International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 558-564
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Antioxidant Effect of Vitamin-E Treatment on Hexavalent Chromium Induced Hepato and Renal Toxicity in Laboratory Chicks

Vandita Kandpal and Dharmendra Kumar*

Department of Zoology, S. V. Govt. P.G. College, Lohaghat, Pin-262524 (Champawat), Uttarakhand, India

Received: 10 Oct 2018 / Accepted: 8 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: oxidation01@gmail.com

Abstract

Protection against metal toxicity has been a centre of attraction for industrial hygienists, public health officials, toxicologists and pharmacologists. Hexavalent chromium compounds have been shown to manifest toxic and carcinogenic effects in humans and animals. Since the discovery of the fact that oxidative damage is one of the mechanisms responsible for their toxicity, the use of antioxidants was considered to be a suitable alternative. Antioxidants restricted the uptake and distribution of chromium in liver and other organs. It is well established that vitamin E act as an antioxidant against toxicity induced by different heavy metals. Keeping this in view, present study has been carried out to investigate the protective effects of vitamin E on renal and hepatic enzymes in chicks against toxicity induced by hexavalent chromium. Developing chicks (Croiler, body weight 100±20 gm) were used as experimental animals. The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and creatinine enzymatic activity parameters were selected for the study. Significant decrease of serum (ALT), (AST), (ALP) and creatinine were observed in purely chromium treated groups, while, these values were significantly increased in vitamin E treated groups as compared to chromium treated group. Hence, it may be concluded that the progressive hepatorenal toxicity of hexavalent chromium can be moderately reduced by administrating vitamin E in laboratory chicks.

Keywords

Antioxidant, hepatorenal toxicity, hexavalent chromium, vitamin E

*****

INTRODUCTION

Every organ can elicit a specific pattern of enzyme release, which remains not elucidated. Specifically, above normal plasma enzyme activities are considered as diagnostic features for several diseases [1]. Release of enzymes usually follows their respective concentration gradients between an organ, such as the liver, and the blood compartments [2,3,4]. In fact, values of serum enzymes activities ("released") are much higher than the apparent disappearance rate constants and they are also consistent with disappearance rates from plasma to aspartate (AST) and alanine (ALT) aminotransferases, after acute liver injury [5]. However, the mechanisms

controlling cellular enzyme release remain poorly understood. Moreover, a drastic increase of serum activities of "liver enzyme markers" ought not necessarily to reflect liver cell death. Therefore, pathological elevations of the plasma activities of liver enzymes do not seem to be simply related to the quantitative release of such enzymes from the liver. Consequently, several enzymatic indices may be determined by differences in the time course of hepatic enzyme release, rather than reflecting true differences in the released quantities of various enzymes [5]. However, the quantitative use of enzymatic data is hampered by the fact that the fractional catabolic rate constants for the elimination of enzyme activities from plasma are unknown [5].

Release of mitochondrial enzymes from the liver is considered to provide strong evidence for hepatic necrosis [6,7] and is also associated with specific forms of liver disease. It has been shown for instance, that glutamate dehydrogenase (GDH) correlates well with the presence and extent of necrosis in alcoholic liver disease [8]. Furthermore, the ratio of mitochondrial and total AST (mAST) has been proposed as a marker for chronic alcoholism [9]. However, both GDH and mAST are widely distributed in various organs and lack specificity as a marker of liver injury. Despite the fact that it was reported that cumulative release of various cytosolic enzymes occurred in proportion to the corresponding activities in human control livers, the mechanism that govern the release of liver enzymes in to the bloodstream are practically unknown. Environmental factors contribute significantly to the pathogenesis of chronic kidney disease. However, these factors, and particularly the toxic effects of heavy metals, have not been completely evaluated. Chromium is a widespread industrial contaminant that has been linked to nephrotoxicity in animal and occupational population studies.

Toxic elements can be very harmful even at low concentration when ingested over a long period. The essential metals can also produce toxic effects when the metal intake is excessively elevated [10]. Chromium is the essential element helping the body to use sugar, protein and fat, at the same time excessive amount is carcinogenic for organisms. Chromium occurs in several oxidation states in environment ranging from Cr^{+2} to Cr^{+6} [11] commonly occurring forms are trivalent Cr^{+2} to Cr^{+6} with both states toxic to humans and plants [12].

