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ACRYLAMIDE-INDUCED SYNAPTIC DEPRESSION AND NEURONAL DAMAGE IN DEVELOPING CHICK EMBRYONIC BRAIN

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ABSTRACT
The neurochemical synaptic transmission is an essential function of the central nervous system and neurotransmitters play a critical role in this process. The present study carried out to investigate the toxic effects of acrylamide in alterations of acetyl cholinesterase (AChE) and various neurotransmitters like Monoamine, epinephrine, nor epinephrine, glutamate and gamma-aminobutyric acid (GABA). After 72h of acrylamide treatment to egg, the tissues were collected from the 11th day (d11) developing chick embryos exposed to different doses (0.2, 0.4, and 0.6mg) of acrylamide toxicity. The present results showed that epinephrine, nor epinephrine, dopamine levels were reduced and increased levels of gamma-aminobutyric acid (GABA) and acetyl cholinesterase (AChE) with increasing doses of acrylamide. These findings concluded that high doses of acrylamide positively associated with neurotoxicity synaptic depression and neuronal damage in a dose-dependent manner.

KEYWORDS: Acryalmide, Acetyl cholinesterase, Chick embryos, Epinephrine, Gamma-aminobutyric acid, neurotransmitter.

INTRODUCTION
Acrylamide (CH_2=CHCONH$_2$) a neurotoxin, highly water soluble organic compound used as industrial chemical and in the production of polyacrylamides. A large number of animal experiments show that acrylamide is carcinogenic and has neuronal and reproductive toxic properties (Pourentezari *et al.,* 2014; Sen *et al.,* 2015, Thyagaraju *et al.,* 2013). Heightened concerns about exposure to acrylamide arose in 2002 when it was discovered in certain foods that are prepared at high temperatures (> 120 °C) (Tareke *et al.,* 2002). It forms, at least in part, due to a Maillard reaction between certain amino acids, such as asparagine, and reducing sugars (Mottram et al., 2002). However, several other pathways and precursors have been proposed to contribute to acrylamide formation.

Acrylamide exposure is associated with signs of impaired neurological performance in central and peripheral nervous systems that include impaired motor function and muscle weakness. Evidence of degenerative lesions in peripheral nerve fibers, as observed by light and electron microscopy, have been detected at oral doses lower than those eliciting clinical signs and other overt indications of functional deficit and acrylamide is a carcinogen with the potential to cause nervous system damage (Arisseto *et al.,* 2007, Venkataswamy *et al.,* 2015). *In vivo* and *in vitro* effects of acrylamide on synaptosomal neurotransmitter uptake and release. Through synaptic vesicle exocytosis is critical for neuronal communication. This exquisitely regulated process involves several steps, including tethering of synaptic vesicles to specialized sites of the presynaptic plasma membrane called active zones, a priming step(s) that leaves the vesicles ready to release, and Ca^{2+}-triggered membrane fusion (Südhof, 2013). Each of these steps can be modulated in a variety of presynaptic plasticity processes that underlie multiple forms of information processing in the brain (Regehr, 2012). Developing brain is of particular interest since it is highly susceptible to neurotoxin insult during the critical window of vulnerability (Rice and Barone, 2000). Given the wide industrial applications and the possible neurotoxic effects associated with the chronic human exposure to acrylamide starting *in vovo*, it is relevant to develop specific therapeutic strategies to abrogate acrylamide -induced fetal neurotoxic effects. The acrylamide neurotoxicity has been known to affect the

nerve terminal and could adduct with the cysteine residues in functionally important presynaptic proteins, resulting in neurotransmitter release inhibition and eventual process degeneration (Lopachin et al., 2006, Thyagaraju *et al.,* 2013). Acrylamide inhibits dopamine uptake in rat striatal synaptic vesicles. Hence the aim of present investigation is to determine levels of various neurotransmitters in acrylamide-induced developing chick embryonic brain.

MATERIALS AND METHODS

The chemicals purchased from indigenous companies were of pure and used for the analysis of various samples of our research.

Source of Fertilized Eggs and Incubation Conditions

Freshly laid *Bobcock* strain zero day old fertilized eggs purchased from Sri Venkateswara Veterinary University, Tirupati, and Sri Balaji hatcheries, Chittoor, Andhra Pradesh were incubated horizontally at 37.5±0.5˚C with a relative humidity of 65% in an egg incubator, we consider day1 (d1) as an incubation period of 24h. The humidity of the incubator was maintained by keeping a trough full of water inside. The water was replaced every alternate day and the water level was maintained to keep the same percentage of humidity throughout the incubation. Eggs were rotated manually four times a day and were examined through the Candler every day for the proper growth and viability (Venkataswamy *et al.,* 2014).

