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ORIGINAL ARTICLE

Synergistic Efficacy of the Combination of Co Q10 and α-tocopherol Against Arsenic Induced Hepatotoxicity in Sprague Dawley Rats

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ABSTRACT

Background: Millions of the people around the world are exposed to higher levels of arsenic via contaminated drinking water which is a threat to human and animal life.
Aim: To determine the possible beneficial effects of co Q10 and tocopherol against arsenic induced liver damage in Sprague Dawley rats**.**
Methodology: In this randomized controlled trial Group I was given standard rat diet with distilled water. Groups II, III, IV and V were administered arsenic 5mg/L mixed in distilled water ad libitum. Additionally, group III and group IV were administered co Q10 and tocopherol 250 mg/kg mixed in rat diet. Group V was given both co Q10 and tocopherol 125 mg/kg each mixed in rat diet. The experiment continued for 15 days. At the end of the intervention, serum was extracted to measure serum LFTs on micro lab. SPSS version 23.0 was used to analyze the data.
Results: There was a significant ($p \leq 0.05$) rise of serum ALT, AST and ALP after arsenic administration in group II animals. Treatment with co Q10 and tocopherol reduced the levels of serum LFTs ($p \leq 0.05$) in group III and IV animals respectively. Whereas, the synergistic effects of both these natural antioxidants in group V animals, reversed these changes to control values.
Conclusion: We concluded that the synergistic effects of both co Q10 and tocopherol were better than their individual effects against arsenic induced hepatotoxicity.
Keywords: Arsenic, Co Q10, Hepatotoxicity and Tocopherol.

INTRODUCTION

Arsenic is a metabolic poison that is widely distributed in our environment. It is a natural component of earth crust where it exists in different forms and among all of them, trivalent arsenic is highly reactive and very toxic to the animals and humans.[1] According to WHO, the current recommended concentration of arsenic in the portable water is 10µg/L[2]. Whereas, millions of people around the globe are exposed to arsenic at levels higher than 50µg/L and majority of them are Asians. Public health surveys have reported that the highest concentration of arsenic in the underground water table is found in India, Bangladesh, Pakistan, Afghanistan and Mayanmar[2]. A meta-analysis held in Pakistan reported that more than 45 million Pakistanis are exposed to higher levels of arsenic, whereas, province Punjab and Sindh are reported to be the arsenic hotspots of Pakistan[3]. Its rapid utilization in the agriculture and in industries are the main causative factors behind the rising concentration of arsenic in the drinking water. There is lack of water purification systems in developing countries. Moreover, arsenic species are not degradable hence, they get accumulated when released into soil and water. This high concentration of arsenic is responsible for multi organs damage that leads to diseases and disabilities[4]. Most known hazardous effects of arsenic include skin lesions, diabetes mellitus, cardiovascular problems and chronic liver and kidney failure[5]. It is proposed that arsenic, being a metalloid, generates oxidative stress inside the body. It is a well characterized inducer of oxidative stress that produces reactive oxygen species e.g. hydron peroxide (H_2O_2), superoxides (O_{2^-}) and peroxyl radicals (OH^-). It also disrupts the structure and function of anti-oxidant enzymes e.g. glutathione reductase and promotes cellular death by apoptosis / autophagy[6].

A growing body of evidence suggests that addition of natural anti-oxidants in our routine diet can shield against arsenic induced oxidative stress. Among various anti-oxidants, tocopherol and co Q10 are potent natural anti-oxidants that can efficiently scavenge the free radicals and protect the normal cellular structure and functions[7].

Coenzyme Q10 is a vitamin like substance found in almost every cell of our body. De novo synthesis of co Q10 occurs in human body. Besides this, dietary sources of co Q10 are fish, meat and nuts etc. Highest concentration of co Q10 is found in liver, heart, kidneys and pancreas. The word co Q10 is named after its chemical structure that contains 10 isoprenoid units attached with a benzoquinone ring. It is also called as ubiquinone/ ubiquinol as it is ubiquitously found in nature[8]. It is located on the inner mitochondrial membrane where it plays vital role in energy production via electron transport chain[9]. Studies have proven multiple health benefits associated with regular intake of co Q10. Co Q10 inhibits abnormal lipid accumulation in the liver cells thus, it prevents the onset of non-alcoholic fatty liver disease[8]. It has been proven that co Q10 can resolve and cure the hepatotoxicity induced by certain drugs e.g. acetaminophen[10]. Heavy metals disturb the oxidative balance in the human body by releasing out their free electrons inside and outside the cellular milieu. Co Q10 acts as a strong anti-oxidant and can scavenge the harmful free radicals. Moreover, it works synergistically with other anti-oxidants radicals e.g. vitamin E in redox cycle and enhance their ability to neutralize the harmful radicals[11].

