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NEUROMODULATORY PROPERTIES OF DIETARY COMPONENTS AGAINST ACRYLAMIDE MODEL OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

Dietary modifications have been implied for major neurodegenerative disorders and are strongly advocated for neurodegenerative disorders. Concentrations of the major dietary components, carbohydrates, proteins and lipids have been associated with extent of pathophysiology of degenerative disorders. Numerous epidemiological and experimental evidences indicate a strong association between dietary protein and cellular redox balance. Accordingly, here we have tested the hypothesis that dietary protein modulates the acrylamide (ACR) induced neurotoxicity among *Drosophila melanogaster*. Adult (10d old) male drosophila (20flies/vial, 3vials/group) were maintained on a diet with varying concentrations of protein (semolina + yeast) (0.5 to 10% w/v, 2ml medium, 1week) and were co-challenged with ACR (2mM). Mortality was recorded daily and locomotor activity was assessed using negative geotaxis at regular intervals. Interestingly, absence of dietary protein resulted in marked mortality among ACR flies which was reduced significantly among flies maintained with dietary protein (5 and 10%). Further, the ACR induced locomotor dysfunction was ameliorated among protein rich groups. In addition, presence of semolina+yeast in the diet reduced the oxidative markers among the whole fly homogenates (hydroperoxides, protein carbonyls, and glutathione). Our data is of the opinion that dietary protein is a key player in the cellular redox and in turn protects neurons.

KEYWORDS: Acrylamide, Neuroprotection, Dietary protein, Oxidative stress.

INTRODUCTION

Oxidative stress mediated by mitochondrial dysfunction is associated with normal aging as well as pathophysiology of numerous diseases/ pathological conditions including cancer, cardiovascular diseases (atherosclerosis, hypertension and ischemia), diabetes, pulmonary diseases and asthma (Birben et al., 2012, Chandran and Muralidhara, 2014). An increase in the levels of oxidatively modified proteins in the neuronal cells is reported in neuropathological complications. Neurodegeneration (NDD) is result of a cocktail of cellular pathways culminating into progressive damage of structure and function of neurons (Prasad and Muralidhara, 2014). Various NDDs are classified and each one is characterized by the loss or functional compromise among specific neuronal populations in the central nervous system (CNS) which results in expression of particular neurobehavioral phenotype (motor activity, mood and cognition). Irrespective of the various pathways and cascades involved in initiation and progression, the general pathophysiology of NDD involves oxidative stress and mitochondrial dysfunction. Uncontrolled generation of free radicals and obvious inadequacy in their detoxification in brain cells results in lipid peroxidation, nucleic acid-base oxidation, protein aggregation leading neuronal demise. In addition, aging is also demonstrated to have significant deleterious effects on brain functions (Seet et al. 2013). Neonatal loss of the ability to regenerate among majority of the neuronal tissues and because degeneration is a continuous process, the amassed effect makes these neurons susceptible to cytotoxicity.

Nutritional deficiencies are related with neuropathy symptoms. Concentrations of dietary components have been assessed for their role in redox homeostasis and cytoprotection among CNS neurons. Limiting carbohydrates has a direct effect on the chief energy deciphering phenomena. Reduced protein consumption is not directly linked to cellular energetics however has been reported to weaken the antioxidant defence and compromised immune responses among neurons *in vitro* and *in vivo (Venkareddy and Muralidhara, 2015)*. Accordingly, we selected a chemical model of neurodegeneration employing drosophila. Drosophila has been established as a most accepted invertebrate in vivo model for toxicological studies. We used acrylamide (ACR) as the neurotoxicant which is known to induce peripheral neuropathy which is usually mediated through neuronal oxidative stress (Erkekoglu and Baydar, 2014).

Hence it was envisaged that dietary protein may be a modulator against major neuronal implications among communities prone to protein malnutrition.

MATERIALS AND METHODS

Chemicals

Acrylamide, xylenol orange, 1,1–dithio nitro–bi–Benzoic acid (DTNB), acetylthiocholine iodide (ATCI), 2,4–Dinitro Phenyl Hydrazine (DNPH), propionic acid, agar agar and other chemicals were of analytical grade which were procured from Sisco Research Laboratory, India. Semolina, and jaggery were from a local grocery shop.