Cr(III) and Cr(IV) are the most stable form and only their relation to human exposure is of high interest [13]. Chromium compounds such as calcium chromate, potassium dichromate, zinc chromate, strontium chromate and lead chromate are highly toxic and carcinogenic in nature. In human's exposure to higher amounts can lead to inhibition of erythrocyte glutathione reductase, which in turn lowers the capacity to reduce methemoglobin and haemoglobin [14]. Once the chromium reaches blood stream it damages blood cells by oxidation reactions leading to heamolysis and subsequently liver and kidney failure. Different experiments have proved that chromate compounds can induce DNA damage in many different ways and lead to formation of DNA adducts chromosomal aberrations, sister chromatic exchanges, alterations in replication and transcription of DNA [15,16]. Because of its mutagenic properties, Cr(VI) is categorized as a Group 1 human carcinogen by the International Agency for Research on Cancer [17,18].

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 concentration [19,20]. One of the most important vitamins for the body is vitamin E. In nature vitamin E comprises eight natural fat-soluble compounds, including four tocopherols [d-alpha-, d-beta-, d-gamma- and d-delta-tocotrienol] [21,22]. Vitamin E is an important antioxidant factor. It is known to possess various physiological functions. A major contributor to non-enzymatic protection against lipid peroxidation is vitamin E, a known free radical scavenger [23,24]. Vitamin E as a lipid soluble, chain-breaking antioxidant [25,26,27,28] plays a major protective role against oxidative stress [23]. and prevents the production of lipid peroxides by scavenging free radicals in biological membranes [29]. Since the discovery of vitamin E in 1922 by H. M. Evans, when it was first described as an anti-sterility agent, many scientist and physicians have sought to elucidate its biochemistry, health benefits and clinical applications [28].

Vitamin E has received wide attention due to its reported hepatoprotective effects in animals, which is primarily due to its ability to attenuate the induced oxidative stress in various tissues and the recovery of impaired hepatic cells [30]. Many studies have reported the antioxidant protective effect of vitamin E against several metals that induced hepatotoxicity, including copper, lead, sodium fluoride, cadmium, mercury and ferrous sulfate [31,32,33,34]. The protective effect of vitamin E against Cr-induced hepatotoxicity was reported in a study where the authors investigated the direct protective effect of vitamin E on Cr (VI)-induced cytotoxicity and lipid peroxidation in primary cultures of rat hepatocytes [35]. Susa et al., (1996) [35] reported that such

protective effects may be associated with the levels of nonenzymatic instead of enzymatic antioxidants. However, this study was conducted on primary cultures of rat hepatocytes.

Vitamin E supplement may be beneficial in reducing and slowing progressive kidney diseases that are significantly accelerated by oxidative stress. Vitamin E therapy is also considered as a mean of correcting plasma antioxidant status and attenuating the cardiovascular disease that accompanies kidney failure. To our knowledge, few studies have been conducted on the protective effects of vitamin E against Cr-induced hepatotoxicity and nephrotoxicity in living organisms. Therefore, the current study aimed to determine whether administration of vitamin E would have protective effects against Cr-induced hepatic and renal toxicity in laboratory chicks.

MATERIAL AND METHODS

Animals – The experiment was carried on Domestic chicks – Croiler Chabro (*Gallus gallus domesticus*). Newly hatched chicks were purchased from the Uttarakhand Village Poultry Project (State Govt. Poultry Farm), Bin, Pithoragarh (Uttarakhand). Selected all chicks were maintained and acclimatized according to the laboratory condition. The animals were housed in battery cages under laboratory conditions at existing room temperature and relative humidity. They were fed on commercial food (Starter, Grower and Finisher) purchased from the local market and tap water *ad libitum.* Healthy male and female chicks (approximately 2-3 weeks old, body weight 100±20 gm) were used in present study. All protocols were approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Science, Bhimtal, Kumaun University, Nainital and the member secretary, CPCSEA, Ministry of Environment, Forest and Climate Change, Government of India (Protocol No.-KUDOPS/89). The animals were kept under standard conditions throughout the experiment to reduce the error. Minimum number of animals was used to obtain reliable results.

Chemicals – Vitamin E (α-tocopherol) was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Potassium dichromate ($K_2Cr_2O_7$) was procured from Glaxo (India). All the reagents and chemicals used in this study were of analytical grade and highest purity procured from standard commercial sources in India.