Vitamin E is a group of lipophilic compounds that consist of 8 different compounds (4 tocopherols and 4 tocotrienols). The word "*tocopherol*" is derived from Greek word meaning "*birth or to carry*". They are regarded as fat soluble vitamins and strong anti-oxidants. Among these compounds, α-tocopherol is most active and potent ROS scavenging compound. Dietary sources of α-tocopherol are vegetable oils, poultry, meat and cereals. It can be taken as synthetic supplements (all rac α-tocopherol). It performs several functions in our body e.g. it regulates the neurotransmitters in our central nervous system, modulates the immune system, controls the expression of several genes and neutralizes the free radicals etc[12]. Deficiency of tocopherol leads to neurological symptoms e.g. ataxia, depression and anxiety etc.[13] Due to these beneficial effects, tocopherol is now used as a drug to treat several clinical conditions e.g. non-alcoholic fatty liver disease, neurodegenerative disorders and cardiovascular dysfunctions etc[14]. Being a master anti-oxidant, it has been proven to be protective against oxidative stress generated by certain drugs (acetaminophen) and heavy metals e.g. cadmium, lead and mercury etc[15].

The objective of the study was to determine the possible beneficial effects of co Q10 and tocopherol against arsenic induced liver damage in Sprague Dawley rats**.**

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METHODOLOGY

According to non-probability convenience sampling technique, total number of 150 healthy male Sprague Dawley rats with body weight of 220-250g and aged 10-12 weeks were sourced from National Institute of Health, Islamabad. All the animals were randomly separated into five groups (each having 30 rats).[16] Animals were kept at the animal house of the Collage of Physicians and Surgeons regional center Islamabad. Before any intervention, rats were kept in a disciplined territory with room temperature 23-27°C and 50-70% humidity along with 12h day and night cycle for habituation for at least one week. During this time period, all the rats were provided with standard pelleted form of diet (that is synthesized at the animal house of NIH) along with water ad-libitum. After one week of habituation, interventions were applied on the animals for a period of 15 days which was approved by the Ethical Research Committee of Army Medical College (letter number ERC/ID/117) according to the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Group I (healthy control group) was fed on standard rat chow and distilled water. Group II (diseased control group) was given standard rat chow and arsenic in a dose of 5mg/L mixed in distilled water ad libitum. Group III was given co Q10 in a dose of 250 mg/kg in rat diet along with arsenic in a dose of 5mg/L mixed in distilled water ad libitum. Group IV was given tocopherol in a dose of 250mg/kg in rat diet along with arsenic in a dose of 5mg/L mixed in distilled water ad libitum. Group V was given both co Q10 and tocopherol in a dose of 125 mg/kg each mixed in rat diet along with arsenic 5mg/L mixed in distilled water ad libitum. At the end of experiment, the animals were euthanized and blood samples were taken by a single intra cardiac puncture. Blood was poured into the gel and clot activator vials that were kept in thermocol boxes with ice packs. After coagulation of blood in the vials, it was centrifuged at 2500-3000 rpm for 10 minutes to extract the serum. Eppendorf tubes were used to store the serum at 4-8 Cin the Multi-Disciplinary Laboratory of the Department of Biochemistry & Molecular Biology, Army Medical College. Stored serum was used for colorimetric estimation of LFTs.

SPSS version 23.0 was used to analyze the data. Mean and standard deviation of the levels of ALT, AST and ALP were calculated. One way ANOVA was applied to measure the statistical significance of difference across the groups. The *p* value of less than or equal to 0.05 was considered significant. Post hoc Tukey's test (HSD) was applied to find the group with significant results.