Neuroprotective properties of dietary components

Culture of *Drosophila melanogaster* and generation of age synchronized flies

D. melanogaster, wild (Oregon K) was procured from the National Stock Facility, Manasagangothri, University of Mysore, Karnataka, India. Flies were maintained at the Drosophotoxicology laboratory of our institute under standard conditions at 22±1°C and 70–80% relative humidity and fed on a standard wheat cream–agar diet made with semolina and jaggery along with yeast granules (Girish and Muralidhara, 2012). Age synchronized adult (9–10 days old) male flies (50/replicate; 3replicates/group) were introduced into glass vials with 2ml medium containing the test compounds (Prasad and Muralidhara, 2012).

Treatment regimen

Age matched adult (10d old) male drosophila (20flies/vial, 3vials/group) were maintained on a diet with varying concentrations of protein (semolina + yeast) (0.5 to 10% w/v, 2ml medium, 1week) and were co-challenged with ACR (2mM). In a separate study, flies were maintained on diet with varying carbohydrate diet (jaggery, 2.5-10%). Mortality was recorded daily and locomotor activity was assessed using negative geotaxis at regular intervals.

Assessment of locomotor activity

The locomotor activity of the flies was assessed using the natural negative geotaxis behavior (Feany and Bender, 2000). Flies introduced into a flat bottom glass tube (length 25cm; diameter 2cm) were tapped down, and observed for 60sec for the climbing activity (20flies/trial; 3trials/replicate). Locomotor activity was expressed as percent flies climbed beyond a height of 10cm in 20 sec.

Estimation of oxidative markers

After the treatment regimen, the flies from each tube were homogenized using phosphate buffer (0.1M, pH 7.4, containing 0.9% NaCl) and centrifuged at 5000 X g (10min, 4^0C) to obtain clear supernatant. Protein content in the samples was estimated using Lowry's method (Lowry et al., 1951). The oxidative markers were estimated according to previous methods. Hydroperoxides were estimated by ferrous oxidation of xylenol orange (Wolff et al. 1994) while protein carbonyls by DNPH (Levine et al., 1990) and glutathione by DTNB method (Chandran and Muralidhara, 2016). Acetylcholinesterase activity was estimated following DTNB oxidation (Ellmann et al., 1961).

Statistics

Data are expressed as mean ± standard error (SE) for each experimental group which were analyzed by one–way analysis of variance followed by post hoc Tukey test for comparison of means among the groups (P=0.05). Groups significantly different were labelled with different letters. All statistical analyses were performed using Graphpad Prism 5.0 software.

RESULTS AND DISCUSSION

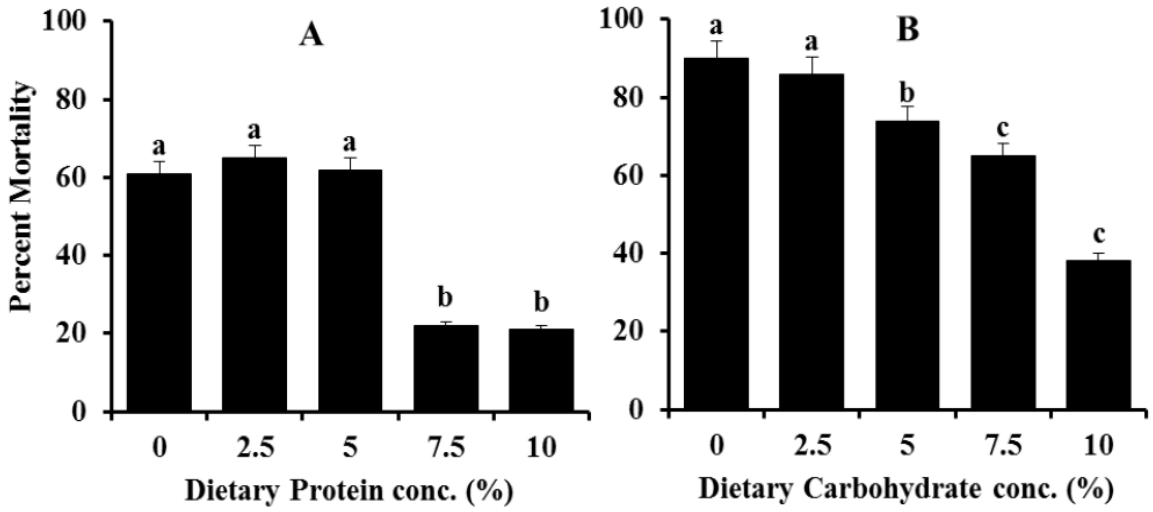

Fig. 1. Acrylamide induced mortality among protein restricted (A) and carbohydrate restricted (B) adult drosophila. Different labels indicate significant difference at P≤0.05.