Experimental Treatments - The selected chicks were divided into three groups (A, B and C) randomly, each containing at least 6 chicks. Chicks of group A were administered with sublethal dose of potassium dichromate ($K_2Cr_2O_7$) (5 mg/100 gm body weight) by gavage on each alternate day for 30 days. Chicks of group B were treated with potassium dichromate ($K_2Cr_2O_7$) as chicks of group A but also administered with vitamin-E (intramuscularly) (0.5 IU/100 gm body weight) on each alternate day for 30 days. Chicks of Group C were administered with saline only to serve as purely control.

Biochemical Analysis - Blood samples were collected from the wing vein using 3ml disposable syringe than directly transferred into a labeled test tube without anticoagulant. Serum was prepared by centrifugation and stored at 0ºC for further analysis. The serum ALT, AST, ALP and creatinine enzymatic activity were determined using commercial kits.

Statistical analysis - The obtained data were expressed as mean ± standard deviation (SD). All data were analyzed statistically using one-way analysis of variance (ANOVA) followed by Student's *t*-test. Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Enzymes markers of liver functions viz. serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) offer reliable information on status of liver function. Present results reveal that activity of ALP, AST and ALT significantly increased in chicks treated with chromium compared with control group. While, the chicks supplemented with vitamin E along with chromium shows significant decrease in liver function enzymes. It means vitamin E reduce the accumulation of chromium in liver by different channelized manner. Similarly, an increase in serum creatinine level was recorded in Cr treated chicks compared to control group. Vitamin E therapy normalizes creatinine up to control level. Results show that enzyme leakage increased in the serum after chromium treatment. Vitamin E succeeded in inhibiting the secretion of enzyme level and control up to the level of normal value. Hence, it protects the liver and kidney by oxidative stress by breaking different chain reactions (Table 01).

The liver is the major organ responsible for metabolism, detoxification and secretary function in the body. Hence, it regulates various important metabolic functions in mammalian systems. Hepatic damage is associated with the distortion of these metabolic functions. The liver tissue is reported to be one of the tissues with a high regenerative capacity [36]. According to Rabelo et al. (2006) [37] hepatocytes exhibit a very good regenerative response to several stimuli, including massive destruction of hepatic tissue by toxins, viral agents,

or surgical extraction. Regeneration of the liver tissues is a result of an organized and controlled response of the liver toward tissue damage induced by toxic agents, chemical agents, trauma, infections, or post-surgery resection which could induce oxidative stress in the liver. Oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity. Oxidative stress is mediated by Reactive Oxygen Species (ROS) generated in liver by a number of mechanisms. ROS are small, highly reactive, oxygen-containing molecules that are naturally generated in small amounts during the body's metabolic reactions and can react with and damage complex cellular molecules such as fats, proteins, or DNA.

Numerous studies have reported toxic and carcinogenic effects induced when animals and humans are exposed to chromium compounds. The toxic effects of chromium involve hepatotoxicity, neurotoxicity and nephrotoxicity. [38]. Chromium compounds are widely recognized as human carcinogens. From the epidemiological studies, it is suggestive that hexavalent chromium causes increased risk of bone, prostate lymphomas, Hodgins etc. reflecting the ability of hexavalent chromium to penetrate all tissues in the body [39]. Due to their extensive use in industry, there is also a need to investigate their toxicity in organ systems and mitigative role of vitamin on their toxicity. Liver with its metabolic detoxifying function is extremely vulnerable to harmful substances. Metal like chromium is known to act as catalyst for the production of free radicals in biological systems. The most important consequences of free radical production are lipid peroxidation increases and change in permeability of cell membrane [40]. Lipid peroxidation is often discussed as a cause of metal induced toxicity [41].

In the present study there was a significant ($p \leq 0.05$) increase in the serum ALT, AST and ALP levels in chicks treated with chromium compared to control group. Previous study noted an elevation in the levels of AST and ALT i.e. in fish [42] and rats [43,44]. Cr toxicity causes elevation of serum hepatic enzymes due to leakage of these enzymes or increase in their production. Maximum leakage of these enzymes occurred in chicks treated with chromium. Co treatment with antioxidant i.e. vitamin E influenced the hepatotoxicity of chromium and reduced it up to the control level. The ability of vitamin E to reverse or prevent chemical agents induced hepatotoxicity was demonstrated by Khalifa et al. (2009), [45] he and co-workers showed that 0.2g kg^{-1} day^{-1} of vitamin E normalized aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels elevated by carbon tetrachloride in rats.

