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Effect of vitamin E supplementation on heavy metal induced renal toxicity in rat model

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Abstract

This experiment was designed to explore the antioxidant effect of vitamin E on oxidative stressed rat's model induced by heavy metal. Heavy metals induce their toxic effects and generate reactive oxygen species. The toxic manifestations of these metals caused imbalance between pro-oxidant and antioxidant homeostasis which is termed as oxidative stress. There are 4 groups of rats were taken, group one is control, second is of mercury induced Hg (10ppm/1000ml of drinking water), third group is of mercury + vitamin E (10ppm/1000ml of drinking water + 25mg /kg body weight) treated rats and fourth group is of vitamin E supplement (25mg/kg body weight). We administered doses of mercury (10ppm/bodyweight) and doses of vitamin E (25mg/kg body weight) simultaneously. To find out the level of oxidative stress assess the certain parameters and biomarkers as ferric reducing antioxidant power (FRAP), Advanced oxidative protein product (AOPP), urea and creatinine level The FRAP content reduced in blood and AOPP, Urea and creatinine level increased in blood, but after the supplementation with vitamin E, the content increased and AOPP (Advanced oxidative protein product) decreased whereas urea and creatinine both normalize. Vitamin E supplementation normalizes the levels of these parameters by putting their ameliorative effect.

Keywords: vitamins E, mercury, oxidative stress, heavy metal, FRAP, AOPP, urea, and creatinine

1. Introduction

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants. Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS. Ratio between oxidized and reduced glutathione (2GSH/GSSG) is one of the important determinants of oxidative stress in the body. Higher production of ROS in body may change DNA structure, result in modification of proteins and lipids, activation of several stress-induced transcription factors, and production of pro inflammatory and anti-inflammatory cytokines. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress''. Regulation of reducing and oxidizing (redox) state is critical for cell viability, activation, proliferation, and organ function. Aerobic organisms have integrated antioxidant systems, which include enzymatic and non-enzymatic antioxidants that are usually effective in blocking harmful effects of ROS.

Metal induced toxicity is very well reported one of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. A growing amount of data provide evidence that metals are capable of interacting with nuclear proteins and DNA causing oxidative deterioration of biological macromolecules1. One of the best evidence supporting this hypothesis is provided by the wide spectrum of nucleobase products typical for the oxygen attack on DNA in cultured cells and animals (Flora *et al.,* 2008) [1].

Mercury is wide spread environmental and industrial pollutants which induces severe alterations in the body tissues of both humans and animals. Mercury toxicity is due to its ability to form stable complexes with the sulfhydryl-cysteine groups of proteins, thus damaging the proteins as well as thiol related enzymes however the binding of mercury to the –SH groups of glutathione or proteins is consider a mechanism of cell defense, as sequesters transports and inactivates reactive metal ions.

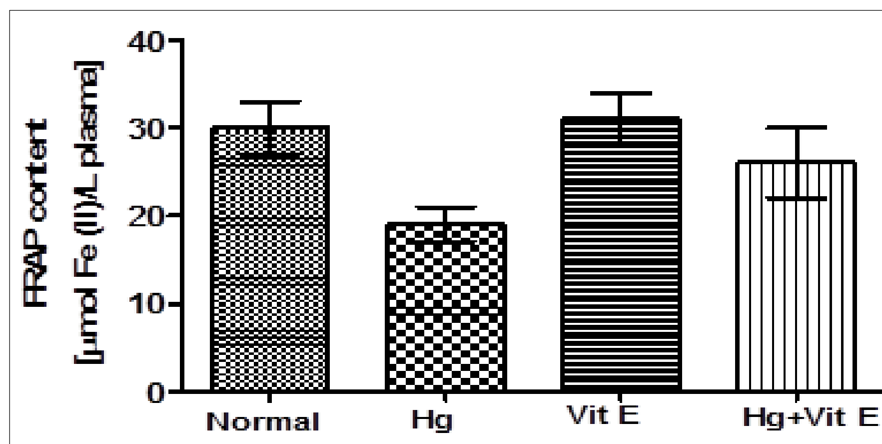
Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations. One of most important Vitamin is a vitamin E. In nature vitamin E comprises eight natural fat- soluble compounds including 4 tocopherols (d-alpha, d-beta, d-gamma and d-delta tocopherol) and 4 tocotrienols (d-alpha, d-beta, d-delta, d-gamma and d-tocotrienol). Vitamin E is an important atioxiant factor it is known to process various physiological functions. A major contributor to non- enzymatic protection against lipid peroxidation is vitamin E, a known free radical scavenger vitamin E as a lipid soluble, chain breaking antioxidant plays a major protective role against oxidative stress and prevents the productions of lipid peroxides by scvanging free radicals in Biological membrane. Vitamin E (α-tocopherol) is a fat-soluble vitamin known potent endogenous antioxidants. A-tocopherol is a term that encompasses a group of potent, lipid soluble, chain-breaking antioxidants that prevents the propagation of free radical reactions.

