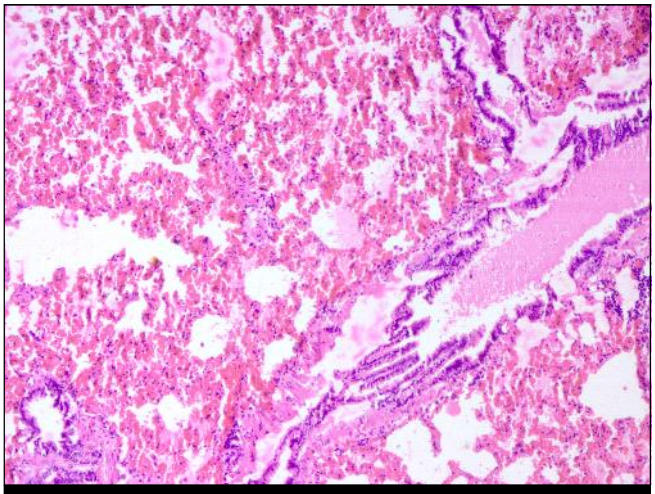

Fig. 4 : Lung from group II rat showing haemorrhage (H and E × 100)

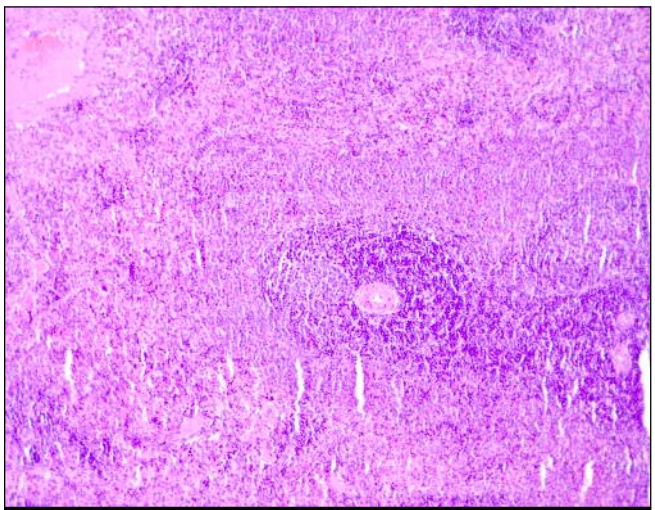

Fig. 5 : Spleen from group I rat showing normal architecture (H and E × 100)

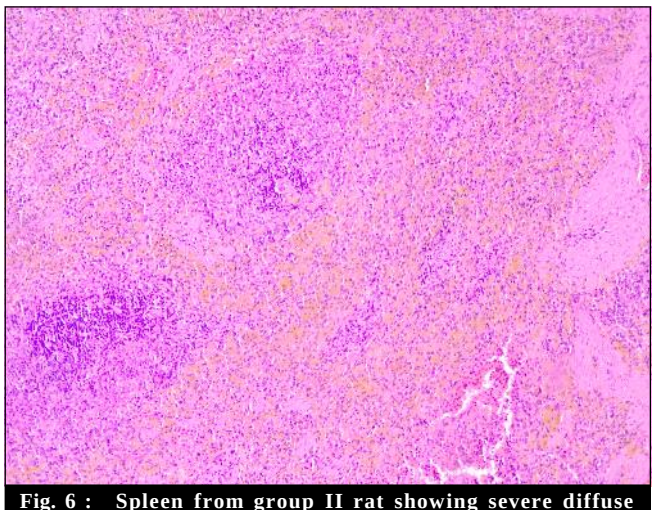

Fig. 6 : Spleen from group II rat showing severe diffuse hemosiderosis with atrophy of white pulp (H and E × 100)

vary considerably between single foodstuffs within food groups, but potato crisps and French fries generally contained high levels of acrylamide compared to any other food groups.

The present study revealed clinical signs were similar to the signs observed by Sharma and Jain (2008) who reported the cause of hair loss from body might be due to ACR deposition in skin and damage to hair follicle. The neurological signs of splaying and dragging especially of the hind limbs were also observed in rats treated with ACR by Hasseeb *et al*. (2013). These neurological signs may be due to the induced lesions in spinal cord as evidenced by Hasseeb *et al*. (2013). In present study, the significant reduction ($p < 0.05$) in body weight of Group II rats is in accordance with the findings reported in rats treated with acrylamide by Rawi *et al*. (2012) who reported that the decrease in body weight may be due to detrimental effect of ACR on growth and development in rats.

In biochemical alterations, the significant decrease ($p < 0.05$) in plasma albumin concentration of group II rats in this study is in accordance with the findings reported in rats treated with ACR by Sadek *et al*. (2011) who reported the marked liver damage due to ACR is responsible for decrease in albumin concentration. The AchE activity decreased significantly ($p < 0.05$) in group II rats, also shown by Rawi *et al*. (2012).

In oxidative stress estimation, the significant increase in LPO level in accordance with the findings reported in rats treated with acrylamide by Sadek (2012). The enhancement of LPO is a consequence of glutathione depletion resulting due to oxidative stress as suggested by Tong *et al.* (2004). The significant decrease in SOD level in conformity with findings of Sadek (2012). The decreased activity of SOD may be due to increased utilization of this antioxidant enzyme with subsequent depletion to counter the increased level of free radicals induced by acrylamide as reported by Sadek (2012).

Grossly the distended urinary bladder in group II rats also reported by Jin *et al*. (2008). The distension of urinary bladder may be attributed to the degeneration of the nerves involved in bladder voiding as advocated by Fullerton and Barnes (1966).

Concerning the present histopathological findings in brain of group II rats were also supported by Rahangadale *et al.* (2012) which might be due to the binding of ACR and glycidamide to dopamine receptors and inhibits activity of kinesin and dyneine resulting in interference with neural intracellular transport as suggested by Tyl and Friedman (2003). Pathomorphological alterations in lung of group II rats, manifestation also coincided with Fraire *et al*. (1992) treated rats with polyacrylamide and reported that changes in lung might be due to irritating effect of polyacrylamide on bronchiolar and alveolar epithelia. Histopathological findings in spleen of group II rats might be due to the destruction of hemoglobin.

The antioxidative and protective effect of vitamin E have been documented previously by Erin *et al*. (1984), London *et al*. (1985), Yousef *et al*. (2003). In the present study, it was also observed that vitamin E administration to rats receiving ACR in rats of group IV produced an appreciable improvement on clinical signs, body weight, biochemical alterations, oxidative stress and pathomorphological findings. Our findings of improved changes in biochemical parameters including, albumin concentration and AchE activity due to vitamin E in group IV rats also coincide with findings of Venkatanarayana *et al.* (2012). Ramirez-Farias *et al.* (2008) reported that short term antioxidant supplementation attenuates lipid peroxidation and protects against liver injury in rats so albumin value of group IV showed increase as compared to group II. In histopathological changes, ameliorative effects of vitamin E on brain coincide with the findings of Pace *et al*. (2003) in rats. The ameliorative effect of vitamin E may be due to its antioxidant property for scavenging free radicals which are toxic for biological membranes as advocated by Yue *et al*. (2010).

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