Many researchers have given explanations for the mechanism that shows hepatoprotective ability of vitamin E. Vitamin E is a lipid soluble antioxidant that scavenges for reactive oxygen species (ROS). This could be associated with its structure as reported. The structure of vitamin E makes it a highly effective antioxidant, readily donating hydrogen from the hydroxyl group on the ring structure to free radicals and rendering them inactive. Vitamin E is fat soluble and is primarily located within the phospholipid's bilayer of the cell membranes where it has a major biological role in protecting polyunsaturated fats and other components of the cell membranes from oxidation by free radicals [46].

Among the eight fat-soluble derivatives, alpha tocopherol predominates in many species and has the highest biological activity with the active site being the 6-hydroxyl group. The side chain is the 2-position facilitates the incorporation and retention of vitamin E in biomembranes, so that the 6-position is optimal for scavenging free radicals and terminating lipid peroxidation [47]. Vitamin E has been reported to express two important function in the membranes: preventing ROS damage in polyunsaturated fatty acids as alipo-soluble antioxidant and acting against damage caused to phospholipids as a membrane stabilizing agent [48]. In addition, vitamin E is known to act by breaking the antioxidant chain that prevents ROS-produced cell membrane damage [49]. Factor et al., (2000) [50] demonstrated that vitamin E can directly reduce ROS production by interfering with the union between the membrane and the NADPH oxidase complex.

Similarly, alkaline phosphatase (ALP) was also included in this study. Alkaline phosphatase has a dimeric structure in serum and exists in same from when released from liver plasma membrane by phospholipase and proteases enzymes [51]. The enzyme anchored to the plasma membrane by a gylcosyl phosphatidylinositol structure and when solubilized from membrane with non-ionic detergent triton X-100 or with butanol treatment at higher pH, the hydrophobic phosphatidylinositol remains covalently attached to C-terminal amino acid residue of the enzyme [52]. Administration of vitamin E with chromium resulted in a significant reduction in the serum level of ALP. Experimental studies by Sokol [53] and Appenroth et al., [54] have reported that antioxidant of vitamin E family have protective effects against metal induced adverse effects in man and laboratory animals. Susa et al., [55] also reported that pre-treatment with vitamin E normalized the

level of non-enzymatic antioxidants such as glutathione and vitamin suppressed by dichromate. Thus, these studies corroborate with the present investigation and confirm the role of vitamin E as a scavenger of free radicals.

In the present study, creatinine was significantly ($p<0.05$) increased in Cr-treated birds compared to control group. Previous studies noted increase in creatinine and urea i.e. in rats [56,57]. Increase in creatinine level indicted renal injury. Increase in creatinine level could be due to interaction of Cr with cell membrane of kidneys resulting in alteration of permeability ultimately functional impairment and loss of integrity [58]. Acceleration in Cr-induced nephrotoxicity could be due to over production of ROS, which damage the membrane components leading to necrosis [59]. From the present results, it is obvious that treating Cr intoxicated chicks with vitamin E significantly protect the creatinine level as compared to control. Vitamin E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions, the resulting antioxidant radicals being a relatively unreactive species [60]. In many studies' vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect [61,62]. Therefore, vitamin E supplementation sufficient to protect the organism from toxic agent and free radical damage is a time-consuming process. It is concluded that vitamin E is an essential component of the kidney for protection of this tissue against peroxidative damage [63].

