The Pharma Innovation Journal 2018; 7(4): 637-640

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(4): 637-640

www.thepharmajournal.com
Received: 19-02-2018
Accepted: 21-03-2018

Jayasudha Yeleti
Department of Veterinary Biochemistry, College of Veterinary Science, CVSc, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

K Aswani Kumar
Department of Veterinary Biochemistry, NTR CVSc, Gannavaram, SVVU, Andhra Pradesh, India

A Gopala Reddy
Department of Veterinary Pharmacology, CVSc, Korutla, PVNRTVU, Hyderabad, Telangana, India

P Eswara Prasad
Department of Veterinary Biochemistry, CVSc, Tirupati, SVVU, Andhra Pradesh, India

Kirankumar Yarramshetti
Department of Veterinary Medicine, CVSc, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

Correspondence
Jayasudha Yeleti
Department of Veterinary Biochemistry, College of Veterinary Science, CVSc, Rajendranagar, PVNRTVU, Hyderabad, Telangana, India

Evaluation of ameliorative effects of omega 3 fatty acids on stress induced alteration of liver antioxidant profile in rats

Jayasudha Yeleti, K Aswani Kumar, A Gopala Reddy, P Eswara Prasad and Kirankumar Yarramshetti

Abstract
The present study was carried out to evaluate the ameliorative effect of omega 3 fatty acids on physical stress in rats. Twenty-four adult male Sprague-Dawley rats were randomly divided in to four groups of six rats in each as group I – control diet, Group II - omega 3 fatty acid supplemented diet, Group III - induced physical stress with control diet, Group IV - induced physical stress with omega 3 fatty acid supplemented diet. Physical stress was induced by overcrowding of 6 rats per standard cage instead of 4 rats. Omega 3 fatty acids were supplemented as fish oil @ 3% in the feed. All rats were euthanized as per CPCSEA guidelines on 29th day and liver tissues were collected. The organs were snap frozen and stored at -20ºC for further analysis of reduced glutathione (GSH), glutathione peroxidase (GPX), superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS) and protein carbonyls in the liver homogenate. Stress group (II) rats showed significant decrease in GSH, SOD, GPx and significant increase in TBARS and protein carbonyl levels emphasizing the fact that overcrowding in Group II rats induced considerable physical stress that resulted in oxidative damage via ROS production. Group IV rats showed significant improvement in GSH, SOD and significant decrease in TBARS, protein carbonyls levels whereas GPx was not altered significantly.
The results of the present study elucidate the physical stress induced ROS production and compromised antioxidant defense in rat liver and ameliorative effect of omega 3 fatty acids present in fish oil on reversing the oxidative stress through alteration in the level of SOD but not GPx.

Keywords: rat; physical stress; omega-3 fatty acids; amelioration

Introduction
Stress is a state of disturbed homeostasis due to internal or external sources like physical or psychological stimuli known as stressors. It results in enhanced release of catecholamines and glucocorticoids due to the activation of sympathoadrenal and hypothalamic-pituitary-adrenal (HPA) axis (Joels *et al.*, 2007) [10].
Stress results in the generation of free radicals and subsequent oxidative damage (Al-rejaie *et al*., 2012) [1]. Scientific evidence revealed that a diet rich in long chain omega-3 fatty acids help in the development of healthy brain, heart and immune system. Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and α-linolenic acid (ALA) are ω-3 EFA that are important for structural and biochemical integrity of all cells, including erythrocytes. Omega-3 fatty acid supplementation has been shown to improve the antioxidant status in both normal rodents and disease models in which oxidative stress is increased (Zararsiz *et al*., 2006) [20].
Studies have shown that intake of fish oil or omega 3 fatty acids (ω-3 EFA or EFA) can have an adaptogenic action in ameliorating the adverse effects of stress (Delarue, 2003 and Bradbury, 2004) [5, 4].

Materials and methods
Experimental animals
Twenty-four adult male Sprague-Dawley rats were obtained from NCLAS (National Centre for Laboratory Animal Sciences), National Institute of Nutrition, Hyderabad. Animals were reared in animal house attached to the Department of Veterinary Pharmacology and Toxicology with 12h–12 h dark and light cycle with a temperature of 25–27 °C. Before conducting the experiment, rats were kept for acclimatization for 1 week. Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Rajendranagar (I/1/12/06-01-2012) approved the experimental protocol.