Treatment

Acrylamide treatment

A group of six eggs (n=6) were maintained for each time point and dose, 0.2, 0.4, and 0.6 mg of acrylamide (10µl) in saline was administered as single dose separately to fertilized chick embryos on day8 (d8) of incubation.

Collection of samples

Brain tissue of 11th day embryo's were dissected out from embryo and homogenized (10% w/v) in phosphate-buffered-saline (0.2 M sodium phosphate buffer with 0.15 M sodium chloride, pH 7.4) in a glass tissue homogenizer with teflon pestle.

Determination of dopamine (DA) concentration

Levels of dopamine determined in brain (Dalpiaz *et al.,* 2007) were calculated using a standard curve and expressed as µg DA/g tissue.

Determination of Acetylcholinesterase

Acetylcholinesterase (AChE) activities were determined as described earlier (Ellman *et al.,* 1961) were expressed as nmoles of acetylcholine substrate hydrolyzed/ min/mg protein.

Determination of Monoamines content

A fluorometric assay for epinephrine (EPI) and norepinephrine (NE) levels determination was carried out according to Ciarolone *et al.,* 1978. The values expressed in µg/g tissue.

Determination of glutamate and GABA concentrations using Enzyme-linked immunosorbent assay (ELISA)

Samples from brain tissues were placed in a pre-cooled glass homogenizer and homogenate buffer (0.1 M PBS, pH 7.0) was added at a ratio of 1:9 (w/v). Homogenates were transferred to centrifuge tubes and preserved at −20°C until use. The optical densities of glutamate and GABA were detected and then their concentrations were calculated by ultraviolet spectroscopy and ELISA assay. In accordance with the ELISA kit for glutamate and GABA (USCNLIFE, Wuhan, China), all experimental procedures were conducted following the manufacturer's instructions. The microplate reader (Biorad used to read the developed color.

Statistical analysis

SPSS version 20 was used for the statistical analysis. The data obtained were calculated by one- way analysis of variance (ANOVA).

RESULTS

Effect of acrylamide dopamine levels

The Fig.1 indicates the dopamine levels in developing chick embryonic brain. After acrylamide treatment an altered present levels of dopamine were observed. A significant decrease (0.4) in dopamine concentration was observed in the brain of chick embryo from the moderate- and high-dose acrylamide groups and presenting an obvious dose-effect relationship

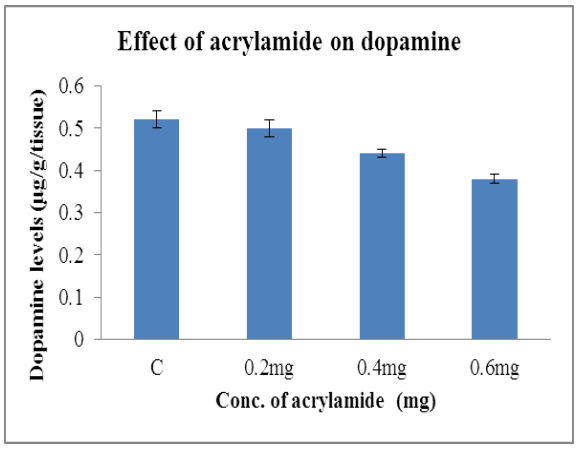

Fig. 1: Effect of acrylamide on dopamine concentrations in the developing chick embryo. Data are expressed as the mean ± SD (*n* = 6).

Epinephrine (EPI) and nor epinephrine (NE) level in the brain tissue

The Fig. 2 and Fig. 3 represent the levels of epinephrine and nor epinephrine in brain. Our results showed that epinephrine and nor epinephrine levels were reduced with increasing doses of acrylamide, brain when chick embryo was administered with 0.6mg acrylamide in comparison with control. The brain epinephrine levels in

0.2, 0.4 and 0.6mg acrylamide treated chick embryo were 25.1±1.2, 20.6±1.1 and 14.1±1.1 and the norepinephrine levels in 02, 0.4, 0.6 mg acrylamide treated chick embryo were 381.14±19.0, 313.1±14 and 259.9±16. The both epinephrine and nor epinephrine levels were decreased 4.38 and 3.8 respectively. when compare to controls.