RESULTS

The comparison of the means of serum ALT, AST and ALP among five groups were shown in table-1. After 15 days of arsenic exposure, significantly elevated levels of serum ALT, AST and ALP were seen in diseased control group (group II) as compared to healthy control group (group I). This clearly indicates the arsenic induced hepatotoxicity ($p< 0.005$) developed in group II animals. Serum levels of these variables in co Q10 treated group (group III) were significantly reduced and more towards normal with *p* values less than 0.005. Similarly, tocopherol treated group (group IV) showed remarkable improvement in serum LFTs ($p< 0.005$). Whereas, further significant decrease of these parameters were seen in group V animals that were given mixed diet (co Q10 + tocopherol).

Inter group comparison by post hoc Tukey's test is shown in the table-2. Results revealed that there is no statistical difference in the levels of serum serum ALT, AST and ALP between group I and group V ($p>0.005$). Similar outcomes are seen in the comparison of results of group III and group IV ($p> 0.005$).

Table-1: Mean ± SD and *p* value of serum ALT, AST and ALP (U/L)

Variables	Group I (n=30)	Group II (n=30)	Group III (n=30)	Group IV (n=30)	Group IV (n=30)	p-value
ALT (17.5-30.2U/L)	26.7 ± 5.65	97 ± 6.40	53.93 ± 8.07	37.8 ± 7.94	32.57 ± 6.33	<0.001*
AST (45.7-80.8U/L)	66.39 ± 11.12	186.14 ± 19.40	91.74 ± 10.05	66.29 ± 10.35	57.83 ± 8.24	<0.001*
ALP (56.8-128U/L)	92.77 ± 16.25	248 ± 11.94	139 ± 12.24	106.5 ± 11.70	69.87 ± 7.76	<0.001*

*Statistically Significant

Table-1 : Comparison of LFTs between the groups by Post hoc Tukey's test

Group II	<0.001*	<0.001*	<0.001*
Group I v/s			
Group III	<0.001*	<0.001*	<0.001*
Group IV	<0.001*	1	<0.001*
Group V	0.12	0.65	<0.001
Group II v/s			
Group III	<0.001*	<0.001*	<0.001*
Group IV	<0.001*	<0.001*	<0.001*
Group V	<0.001*	<0.001*	<0.001*
Group III v/s			
Group IV	<0.001*	<0.001*	<0.001*
Group v	<0.001*	<0.001*	<0.001*
Group IV v/s			
Group V	0.33	0.07	<0.001*

*Statistically Significant

DISCUSSION

Rising concentration of arsenic has become a global issue. Millions of the people around the globe are exposed to higher levels of arsenic which is adversely influencing the human and animal life. Use of arsenic in various industries and in agriculture have aggressively expanded the arsenic contaminated regions in the world[17]. Arsenic has 2 forms in nature e.g. organic and inorganic. Organic forms of arsenic cannot get absorbed into the body hence, they are not harmful. Inorganic arsenic exists as pentavalent and trivalent form. Pentavalent form is unstable and converts into lethal trivalent form. Trivalent arsenic can damage almost all the organs and systems of human body. It undergoes biotransformation in the liver cells by arsenic methyltransferase enzyme into organic arsenicals e.g. mono and dimethyl arsenic acid. Mthylation of arsenic facilitates its excretion via kidneys. Thus, liver and kidneys are at a greater risk of arsenic poisoning[18].

Serum ALT, AST and ALP are diagnostic and prognostic indicator of hepatocyte well-being. These enzymes leak out of the liver cells and accumulate into the blood in case of hepatocytes injury (necrosis) caused by toxic compounds. The abnormally high levels of serum ALT, AST and ALP in group II rats of this study are due to arsenic induced hepatotoxicity and these results are in accordance with the results of the studies of K Renu and C Wand *et al* which has highlighted that exposure to arsenic rapidly develops hepatic cell damage[19].

The exact biochemical mechanism of arsenic toxicity is still ambiguous. Yet, the diverse array of observations conclude that arsenic, being a metalloid, releases out free radicals and alters the cellular redox state. Reactive oxygen species generated by arsenic are superoxides (O_2^-), peroxides (H_2O_2) and hydroxyl radicals (OH)[19]. It spontaneously binds with the sulfhydryl group of various proteins and disrupts their structure and function. There is a reciprocal relation between anti-oxidant levels and oxidative stress in a cell. Arsenic rapidly declines the serum anti-oxidant levels e.g. glutathione proxidase, catalase and superoxide dismutase and render the cell exposed to reactive oxygen species[20]. Recovery from chronic arsenic insult becomes nearly impossible and ultimately leads to cell death. Thus, oxidative stress is the key cellular pathway underlying arsenic induced toxicity. Moreover, arsenic alters the structure and function of *DNA methyltransferase* (DNMT) enzyme, inhibits the expression of several genes e.g. gene that codes for antioxidant enzymes.[21]