Experimental protocol

The rats were randomly divided into 4 groups of 6 rats in each after the acclimatization period of 1 week. The four groups were (1) control animals without stress (G-I); (2) rats with induced physical stress (G-II); (3) unstressed rats fed with omega fish oil as a source of omega-3 fatty acid (G-III) and (4) stressed rats fed with omega-3 fatty acid supplemented diet (G-IV). In G-II and G-IV, the physical stress was induced by keeping six rats in a standard cage instead of four rats and in G-III, G-IV fish oil was added in the feed @ 3%. They were maintained as per the CPCSEA guidelines under strict supervision. Feed and water were provided ad libitum.

Liver antioxidant profile

Liver antioxidant parameters including GSH, GPx, SOD, TBARS and Protein carbonyls were estimated by following standard methods using double beam UV/Vis spectrophotometer (Tech Comp UV 7500; Tech Comp Ltd. Kowloon Bay, Hongkong). Thiobarbituric acid reacting substances (TBARS) were estimated by the method of Balasubramanian *et al*. (1988) [13]; Superoxide dismutase (SOD) by the method of Madesh and Balasubramanian (1998) [13]; Reduced glutathione (GSH) by the method of Moron *et al*. (1979) [16]; Protein carbonyls by the method of Levine *et al*. (1990) [12] and Glutathione peroxidase (GPx) by the method of Paglia and Valentine (1967) [17].

Statistical analysis

The data were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 15. Differences between means were tested using Duncan's multiple comparison test and significance level was set at $P<0.05$.

Results

The concentration of TBARS (n moles MDA/mg protein) and protein carbonyls (n moles/mg protein) in liver were significantly higher in group II when compared to all other groups. The concentration of TBARS in group III was significantly low when compared to all other groups. The concentration of GSH (µ moles /mg protein), mean activity of GPx (U/mg protein) and SOD (U/mg protein) in liver were significantly lower in group II as compared to all other groups. The GSH concentration & SOD activity in group III showed a significantly higher value among all the four groups. The values of GPx activity were statistically similar in group I, III & IV.

Table: Mean values of TBARS, GSH, GPx, SOD and Protein Carbonyls in experimental groups of rats on 29th day

Group	TBARS (n moles MDA/mg protein)	GSH (µ moles /mg protein)	GPx (U/mg protein)	SOD (U/mg protein)	Protein Carbonyls concentration (PC; n moles/mg protein)
I	0.59 ± 0.00^{B}	11.00 ± 0.01^{B}	0.18 ± 0.02^{A}	12.34 ± 0.16^{B}	2.63 ± 0.15^{B}
II	0.69 ± 0.00^{A}	10.80 ± 0.00^{C}	0.13 ± 0.01^{B}	11.04 ± 0.12^{C}	3.09 ± 0.10^{A}
III	0.54 ± 0.00^{D}	11.35 ± 0.05^{A}	0.19 ± 0.01^{A}	13.40 ± 0.06^{A}	2.63 ± 0.13^{B}
IV	0.59 ± 0.01^{C}	11.11 ± 0.06^{B}	0.19 ± 0.02^{A}	12.39 ± 0.15^{B}	2.63 ± 0.15^{B}

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly ($p<0.05$).

