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IMPACT OF FUNCTIONAL FOODS (ALPHA LIPOIC ACID & PROBIOTIC) ON ACETAMINOPHEN & HYPOBARIC PRESSURE INDUCED OXIDATIVE STRESS RELATED UREMIC MALE RATS

Dr. Dilip K Nandi[1]* and Dr. Banadeb Sinha[2]

[1]Department of Microbiology, Nutrition, and Human Physiology, Raja N L Khan Women's College, Midnapore, Pin-721102, West Bengal, India.
[2]Department of Biochemistry, Midnapore Medical college & Hospital, West Bengal, India.

***Author for Correspondence: Dilip Kumar Nandi**
Department of Microbiology, Nutrition, and Human Physiology, Raja N L Khan Women's College, Midnapore, Pin-721102, West Bengal, India.

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ABSTRACT
Hypobaric pressure & acetaminophen overdoses seems to be favorable with increased oxidative damage which could be the consequence of the increased activity of ROS generating and decreased activity of antioxidant system that ultimately results in uremia. The present study is aimed to evaluate the impact of ALA & probiotic supplementation on stress induced uremia considering different biochemical parameters like urea, creatinine, MDA, SOD, catalase, RBC & hemoglobin. It was noted that serum urea, creatinine, MDA both are increased in uremic group & decreases SOD & catalase level. Acetaminophen overdoses causes the formation of NAPQI that decreases the glutathione level & causes oxidative stress. Hypobaric pressure causes hypoxia induced damage to endothelial cells, activation of cytokines, chemokines and cell adhesion molecules may orchestrate the lung inflammatory response. It cannot be ruled out that reactive oxygen (ROS) & ultimately leads to oxidative stress injury. But above theses values are resettled in treatment group & minimize the stress induced uremia. It has great societal impact on community people as it is an alternative, cost effective, affordable harmless therapy which can minimize the oxidative stress induced uremia.

KEYWORDS: *Alpha-lipoic acid; uremia; Probiotic; catalase; Oxidative stress.*

INTRODUCTION

At present kidney diseases appear to be a major problem across the globe. As per global and regional overview, 17,83,000 patients are suffering from End Stage Renal Disease of which dialysis has been given to 13,71,000 patients and kidney has been transplanted in 4,12,000 patients suffering from renal failure, live under particularly pro-oxidative conditions causing uremia.[1,2] At therapeutic doses, acetaminophen is metabolized via glucuronidation and sulfuration reactions occurring primarily in the liver, and results in water-soluble metabolites that are excreted via the kidney. As a result of the metabolic conversion of acetaminophen by the microsomal P-450 enzyme system, a highly reactive intermediate, N-acetylpbenzoquinone imine (NAPQI) is produced. NAPQI directly reacts with glutathione (GSH) and at overdoses of acetaminophen, the depletion of cellular GSH occurs. This allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to renal injury. Previous studies demonstrated that acute APAP overdose increased lipid peroxidation, endoplasmic reticulum stress. Paracetamol (also known as acetaminophen) poisoning is due to metabolic activation of APAP by renal P450 mixed-function oxidases, similar mechanism was proposed for the nephrotoxicity.[3,4] The gastrointestinal tract of the human and other mammals is populated by a vast and diverse group of microbes in a composite manner.[5] Bacteria are the major population of the alimentary tract and this native microflora is commonly designated the gut microflora.[6] Microbial flora present in the microenvironment of the gastrointestinal tract performs several important and essential activities of the host[7] such as the breakdown of undigested food, metabolism of drugs, enhanced absorption of foodstuffs, synthesis of vitamins, creation of resistance against pathogenic bacteria by colonization resistance, stimulation of host immunity and induction of intestinal maturation.[8,9]

The microflora is also highly sensitive to oxygen tension[10,11] and correlated with the atmospheric pressure. Individuals who are exposed to high altitude (HA) are characterized by hypobaric hypoxia environmental conditions that induce several physiological changes like body weight, hematological changes[12] including gastrointestinal disorders in

human[13] Acute mountain sickness (AMS) is a frequent complication for military personnel, veterans, athletes, and travelers at high altitudes.