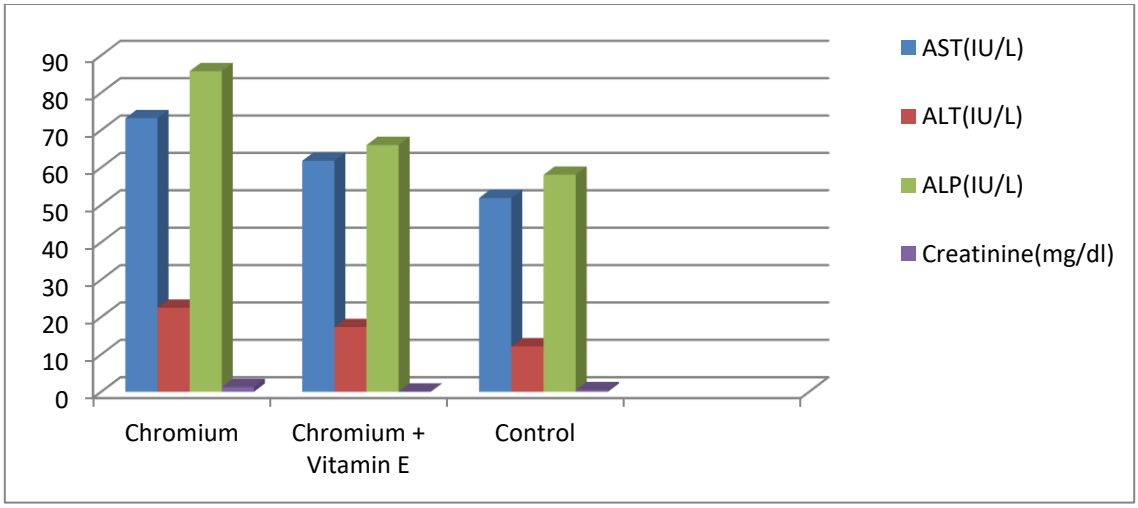
Based on the results obtained in the present investigation and above-mentioned previous studies, it can be suggested that administration of vitamin E alleviate the liver and kidney function enzymes from the oxidative stress produced by exposure to chromium. Collectively, this study demonstrated that toxicity of chromium has a potential hepatotoxic and nephrotoxic influence in chicks. The increase in the liver and kidney function enzymes induced by administration of chromium was improved under the treatment effect of vitamin E. The hepatoprotective and nephroprotective effects of vitamin E are due to its antioxidant activity. To strengthen these findings, further studies are required to clarify the mechanism actions of vitamin E as a therapeutic agent against the hepato and nephrotoxic influence of hexavalent chromium.

Table 01. Effect of vitamin E on AST, ALT, ALP and creatinine in chromium fed chicks

Parameters	Group A (Chromium)	Group B (Chromium + vitamin E)	Group C (Control)	F- ratio	Level of significance
AST (IU/L)	73.18±3.99	61.78±2.42	51.83±5.77	18.62	**
ALT (IU/L)	22.49±2.65	17.30±0.89	12.15±1.66	23.17	**
ALP (IU/L)	85.76±5.37	66.0±13.91	58.04±4.88	7.32	**
Creatinine (mg/dl)	1.26±0.25	0.40±0.1	0.43±0.06	28.59	**

Results are expressed as mean ± SE, ** indicates significant at p< 0.05

Figure 01: Graphical representation of therapeutic effect of Vitamin E on hepatic and renal enzymes in chromium fed chicks



CONCLUSION

Present study reveals that the liver enzyme markers of chromium treated chicks showed a significant increase in the overall mean values of AST, ALT and ALP at significant ($p<0.05$) level in comparison to control chicks. Similarly, a significant increase is also observed in serum creatinine in Cr treated chicks. While, the chicks supplemented with vitamin E along with chromium shows significantly improve in liver function enzymes and creatinine. Hence it may be concluded that the progressive hepatotoxic and nephrotoxic effects of hexavalent chromium @5mg/100gm body weight in the form of potassium dichromate ($K_2Cr_2O_7$) can be moderately reduced by supplying vitamin E @0.05 IU/ 100 gm body weight intraperitoneally in laboratory chicks.

REFERENCES

[1] Verrill H.L., Pickard N.A. and Gruemer H.D. *Mechanism of cellular enzyme release. I. Alteration in membrane fluidity and permeability. Clinical Chemistry. 23(12)*: 2219-2225, (1997).

[2] Schmidt E. and Schmidt F.W. *Release of enzymes from the liver. Nature. 213(5081)*: 1125-1126, (1967).

[3] Batt A.M. and Ferrari L. *Manifestations of chemically induced liver damage. Clinical Chemistry. 41(12)*: 1882-1887, (1995).

[4] Dufour D.R., Lott J.A., Nolte F.S., Gretch D.R., Kolf R.S. and Seeff L.B. *Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clinical Chemistry. 46(12)*: 2027-2049, (2000).

[5] Peltenburg H.G., Hermens W.T., Willems G.M., Flendrig J.G. and Schmidt E. *Estimation of the fractional catabolic rate constants for the elimination of cytosolic liver enzymes from plasma. Hepatology. 10(5)*: 833-839, (1989).

[6] Schmidt E. and Schmidt F.W. *Aspekte der Enzym-Diagnostik. Die Medizinische Welt. 21*: 805-816, (1970).

[7] Frederiks W.M., Vogels I.M.C. and Fronik G.M. *Plasma ornithine carbamyl transferase level as an indicator of ischaemic injury of rat liver. Cell Biochemistry and Function. 2(4)*: 217-220, (1984).