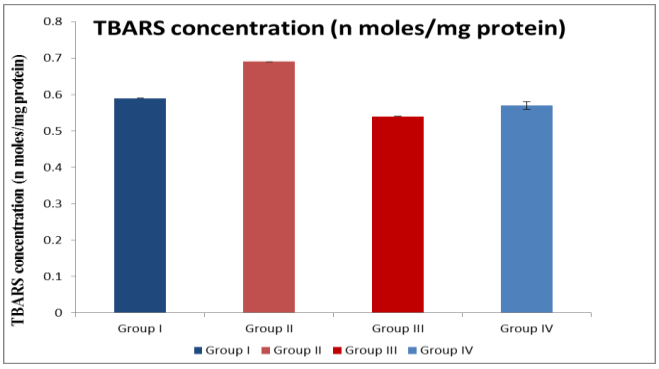

Fig 1: Mean values of TBARS concentration in experimental groups of rats

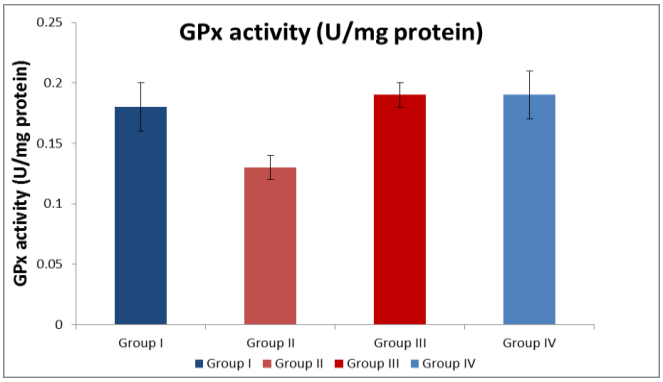

Fig 3: Mean Values of GPx activity in experimental groups of rats

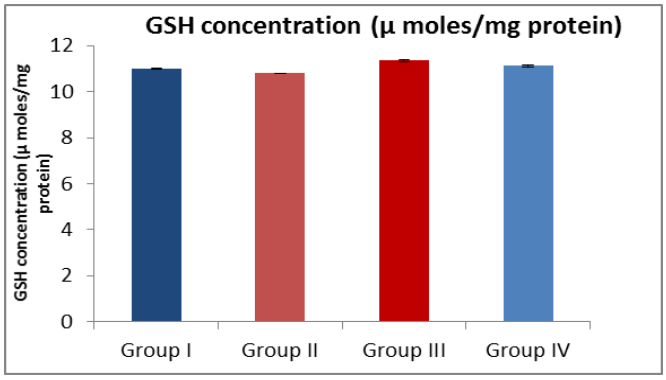

Fig 2: Mean values of GSH concentration in experimental groups of rats

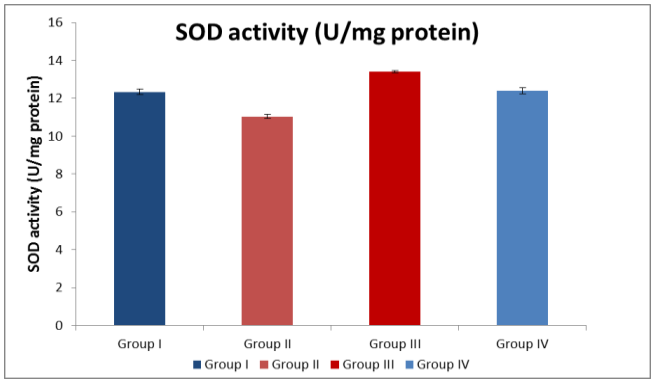

Fig 4: Mean values of SOD activity in experimental groups of rats

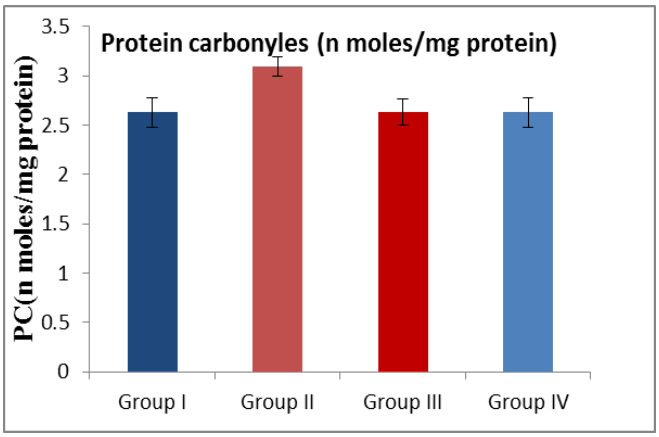

Fig 5: Mean values of Protein Carbonyls concentration in experimental groups of rats

Discussion

The activities of antioxidant enzymes are reduced in stressed individuals due to free radical induced DNA damage that results in impaired expression of antioxidant enzyme genes and associated decrease in the concentration of antioxidant enzyme proteins. The stress marker TBARS in the rat liver homogenate was significantly higher in group II as compared to group I in the present study, which is in agreement with the findings of Bindu (2014) [3], who reported significantly higher concentration of MDA in the liver tissue of rats exposed to overcrowding stress. The stress marker TBARS level showed a significant decrease in fish oil supplemented group III as compared to control group. Iraz *et al.* (2005) [7] reported similar results of lower mean MDA levels in erythrocytes of rats that received dietary supplementation of fish oil when compared to control rats.