METHODS AND MATERIALS

Selection & Animals care

The study was conducted on 30 healthy, adult, male albino rats of Wister strain (Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata 54) having a body weight of 100 ±15 g. They were acclimatized to laboratory condition for 2 weeks prior to experimentation. Animals were housed three rats/cage in a temperature-controlled room (22± 2^0C) with 12–12 h dark–light cycles (8.00–20.00 h light, 20.00– 8.00 h dark) at a humidity of 50 ±10%. They were provided with standard food and water ad libitum. Animal care was provided according to the Guiding Principle for the Care and Use of Animals.[14]

Grouping of animals and experimental procedure

The rats were divided into four equal groups as follows

Group I or control – Six animals were subjected to control group. They were housed at room temperature (25± 3^0C) and feed normal diet and water ad libitum.

Group II or acetaminophen induced uremic rats – Six animals were randomly placed in cage with normal diet and injected with acetaminophen at the conc. of 500 mg with de-ionized water 5 mL/kg of body weight/day for 10 days to achieve uremia.

Group III–This group of animals exposed at hypobaric pressure for 10 days

Group IV-Animals of this group were treated as group II & co-administered as Alpha-lipoic acid at the dose (100)mg/ kg body weight/day) for 10 days

Group-V Animals of this group were treated as group III & co-administered as commercial available probiotic supplementation with Lactic acid bacteria at the dose (1×109 cfu/day/kg body weight) for 10 days.

Animals sacrificed and plasma and organ collected

This experimental design was continued for 24 days. After 24 days, the animals were sacrificed and blood & tissue was collected from the aorta after which the kidneys were collected for different biochemical analysis.

Parameters

Hematological parameter

After the total experimental period, animals were sacrificed by diethyl ether anesthesia. Blood samples were collected by hepatic artery puncture under diethyl ether anesthesia using 21 gauge needles mounted on a 5 ml syringe into heparin coated sample bottles for analysis of hematological parameters like total RBC by haemocytometer and hemoglobin (Hb) by standard kit method (Merck, Japan). Biochemical assay of catalase (CAT) activity[11], superoxide dismutase (SOD)[12] and estimation of Lipid Peroxidation from the levels of Malondialdehyde (MDA)[13] and blood Urea and Creatinine[14] also measured.

2.5 Statistical Analysis

Analysis of variance (ANOVA) followed by a multiple two-tail 't' test with Bonferroni modification was used for statistical analysis of the collected data. Difference were considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

Acetaminophen over dose is often linked to many metabolic disorders including serum electrolyte, urea & creatinine derangements. Increased concentration of serum urea & creatinine are considered for investigating drug induced nephrotoxicity in animals & man.[15] The fundamental function that blood cells perform ,together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoietic system unique as a target organ. Erythrocytes, leuckocytes & platelets are produced at a turnover rate of about 1 - 3 million per second in a healthy human adult & these values can be distorted in certain physiological & pathological states during hemolytic anemia & suppressive inflammation.[16]

Most of probiotic microorganisms belong to Lactic Acid Bacteria (LAB), such as *Lactobacillus spp.*, *Bifidobacterium sp.* and *Enterococcus sp.* Among lactic acid bacteria, *Lactobacillus* has attracted a lot of attention for their potential probiotic effects in human health[17] like improve humoral immune responses**[18],** altered cell numbers within White Blood Cell (WBC) subsets and enhanced phagocytic capacity in the peripheral granulocyte population. Plasma endotoxin concentrations were decreased during probiotic feeding and Red Blood Cells (RBCs) were decreased susceptibility to osmotic pressure. Individuals who are exposed to high altitude (HA) are characterized by hypobaric hypoxia environmental conditions that induce several physiological changes like body weight, hematological changes.[19] In this present study it was observed that total RBC count & hemoglobin concentration both are decreased in acetaminophen treated group (group II) in compared with control group (group I) but these values are significantly ($p<0.05$) increased in hypobaric pressure induced rats (group III)**.** But co-administration of ALA improves the RBC count & hemoglobin level. Probiotic supplementation on group III decreases theses parameters & it is near to control level **(Fig: 5& 6)**.