2. Methodology

The experiment was carried out on 16 male Albino rats between 100-200 gm. They were divided into four groups (n=4). They were housed in a temperature controlled room (25 ± 5 °C) with 12h light- dark cycles. All rats were fed with normal laboratory diet in the form of nutrient rich pellets containing total energy as fat, protein, and carbohydrates, with free access to drinking water. The rats were divided into four groups. Blood samples were collected by cardiac puncture into10unit/mL heparin rinsed anticoagulant syringes and then red blood cells were pelleted by centrifugation at 800g for 10min at 4∘C. The plasma was

immediately frozen at − 80∘C until use for biochemical assays, buffy coat and upper 15% of packed red blood cells (PRBCs) the RBC were washed twice with cold phosphate buffered saline (PBS). The protocol of study was approved by the Animal Care and Ethics Committee of University of Allahabad. Erythrocyte 'ghosts' from leukocyte -free RBCs were prepared by following the method of Dodge, Mitchell and Hanahan (1963) with slight modifications. Briefly, washed and packed erythrocytes were lysed by adding 10 volume of 5mM phosphate buffer pH 7.4(at 4),after leaving on ice for 30 minute erythrocytes membrane were packed by centrifugation at 20,000 x g for 10 min at 4c and the hemoglobin containing supernatant was removed. the erythrocyte membrane were then washed three times by suspending in fresh buffer followed by centrifugation under the same condition, finally the membranes were suspended in hypotonic 5mM buffer followed by centrifugation under the same condition and then suspended in 5mM phosphate buffer pH 7.4.protein was estimated in red cell membrane preparation following the method of Lowery, Rose rough, Farr, and Randall (1951) using the bovine serum albumin as a standard. The total antioxidant potential of the plasma was determined using a modification of the ferric reducing ability of plasma (FRAP) assay reported by Benzie and Strain (1996). FRAP reagent was prepared from 00mmol L-1 acetate buffer, pH 3.4, 20mmol L-1 ferric chloride and 10 mmol L-1 2,4,6 tri-pyridyl-s-triazine made up in 40mmol L-1hydrochloric acid. All three solutions were mixed together in the ratio10:1:1(v/v/v) respectively. 3ml of FRAP reagent were mixed with 100µl of plasma and the contents were mixed thoroughly. the absorbance was read at 593 nm at 30s intervals for 4 min. Aquous solution of known fe (II)concentration in the range of 100-1000µmol L-1 was used for calibration. Using the regression equation the FRAP values (µmol fe (II)/L) of the plasma was calculated. Determination of AOPP levels was performed by modification of the method of (Witko-Sarsat *et al*., 1996). 2 ml of plasma was diluted in 1:5 in PBS, 0.1 ml of 1.6 M potassium iodide was then added to each tube, followed by 0.2 ml acetic acid after 2 min. the absorbance of reaction mixture was immediately read at 340 nm against blank containing 2ml of PBS, 0.1 ml of KI, and 2 ml of acetic acid. The chloramine –T absorbance at 340 nm being linear within the range of 10-100mmol L-1, AOPP concentrations were expressed as µmol L-1 chloramine-T equivalents. All data are presented as means ± SEM. Statistical analyses were conducted using the software PRISM version 5.01. T-test was used to assess relationships between parameters and differences among treatments. Multiple comparisons were done by employing one way analysis of variance (ANOVA) and subjecting Bonferroni posttest on the data.

3. Results

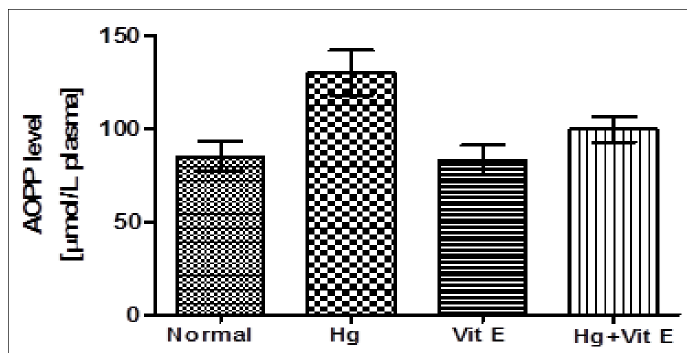


Values are expressed as mean ±SD

Significant ($P < 0.05$) difference was obtained

Fig 1: Effect of vitamin E supplementation on FRAP content in heavy metal induced rats.