[8] Van Waes L. and Lieber C.S. *Glutamate dehydrogenase: a reliable marker of liver cell necrosis in the alcoholic, British Medical Journal. 2(6101)*: 608-614, (1977).

[9] Nalpas B., Vassault A., Charpin S., Lacour B. and Berthelot P. *Serum mitochondrial aspartate aminotransferase as a marker of chronic alcoholism: diagnostic value and interpretation in a liver unit, Hepatology. 6(4)*: 608-614, (1986).

[10] Celik U. and Oehlenschlager J. *High contents of cadmium, lead, zink and copper in popular fishery products sold in Turkish supermarkets, Food control. 18*:258-261, (2007).

[11] Rodriguez M.C., Barsanti L., Pasarelli V., Evangelista V. and Gualtieri P. *Effects of chromium on photosynthetic and photoreceptive apparatus of the alga Chlamydomonos reinhardtii. Environ Res. 105(2)*: 234-239, (2007).

[12] Mohanty M. and Kumar Patra H. *Effect of iconic and chelate assisted hexavalent chromium on mung bean seedlings [Vigna radiate (L.) Wilczeck,] (Vark-851) during seedling growth. JSPB. 9(2)*: 232-241, (2013).

[13] Zhitkovich, A. *Importance of chromium DNA adducts in mutagenicity and toxicity of chromium (VI). Chem Res Toxicol. 18(1)*: 3-11, (2005).

[14] Koutras G.A., Schneider A.S., Hattori, M. and Valentine, W.N. *Studies on chromated erythrocytes. Mechanisms of chromate inhibition of glutathione reductase. Br. J. Heamatol. 11(3)*: 360-369, (1965).

[15] O'Brien T., Xu J. and Patierno S.R. *Effects of glutathione on chromium induced crosslinking and DNA polymerase arrest. In molecular Machanism of Metal toxicity and carcinogenesis. Springer US.* 173-182, (2001).

[16] Matsumoto S.T., Mantovani M.S., Malaguttii M.I.A., Dias A.L., Fonseca I.C. and Marin-Morales M.A. *Genotoxicity and mutagenicity of water contaminated with tannery effluents as evaluated by the micronucleus test and commet essay using the fish oreochromis niloticus test and chromosome aberrations inonion root tips. Genet. Mol. Biol. 29(1)*: 148-158, (2006).

[17] Dayan A.D. and Paine A.J. *Mechanism of chromium toxicity, carcinogenicity and allergenicity. Human and experimental toxicology. 20(9)*: 439-451, (2001).

[18] Zhang Y.W., Robert Thompson Han Zhang and Huaxi Xu. *APP Processing in Alzeihmers disease. Molecular Brian. 4:3*, (2011).

[19] Cadenas S. and Cadenas A.M. *Fighting the stranger-antioxidant protection against endotoxin toxicity. Toxicology. 180*: 45-63, (2002).

[20] Kanter M., Coskun O., Armutcu F., Uz Y.H. and Kizilay G. *Protective effects of vitamin C, alone or in combination with vitamin A, on endotoxin-induced oxidative renal tissue damage in rats.Tohoku J. Exp, Med. 206*: 155-162, (2005).

[21] Malafa M.P., Fokum F.D., Mowlavi A., Abusief M. and King M. *Vitamin E inhibits melanoma growth in mice. Surgery. 131*: 85-91, (2002).

[22] Songthaveesin C., Saikhun J., Kitiyanant Y. and Pavasuthipaisit K. *Radio-protective effect of vitamin E on spermatogenesis in mice exposed to gamma-irradiation: a flow ctyomrtric study. Asian J. Androl. 6*: 331-336, (2004).

[23] Fraga C.G. Arias R.F., Llesuy S.F., Koch O.R. and Boveris A. *Effect of vitamin E and selenium-deficiency on rat liver chemiluminescence. Biochem. J. 242*: 383-386, (1987).

[24] Rikans L.E., Moore D.R. and Snowden, C.D. *Sex-dependent differences in the effects of aging on antioxidant defense mechanism of rat liver. Biochim. Biophys. Acta. 1074*: 195-200, (1991).

[25] Halliwell B. *Oxidants and the central nervous system: some fundamental questions. Is oxidant relevant to Parkinson's disease, Alzheimer's disease, traumatic injury or stroke? Acta Neurol. Scand. 126*: 23-33, (1989).