Acetaminophen over dose is often linked to many metabolic disorders including serum electrolyte, urea and creatinine derangements. Increased concentration of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animals and man **(20).** In this study it was observed that urea & creatinine level is significantly higher in group II & group III rats in comparison with control group. But ALA & probiotic supplementation significantly restored

theses toxic substances & it is near to control level **(Fig: 1& 2).**

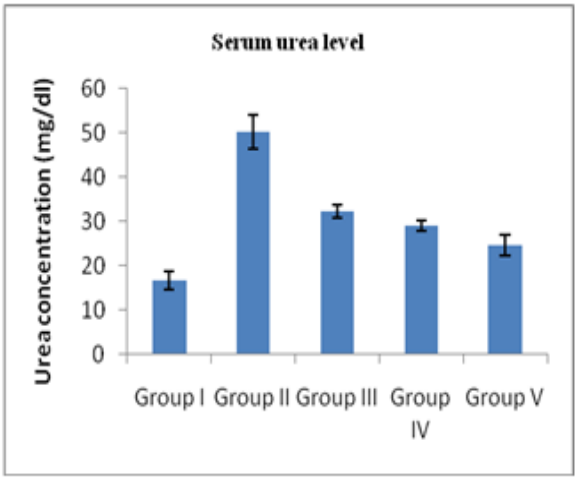

Fig: 1. Effect of ALA & Probiotic on plasma urea level on acetaminophen & hypobaric pressure induced uremic rats.

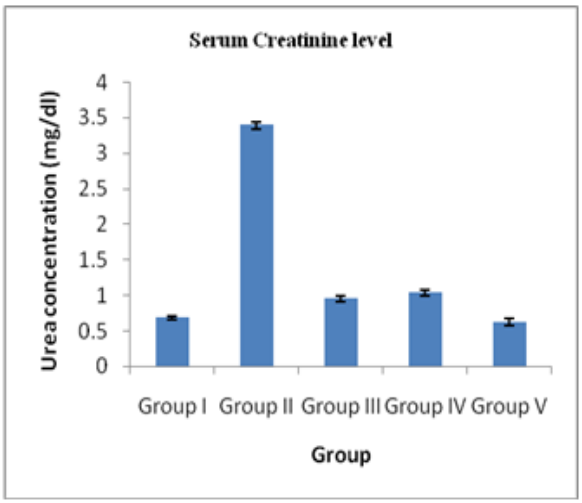

Fig: 2. Effect of ALA & Probiotic on plasma creatinine level on acetaminophen & hypobaric pressure induced uremic rats.

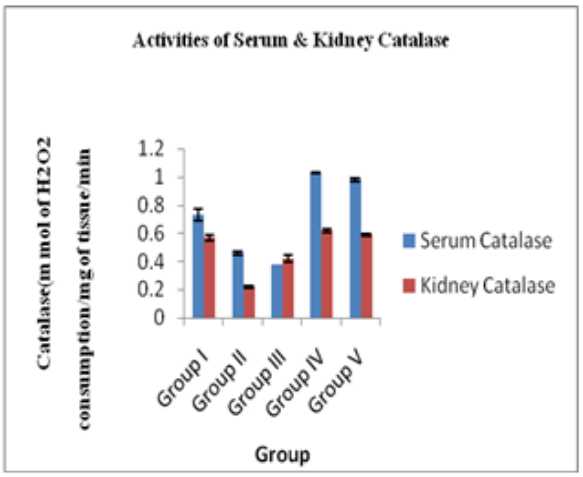

Fig: 3. Effect of ALA & Probiotic on serum & kidney Catalse activities on acetaminophen & hypobaric pressure induced uremic rats.

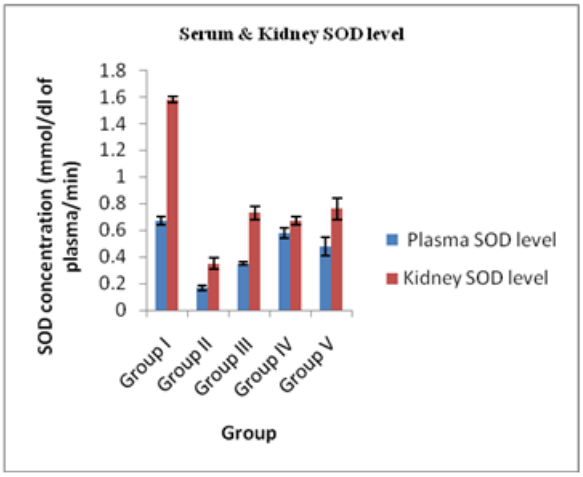

Fig: 4. Effect of ALA & Probiotic on serum & kidney SOD level on acetaminophen & hypobaric pressure induced uremic rats.