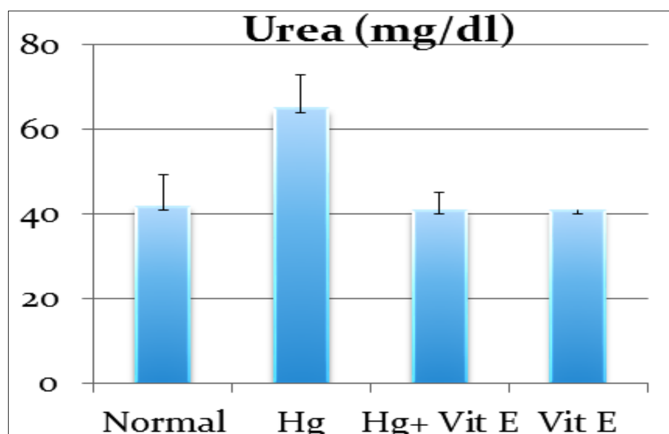
Ferric reducing antioxidant power (FRAP) found to be normal in controle rats, heavy metal (mercury) induction lowering the FRAP content, when heavy metal was induced along with vitamin E the FRAP content returned near to normal, whereas in Vtamin E supplemented rats the plasma FRAP content level returned near to normal.



Values are expressed as mean ± SD
Significant ($P < 0.05$) difference was obtained

Fig 2: Effect of vitamin E supplementation on AOPP level in heavy metal induced rat

AOPP level was found to be normal in the controle rats, heavy metal(mercury) caused a rapid increase in AOPP level when heavy metal was induced along with Vitamin E the AOPP level returned near to normal, whereas in Vitamin E supplemented rats the AOPP level in plasma returned to normal.



Values are expressed as mean ± SD
Significant ($P <0.05$) difference was obtained

Fig 3: Effect of supplementation of vitamin E on urea level in heavy metal induced rats.

Urea level in plasma was found to be normal in the control rats; heavy metal (mercury) induction caused a rapid increase in urea level, when heavy metal was induced along with Vitamin E the urea level returned near to normal, whereas in Vitamin E supplemented rats the urea level returned to normal.

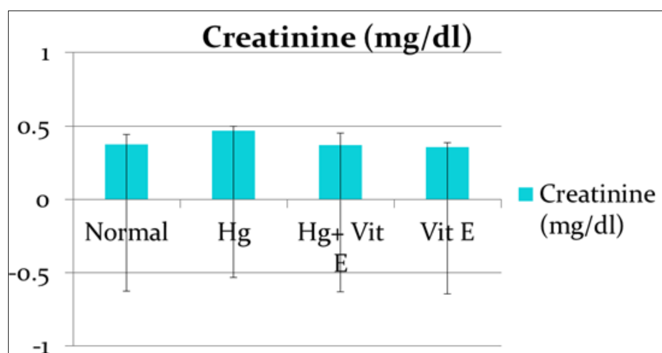


Fig 4: Effect of supplementation of vitamin E on creatinine level in heavy metal induced rats.

Plasma creatinine level was found to be normal in controle rats; heavy metal (mercury) induction caused a rapid increase in creatinine level in plasma, when heavy metal was induced along with Vitamin E the creatinine level returned near to normal, whereas in Vitamin E supplemented rats the plasma creatinine level returned to normal.

4. Conclusion

The above results provide an insight onto the role of reactive species in metal-induced toxicity (mercury toxicity) and their prevention to antioxidant like vitamin E.

Exposure to mercury caused oxidative stress which results in reduction in antioxidants activities and variation in biochemical parameters and biochemical biomarkers.

However, Vitamin E treatment has protective effect on these alterations caused by mercury by increasing urea and creatinine level and by reducing the FRAP and AOPP level in blood plasma. Further the conclusion is that supplementation of vitamin E is beneficial and provides nephroprotective role by reducing the urea and creatinine level in blood plasma which are the biomarkers which show the sign of an oxidative damage whereas vitamin E balance the level of FRAP and AOPP content So this study recommends the intake of vitamin E by eating a well-balanced diet high in vitamin E. it is a well-known immunity enhancer and has protective role in our study.

5. References

1. Flora SJS, Mittal M, Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therap., Division of Pharmacology & Toxicology. 2008.