The concentration of protein carbonyls in liver revealed a significantly higher value in stress group (G-II) as compared to control group (G-I). This might be due to the compromised antioxidant defenses resulting in increased production of ROS. This higher value of protein carbonyls in stress group is in accordance with the findings of Ji *et al*. (1990) [9] and Sohal *et al.* (1995) [19], who reported that carbonylation level increases as a direct consequence of a diminished activity of the primary antioxidant defense systems. The concentration of protein carbonyls in group III showed a comparable value to control group. This is in accordance with the findings of Patten *et al*. (2013) [18] who reported that omega-3 supplementation induced a non-significant trend toward a decrease in the levels of protein carbonyls in ethanol-exposed *Sprague-dawley* rats.

The stress group (G-II) had a significantly lower reduced glutathione (GSH) in liver as compared to control group (G-I), which is in agreement with the findings of Bindu (2014) [3], Khataibeh *et al*. (2013) [11] and Jafari *et al*. (2014) Khataibeh *et al*. (2013) [11] reported a significant decrease in liver GSH concentration in rats exposed to acute restraint stress as compared to non-stress control rats. Jafari *et al*. (2014) [8] reported reduced GSH level in rats due to physical stress on the 1st and 15th day of the experiment as compared to the control rats, which was restored to normal level in stressed group after 30 days. The decreased GSH level might be due to the presence of ROS produced by the stress, the increased activity of Glutathione S-Transferase and limited GSH synthesis (Jafari *et al*., 2014) [8]. The reduced glutathione concentration in group III showed a significant increase as compared to control group. These findings are in agreement with the findings of Meganathan *et al*. (2011) [15]. Probably, the increase in GSH level in group III might be due to antioxidant action of omega-3 fatty acids, which emphasizes the ameliorative action of omega-3 fatty acids on oxidative stress by maintaining GSH homeostasis (Meganathan *et al*., 2011) [15].

The mean activity of GPx in the liver homogenate of group II showed significant decrease when compared to that of groups I, III and IV. However, there was no change in GPx activity in group III as compared to that of control group. This confirms the findings of Mattei *et al*. (2011) [14], who reported no significant variation in GPx activities in rat forebrains with experimental treatment viz., control, astaxanthin fed group, fish oil fed group and combined ASTA/Fish oil fed group. The results of the present study indicate that GPx activity is significantly lowered in physical stress induced group but omega 3 fatty acids present in fish oil had no beneficial action in restoring the adverse effect of physical stress.

The activity of SOD in liver revealed a significant decrease in group II as compared to group I. This is in agreement with the findings of Bindu (2014) [3] and Khataibeh *et al*. (2013) [11]. Khataibeh *et al.* (2013) [11] reported significant decrease in the activity of SOD in rats with restraint stress as compared to control rats. The activity of SOD in group III showed a significant increase as compared to control group. This was in agreement with the findings of Hussein *et al*. (2014) [6], who reported that fish oil supplementation, significantly increased SOD in treated diabetic group as compared to diabetic group without fish oil supplementation, although it was still significantly lower as compared to non-diabetic control group in albino rats (Hussein *et al*., 2014) [6]. The increase in the levels of SOD is presumably because of antioxidant action of omega-3 fatty acids that helps to attenuate oxidative stress (Meganathan *et al*., 2011) [15].

Conclusion

The findings of the present study conclude that physical stress enhanced the generation of reactive oxygen species with subsequent depletion of antioxidant defenses and eventual oxidative stress resulted in damage to liver. Increased levels of TBARS, protein carbonyls and decreased levels of GSH, GPx and SOD are indicative of stress in Group II rats. Supplementation of omega-3 fatty acids (fish oil) in the diet was found to be beneficial in countering the oxidative damage of physical stress by increasing antioxidant defense through elevation SOD activity but not GPx.