So, we concluded that the hepatotoxicity observed in this study is caused by arsenic induced oxidative stress. Co Q10 is a known lipophilic natural anti-oxidant required for the proper

functioning of our body. It is a member of ubiquinone family. The benzoquinone head of co Q10 is a critical component of its structure as it can accept and donate the free electrons. In certain clinical conditions e.g. neurodegeneration, cardiovascular diseases, mitochondrial diseases and muscular dystrophies, the serum concentration of co Q10 is found to be low. Many studies have showed that oral supplementation of co Q10 in the diet of these patients, help the cells to optimize their functions that require co Q10. Being a strong anti-oxidant, co Q10 has a potential to neutralize the free radicals and enhance the activity of principal anti-oxidant enzymes of our body e.g. superoxide dismutase. It also activates the other anti-oxidants e.g. vitamin E while scavenging the ROS.

K.V Kumar concluded in his study that co Q10 in a certain dose (100mg per day) is an effective adjunct to the conventional medical therapy for the treatment of cardiovascular disorders. It works synergistically with lipid lowering drugs and minimizes the oxidative stress in these patients.[20] Co Q10 in group III animals of this study had showed promising effects against arsenic induced hepatotoxicity (p=0.000) and these results are similar to the results of the study of MH Song demonstrated that co Q10 protects the liver cells by minimizing the lipid peroxidation and enhancing the anti-oxidant activity in laboratory rats[21].

Tocopherol, the active form of vitamin E is a fat soluble vitamin and a potent anti-oxidant. It get incorporated into cell membrane where it prevents lipid peroxidation and generation of free radicals. α tocopherol contains a weak –OH bond that can easily loses the H atom. This H atom is donated to free radicals (peroxyl radicals) to buffer their damaging effects on a cell.

ROO•+Tocopherol-OH → ROOH+Vit E-O•

Equation 1: Free radical + tocopherol → Neutralized radical + Phenoxy radical

Thus, vitamin E not only itself scavenges the free radicals, but, it also spares the other anti-oxidants e.g. vitamin A and vitamin C. Moreover, it enhances the concentration of other anti-oxidants e.g. reduced glutathione[22].

The serum levels of ALT, AST and ALP are reduced due to tocopherol administration in group IV animals (p=0.000). These effects of tocopherol are owned to its ROS scavenging abilities and these effects are in line with the hepatoprotective effects of vitamin E observed in the study of M. Zubair and J Fang[23].

Combine effects of anti-oxidants are more beneficial as compare to their individual effects. As described earlier, tocopherol is converted into tocopheroyl radicals after donating its H atom to peroxyl radicals. Other anti-oxidant e.g. co Q10, if present in that milieu oxidizes tocopheroyl radicals back into their active form in two steps reaction. This process is known as redox cycle.

Tocopherol -O• + QH2 → Tocopherol -OH + QH·

Tocopherol -O• + QH·→ Tocopherol -OH + Q

Equation 2 Phenoxy radical+Co Q10 (ubiquinol)→Active Tocopherol + co Q10

Benefits of combined anti-oxidant therapy can be manifested by the intergroup comparison shown in table-2 which demonstrated that there was no statistical difference in the values of ALT, AST and ALP between healthy group and group V animals. The combine therapy of co Q10 and tocopherol has reversed the values of LFTs close to the normal values. Bundles of other studies have highlighted that the collaborative effects of tocopherol and co Q against are better than their individual effects (Sharma et al., 2018, Sharma et al., 2021)

CONCLUSION

We concluded that the synergistic effects of both co Q10 and tocopherol were better than their individual effects against arsenic induced hepatotoxicity.

Authors' Contribution: **UZM&AR:** Conceptualized the study, analyzed the data, and formulated the initial draft, **MJY&HGK:** Contributed to the histomorphological evaluation, **AM&TL:** Contributed to the analysis of data and proofread the draft.

Limitations: The study has few limitations as well. The size of the sample was not enough to generalize the results. Limited resources were available.

Conflict of Interest: None to declare

Financial Disclosure: None

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