[26] Halliwell B. *Reactive oxygen species and the central nervous systems. Free radicals in the brain. In: Packer, L., Prilipko, L., Christen, Y. (Eds.), Aging, Neurological and Mental Disorders. Springer-Verlag, Berlin.* 21-40, (1992).

[27] Kagan V.E., Bakalova R.A., Koynova G.M., Tyurin V.A., Serbinova E.A. and Petkov W. *Antioxidant protection of the brain against oxidative stress. Free radicals in the brain.* In: Packer L., Prilipko L., Christen Y. (Eds.), *Aging, Neurological and Mental Disorders. Springer-Verlag, Berlin.* 49-61, (1992).

[28] Packer L., *Free radical scavengers and antioxidants in prophylaxy and treatment of brain disease. Free radicals in the brain.* In: Packer, L., Prilipko, L., Christen, Y. (Eds.)., *Aging, Neurological and Mental Disorders. Springer-Verlag, Berlin.* 1-20, (1992).

[29] Suga T., Watanabe, T., Matsumoto, Y. and Horie, S. *Effects of long-term vitamin E deficiency and restoration on rat hepatic peroxisomes. Biochim. Biophys. Acta. 794*: 218-224. (1984).

[30] Bharrhan S., Chopra K. and Rishi P. *Vitamin E supplementation modulates endotoxin-induced liver damage in a rat model. Am. J. Biomed. Sci. 2*: 51-62, (2010).

[31] Al-Attar A.M. *Vitamin E attenuates liver injury induced by exposure to lead, mercury, cadmium and copper in albino mice. Saudi J. Biol. Sci. 18*:395-401, (2011).