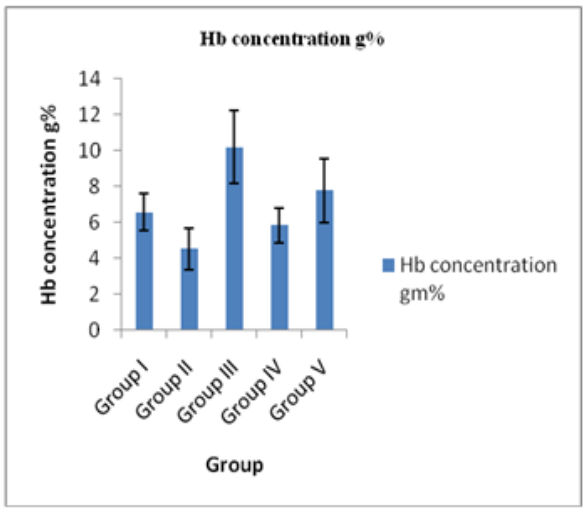

Fig: 5. Effect of ALA & Probiotic on Hemoglobin concentration (g%) on acetaminophen & hypobaric pressure induced uremic rats.

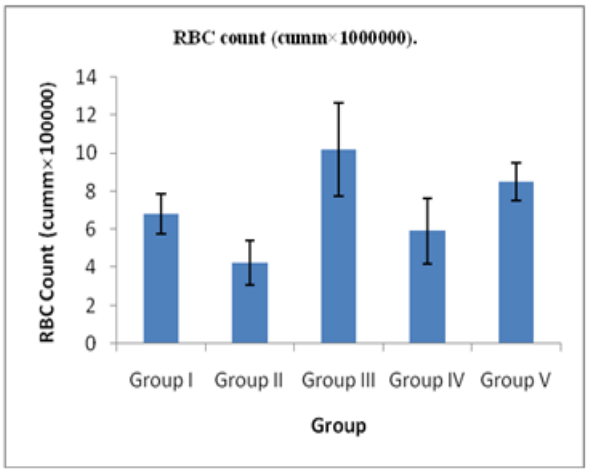

Fig: 6. Effect of ALA & Probiotic on Total RBC count on acetaminophen & hypobaric pressure induced uremic rats.

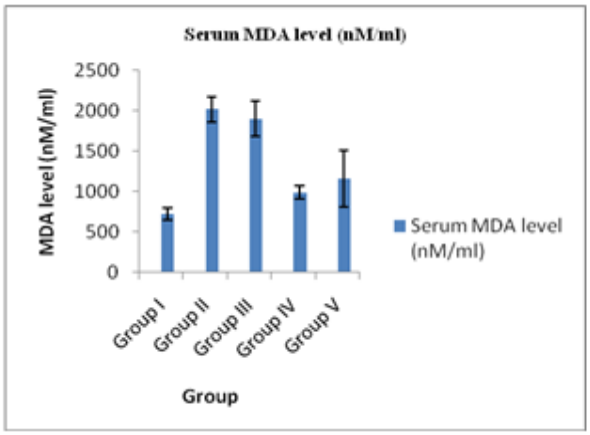

Fig: 7. Effect of ALA & Probiotic on serum MDA level on acetaminophen & hypobaric pressure induced uremic rats.

The short exposure (5 days) to an altitude of 7576 m caused increased lipid per oxidation level in plasma of rats. Beside hypoxia induced damage to endothelial cells, activation of cytokines, chemokines and cell adhesion molecules may orchestrate the lung inflammatory response. It cannot be ruled out that reactive oxygen (ROS) and nitrogen species (RONS) are also involved and may even play a causative role in AMS, HAPE and HACE. ROS can contribute to 'opening up' of the blood- brain barrier, allowing neurotoxins, endotoxin and inflammatory cells to enter the brain. Hypoxia is thus a life threatening stress that has to be dealt with at both cellular and systemic levels.[12] In this study it was found that there were significant decreases in anti-oxidative enzymes & increases in MDA level both acetaminophen & hypobaric pressure treated group. Our present work showed that there was significant ($p<0.05$) increase in MDA level & decrease in anti-oxidant enzymes level like SOD & Catalase in serum & kidney in both group II & group III rats due to oxidative stress related injury. But ALA & probiotic supplementation decreases significantly ($p<0.05$) the MDA level & significantly increases SOD & Catalase level in serum & kidney when compare with group II & group III rats **(Fig: 3.4. &7).**

CONCLUSION

From this study it may be concluded that supplementation of ALA & probiotic prevent the acetaminophen & hypobaric pressure induced oxidative stress related uremia. It has great societal impact on community people as it is an alternative, cost effective, affordable therapy which can minimize the oxidative stress induced uremia.

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