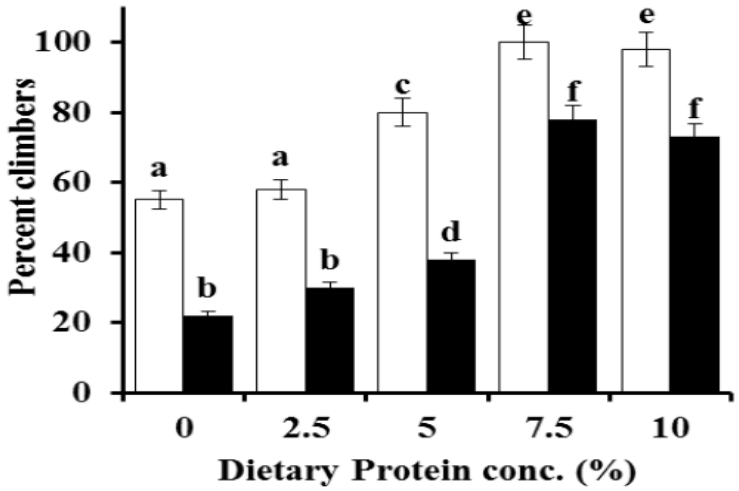

Fig. 2. Acrylamide induced locomotor phenotype among protein restricted flies. Different labels indicate significant difference at P≤0.05.