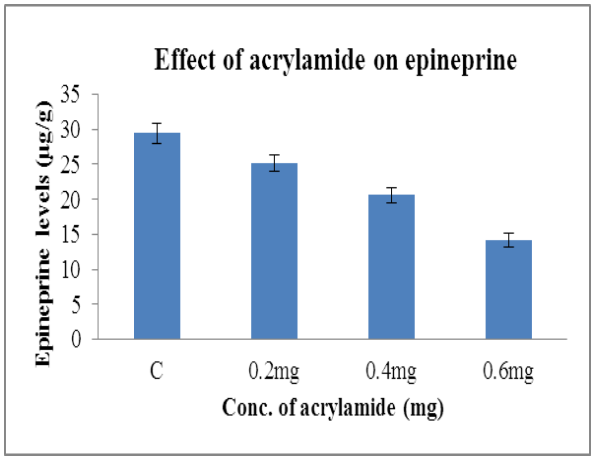

Fig. 2: Effect of acrylamide on Epinephrine concentrations in the developing chick embryo. Data are expressed as the mean ± SD (*n* = 6).

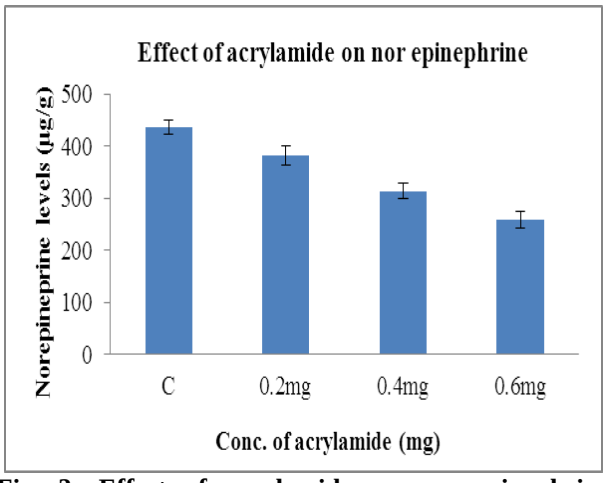

Fig. 3: Effect of acrylamide on nor epinephrine concentrations in the developing chick embryo. Data are expressed as the mean ± SD (*n* = 6).

Effect of acrylamide on glutamate and GABA

In developing chick embryo glutamate and GABA levels were determined in the brain tissue and results were represented in Fig. 4 and Fig. 6. A significant decrease in glutamate concentration (1.61) was observed in the brain of chick embryo from the moderate and high dose acrylamide group, where the concentrations of GABA were significantly increased (1.2) compared with untreated groups.

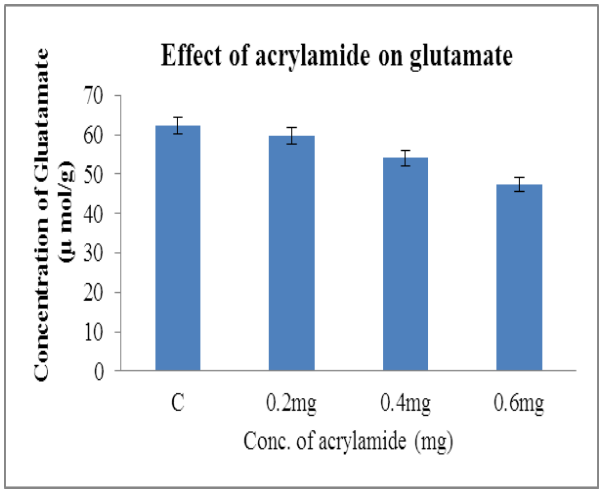

Fig. 4: Effect of acrylamide on glutamate levels in the developing chick embryo.
Data are expressed as the mean ± SD (*n* = 6).

Acetylcholine esterase (AChE) in brain tissue

In developing chick embryonic brain tissues, the levels of AChE (mU/ml) were determined and the results showed that the acrylamide induced a significantly elevates the levels of AChE. The results were represented in Fig. 5. 1.5 highest activities were noted in 0.6mg acrylamide dose group (14.8±0.6) when compared to controls (5.92±0.1). The increased activity of AchE with dose dependent manner was found in the developing chick embryonic brain.

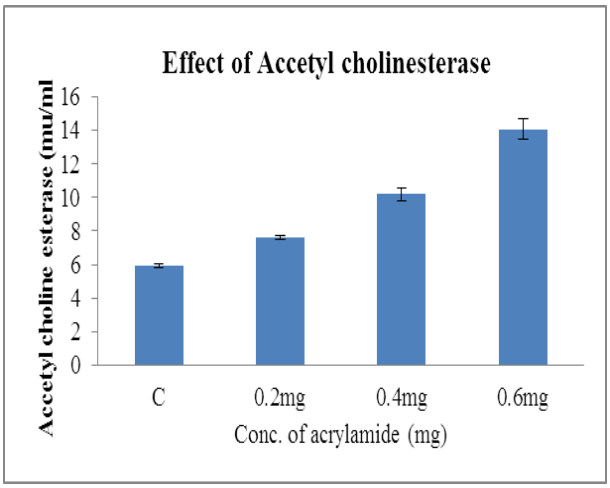

Fig. 5: Effect of acrylamide on Acetyl cholinesterase concentrations in the developing chick embryo. Data are expressed as the mean ± SD (*n* = 6).