[32] Chinoy N., Sharma A., Patel T. Memon R. and Jhala D. *Recovery from fluoride and aluminium induced free radical liver toxicity in mice. Fluoride. 12*: 14-16. (2004).
[33] Gaurav D., Preet S. and Dua K. *Chronic cadmium toxicity in rats: treatment with combined administration of vitamins, amino acids, antioxidants and essential metals. J. Food Drug Anal. 18*:464-470, (2010).
[34]. Osfor M.M., Lbrahim H.S., Mohamed Y., Ahmed A., Abd El Azeem A. and Hegazy M. *Effect of alpha lipoc acid and vitamin E on heavy metals intoxication in male albino rats. J. Am. Sci. 6*:6-63, (2010).
[35] Susa N., Ueno S., Furukawa, Y. and Sugiyama, M. *Protective effect of vitamin E on chromium (VI)-induced cytotoxicity and lipid peroxidation in primary cultures of rat hepatocytes. Arch. Toxicol. 71*: 20-24, (1996).
[36] Khan A.Z. and Mudan S.S. *Liver regeneration: Mechanisms, mysteries and more. ANZ J. Surg. 77*: 9-14, (2007).
[37] Rabelo M.R., Ramalho F.S., Ramalho L.N., Tde S.C. and Brando D.F. *A molecular view of liver regeneration. Acta Cir. Bras. 21*: 58-62, (2006).
[38] Valko M., Morris H. and Cronin M.T. *Metals toxicity and oxidation stress. Curr. Med. Chem. 12*: 1161-1208, (2005).
[39] Costa M. *Toxicity and carcinogenicity of Cr (VI) in animal models and humans. Crit. Rev. Toxicol. 27*:431-442, (1997).
[40] Panda S.K. *Heavy metal phytotoxicity induces oxidative stress in a moss, Taxithelium Sp. Curr. Sci. 84:5*, (2003).
[41] Ames B.N., Holstein M.C. and Catheart R. *Lipid peroxidation and oxidative damage to DNA. In Lipid peroxides in biology and medicine (Yogi, K. Ed.), Academic Press, N. Y*. 325-351, (1982).
[42] Shaheen, T. and Akhtar, T. *Assesment of chromium toxicity in Cyprinus carpio through hematological and biochemical blood markers. Turk. J. Zool., 36*: 682-690, (2012).
[43] Kim H., Lee S. and Jang B. *Subchronic inhalation toxicity of soluble hexavalent chromium trioxide in rats. Archiv. Toxicol., 78*: 363-368, (2004).
[44] Mehany H. A., Abo-youssef A. M., Ahmad L. A., Arafa E. A. and Abd El-Latif H. A. *Protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats. J. Basic. Appl. Sci., 2*: 96-102, (2013).
[45] Khalifa M.A., Maksymov V. and Rowsell C. *Retroperitoneal margin of the pancreaticoduodenectomy specimen: Anatomic mapping for the surgical pathologist. Virchows Arch, 454*: 125-31, (2009).
[46] Hicman I. and Macdonald, G. *Is vitamin E beneficial in chronic liver disease? Hepatol. 46*: 288-290, (2007).
[47] Bjorneboe A., Bjorneboe G. and Drevoh C.A. *Absorption, transport and distribution of vitamin E. J. Hutr. 120:* 233-242, (1990).
[48] Bradford A., Atkinson J., Fuller N. and Rand R.P. *The effect of vitamin E on the structure of membrane lipid assemblies. J. Lipid. Res, 44*: 1940-1945, (2003).
[49] Brigelius-Flohe R. and Traber M.G. *Vitamin E: function and metabolism. FASEB J., 13*: 1145-1155, (1999).
[50] Factor V.M., Laskowska D., Jensen M.R., Woitach J.T. and Popescu N.C. *Vitamin E reduces chromosomal damage and inhibits hepatic tumor formation in a transgenic mouse model. Med. Sci., 97*: 2196-2201, (2000).
[51] Hertmann A. H., Bircher J. and Creutzfeldt W. *Superiority of the Child–Pugh Classification to Quantitative Liver Function Tests for Assessing Prognosis of Liver Cirrhosis. Scandinavian Journal of Gastroenterology. 24*: 269-276, (1989).
[52] Hawrylak K. and Stinson R. A. *The solubilization of tetrameric alkaline phosphatase from human liver and its conversion into various forms by phosphatidylinositol phospholipase C or proteolysis. J. Biol. Chem. 263*: 14368-14373, (1988).
[53] Sokol R.J. *Antioxidant defense in metal-induced liver damage. Semin. Liver Dis. 16*: 39-46, (1996).
[54] Appenroth D., Karge E., Kiessling G., Wechter W.J. Winnefield K. and Heck C. *LLU-alpha an endogenous metabolite of gamma tocopherol, is more effective against metal nephrotoxicity in rats than gamma-tocopherol. Toxicology, 112*: 255-265, (2001).
[55] Susa N., Ueno S., Furkawa Y. and Sugiyama M. *Protective effect of vitamin E on chromium (VI)-induced cytotoxicity and lipid peroxidation in primary cultures of rat hepatocyte. Toxicology, 71*: 20-24, (1996).
[56] Pedraza-Chaverri, J., Yam-Canul P., Chirino Y.I, Sa´nchez-Gonza´lez D.J., Martı´nez-Martı´nez, C.M., Cruz C. and Medina-Campos, O.N. *Protective effects of garlic powder against potassium dichromateinduced oxidative stress and nephrotoxicity. Food. Chem. Toxicol., 46*: 619–627, (2008).
[57] Mehany H.A., Abo-youssef A.M., Ahmed L.A., Arafa E.A. and Abd El-Latif H.A. *Protective effect of vitamin E and atorvastatin against potassium dichromate-induced nephrotoxicity in rats. J. Basic. Appl. Sci., 2*: 96–102, (2013).
[58] Parveen K., Khan M.R. and Siddiqui W.A. *Pycnogenol® prevents potassium dichromate ($K_2Cr_2O_7$)-induced oxidative damage and nephrotoxicity in rats. Chemico-Biol Interact., 181*: 343–350, (2009).
[59] Liu K.L. and Shi X. *In vivo reduction of chromium (VI) and its related free radical generation. Mol. Cell. Biochem., 222*: 41–47, (2001).
[60] Pascoe G., Olafsdottier F. and Read D. *Vitamin E protection against chemical-induced cell injury. I. Maintenance of cellular protein thiols as a cytoprotective mechanism. Arch. Biochem. Biophys. 256*: 150-158, (1987).
[61] Aldana L., Tsutsumi V., Craigmill A., Silveira M.I. and De Mejia E.G. *Alpha-Tocopherol modulates liver toxicity of the prethroid cypermethrin. Toxicol. Lett. 125*: 107-116, (2001).
[62] Jhon S., Kale M., Rathore N. and Bhatnagar D. *Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. J. Nutr. Biochem. 12*: 500-504, (2001).
[63] Champe P.C. and Harvey R.A. *Vitamin E. In: Barnes, D., Robinson, S., Hoeltze, L.E., Baldwin, T.J.B. (Eds.). Lippincott's Illustrated Review Biochemistry. Lippincott Com. Philadelphia.* 311-331 (Chapter 27: Vitamins), (1987).