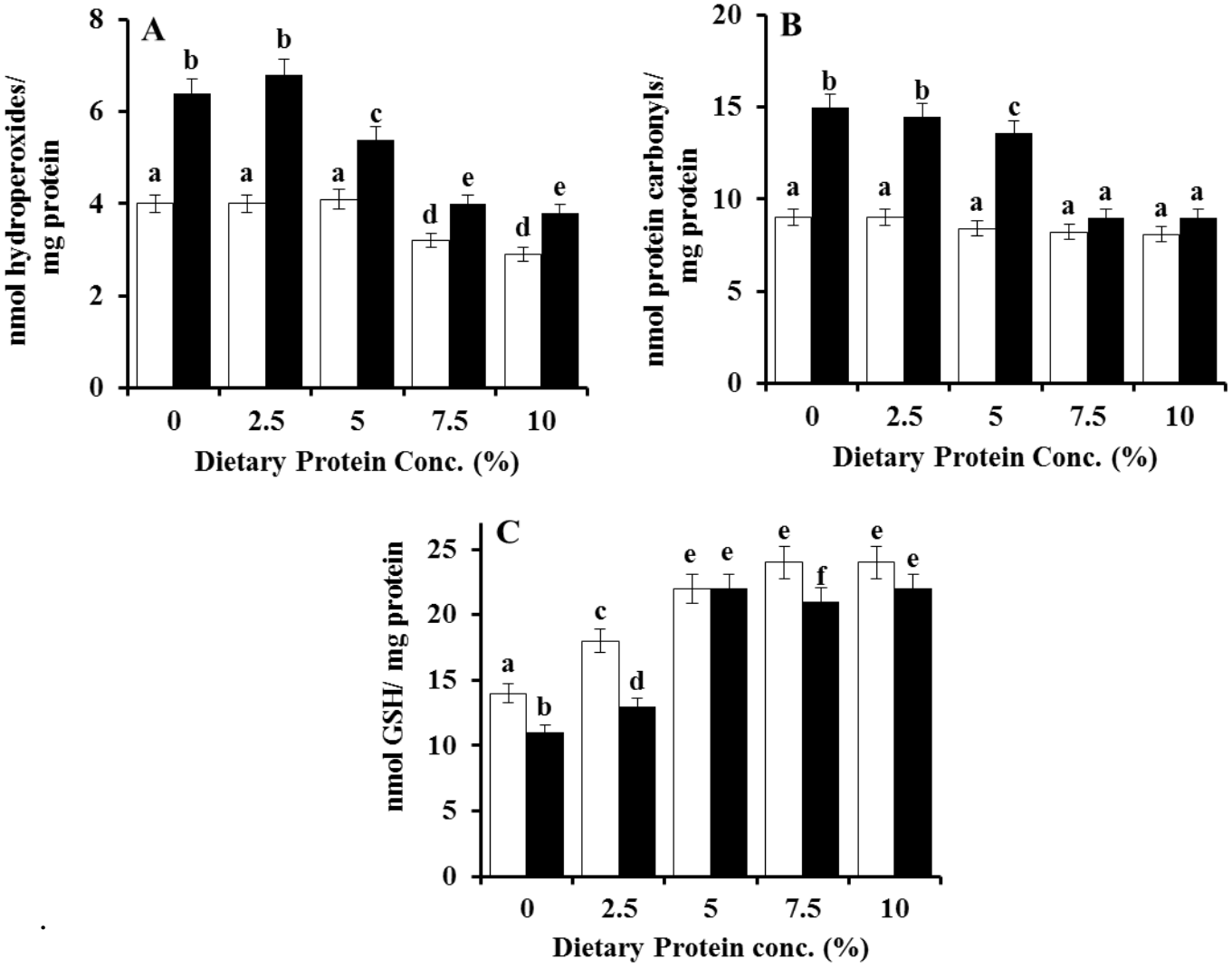

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Fig. 3. Acrylamide induced oxidative markers viz., hydroperoxides (A), protein carbonyls (B) and glutathione (C) among whole body homogenates of flies maintained on protein restricted diet. Different labels indicate significant difference at P≤0.05.

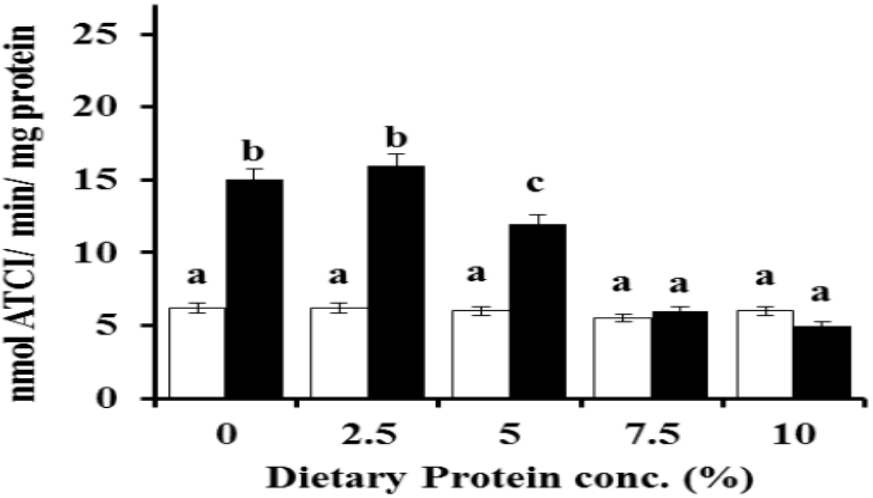

Fig. 4. Acrylamide induced acetylcholinesterase activity among whole body homogenates of flies maintained on protein restricted diet. Different labels indicate significant difference at P≤0.05.

Changes in dietary carbohydrates or proteins did not induce mortality among flies. Reduction or complete absence of dietary protein (semolina+yeast) markedly increased (from 21% to 63%) ACR-mortality **(Fig. 1A).** Interestingly reduction in the dietary carbohydrates in terms of jaggery increased (from 30% upto 86%) ACR-induced mortality among adult drosophila **(Fig. 1B).** Further, carbohydrate-less diet enhanced the ACR induced an obvious fatigue and increased locomotor dysfunction among adult drosophila (data not presented). Due to the fatigue with smallest change in dietary carbohydrate we chose to continue only with protein modulatory studies. In addition to increased mortality, the survivors among limited dietary protein groups performed bad in the climbing assay suggesting an enhanced ACR neurotoxicity **(Fig. 2).**

The fly homogenates were assessed for oxidative markers. There was a marked elevation (40%) of hydroperoxides among ACR groups receiving less than 5% dietary protein (Fig 3.A). Further, the levels of oxidatively modified protein levels were significantly increased with ACR exposure upto 35% among low protein flies (Fig. 3B). In addition, the glutathione levels were increased among high protein diet groups on exposure to ACR which otherwise will reduced (Fig. 3C). The levels of oxidative markers among the high protein diet groups strongly support the hypothesis that protein in the diet has a direct implication on the redox homeostasis.

The AChE activity was significantly enhanced with ACR exposure among the flies maintained on lower dietary proteins. However, the AChE activity levels were normalized with normal dietary protein (Fig. 4). This observation suggests a strong association between the normal protein diet and significantly improved stress behaviour among flies (data not presented). Reduced AChE activity is implicated in reduced dementia and stress (Lee et al., 2011).

Our data suggests that dietary protein significantly deduces the cellular redox homeostasis and neuronal health among drosophila. Further, the dietary protein also protects against acrylamide neurotoxicity in terms of redox markers and locomotor function. Further experiments are planned to assess the molecular mechanisms through which the dietary components render neuroprotection.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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