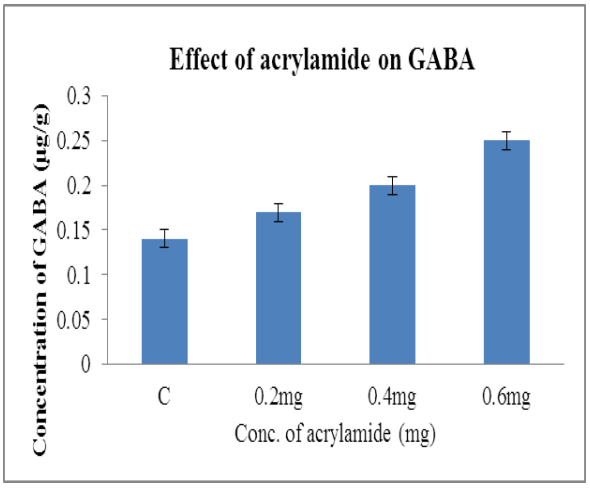

Fig. 6: Effect of acrylamide on GABA levels in the developing chick embryo.
Data are expressed as the mean ± SD (*n* = 6).

DISCUSSION

The dopamine, epinephrine and norepinephrine are the products of phenylamine and as hydroxylation phenylamine is metabolised to above compounds. Another aminoacid, Glutamic acid, as decarboxylation is converted in to gama amino butyric acid and an enzyme which play an role in neurotransmission was acetylcholine esterase. This enzyme converts acetylcholine into choline and acetyl molecules. All these five compounds dopa, epinephrine, norepinephrine, gama amino butyric acid and acetyl molecule play a significant role in neurotransmission in the presence of Ca^{2+} ion. Serotonin is another product involved in neurotransmission is a product of tryptophan.

Acrylamide can induce neurotoxicity through multiple exposure routes, and although the precise toxic mechanism(s) remain unclear, the cumulative effect is generally neurotoxicity (Pennisi *et al.*, 2013). There is typically a prolonged latency period between the exposure and symptom development, which is likely related to the fact that nerve damage must exceed a threshold before neurological symptoms become apparent. The present study indicated the neurotoxic effects of acrylamide and its effects on the neurotransmitters (Dopamine, EPI, NE, Glutamate and GABA) and also in the AChE activity. The mechanism of neuronal communication involves the release of neurotransmitters that bind to specialized receptors on the target cell, changing its activity (Valenzuela *et al.*, 2011). Results from the present study showed that; intoxication of chick embryo with acrylamide produced significantly altered levels Dopamine, EPI, NE, Glutamate and GABA contents in tested brain.

A decrease in acetylcholine levels in the synaptic cleft contributes to progressive cognitive impairment and possibly other neurological dysfunctions as seen in diabetic neuropathy or other neurodegenerative disorders (Schmatz *et al.*, 2009 and Bernhardi *et al.*, 2005). An increase in AChE activity has been found to inhibit cell proliferation and promote apoptosis (Jin *et al.*, 2004). Acrylamide induced significant elevation of neurotransmission markers such as AChE activity levels in the brain. The acrylamide also caused a significant decrease in NE and SER in brain tissue; this may be due to axonal and nerve terminal degeneration which caused alternations in the synthesis of transmitter, storage uptake, release and reduction in synaptic vesicle. The mechanism underlying acrylamide-induced neuronal injury remains unclear. An earlier study has suggested that it might be related to acrylamide-induced apoptosis (Li *et al.*, 2008). Acrylamide can suppress metabolism and axonal transport in neurons, causing deficiency of nutritional factors (Honig and Rosenberg, 2000). Acrylamide also caused a decrease in adenosine triphosphate level of cerebrum, which in turn weakened the ability of neurons to reuptake GABA. The abnormally high GABA concentration in the synaptic cleft may over-stimulate the corresponding receptor in the postsynaptic membrane, causing depression of normal neuron activity.

CONCLUSION

On the basis of results obtained as the effect of acrylamide on brain, it is concluded that the synaptic depression involved is slow and depend on long-term neuronal damage due to decrease in dopamine and increase in GABA levels in brain. A further decrease in epinephrine and nor epinephrine in brain tissue is due to axonal and nerve terminal degeneration caused acrylamide induced alternations in the synthesis of transmitter, storage uptake, release and reduction in synaptic vesicle in developing chick embryonic brain.

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