Ethical compliance

The study was in compliance with the Institute Animal Ethics Committee, CVSc, Rajendranagar

References

1. Al-rejaie S, Abuohashish H, Ahamad M, Alroujayee A, Alkhamees O. Immobilization stress-induced oxidative damage and its amelioration with green and black Tea. Journal of Pharmacology and Pharmacotherapeutics. 2012; 6:538-545.
2. Balasubramanian KA, Manohar M, Mathan VI. An unidentified inhibitor of lipid peroxidation in intestinal mucosa. Biochimica *et al*. Biophysica Acta. 1988; 962:51-58.
3. Bindu MP. Effect of different types of stress on lipid metabolism and antioxidant status in albino rats. Thesis submitted to University of Calicut, 2014.

4. Bradbury J, Myers SP, Oliver C. An adaptogenic role for omega-3 fatty acids in stress; A randomised placebo controlled double blind intervention study. Nutrition Journal. 2004; 3:20.
5. Delarue JOCPRL, Matzinger O, Binnert C, Schneiter P, Chiolero R, Tappy L. Fish oil prevents the adrenal activation elicited by mental stress in healthy men. Diabetes and Metabolism. 2003; 29:289-295.
6. Hussein JS, El-khayat Z, Morsy S, Oraby F, Singer G. The effect of fish oil on oxidant/ antioxidant status in diabetic rats through the reduction of arachidonic acid in the cell membrane. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6:196-199.
7. Iraz M, Erdogan H, Ozyurt B, Ozugurlu F, Ozgocmen S, Fadillioglu E. Omega-3 essential fatty acid supplementation and erythrocyte oxidant/ antioxidant status in rats. Annals of Clinical and Laboratory Science 2005; 35:169-173.
8. Jafari M, Salehi M, Zardooz H, Rostamkhani F. Response of liver antioxidant defense system to acute and chronic physical and psychological stresses in male rats. EXCLI Journal 2014; 13:161-171.
9. Ji LL, Dillon D, Wu E. Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. American Journal of Physiology. 1990; 258:918-923.
10. Joels M, Krugers H, Karst H. Stress-induced changes in hippocampal function. Progress in brain research. 2007; 167:3-15.
11. Khataibeh M. Cinnamon modulates biochemical alterations in rats loaded with acute restraint stress. Journal of Saudi Chemical Society. Journal name, 2013.
12. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG *et al*. Determination of carbonyl content in oxidatively modified proteins. Methods in Enzymology 1990; 186:464-478.
13. Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics 1998; 35:184-188.
14. Mattei R, Polotow TG, Vardaris CV, Guerra BA, Leite JR, Otton R *et al*. Astaxanthin limits fish oil- related oxidative insult in the anterior forebrain of *Wistar* rats: Putative anxiolytic effects? Pharmacology, Biochemistry and Behavior. 2011; 99:349-355.
15. Meganathan M, Gopal KM, Sasikala P, Mohan J, Gowdhaman N, Balamurugan K *et al*. Evaluation of hepatoprotective effect of omega-3 fatty acid against paracetamol induced liver injury in Albino Rats. Global Journal of Pharmacology. 2011; 5:50-53.
16. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S transferase in rat lung and liver. Biochimica et Biophysica Acta. 1979; 582:67-68.
17. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine. 1967; 70:158-169.
18. Patten AR, Brocardo PS, Christie BR. Omega-3 supplementation can restore glutathione levels and prevent oxidative damage caused by prenatal ethanol exposure. Journal of Nutritional Biochemistry. 2013; 24:760-769.
19. Sohal RS, Agarwal S, Sohal BH. Oxidative stress and aging in the Mongolian gerbil (*Merione sunguiculatus*). Mechanisms of Ageing and Development. 1995; 81:15-25.
20. Zarasiz I, Kus I, Akpolat N, Songur A, Ogeturk M, Sarsilmaz M. Protective effects of omega-3 essential fatty acids against formaldehyde-induced neuronal damage in prefrontal cortex of rats. Cell Biochemistry and Function. 2006; 24:237-244.