Original Article

Effect of Uric Acid on Vitamin C and E in Induced Hyperuricemic Model

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ABSTRACT

Objective: To measure the level of uric acids and find out the effect of uric acid on vit C and E in induced hyperuricemic model.

Study Design: comparative study

Place and Duration of Study: This study was conducted at Baqai Medical University, Karachi from June 2010 to January 2011.

Materials and Methods: Forty male albino rats with an average weight of 180±2g were selected. The rats were grouped. The animals were fed on stardards diet and given tap water ad libitum until treatment. The protrocols for experiment was according to institute of laboratory animal resources on life sciences,US National research council,1996 and institutional animal ethical committee(IAEC) of baqai medical university, Karachi. Albino rats were divided into four groups. Group A(10) – control given only standard diet, group B(10) fed on 60%fructose with standard diet , group C(10) fed on fructose, standard diet and intraperitonially oxonic acid 250mg/kg and group D (10) only on injection intraperotonially oxonic acid 250mg/kg. At the end of study 10 ml of blood was drawn from heart of rats. Then blood was estimated for vitamin C, E and uric acids done by kit methods randox-manual /Rx monza UA230/UA 233.

Results: The concentration of vitamin C and E were significantly lowered as compared to uric acid concentration in the group B,C and D.

Conclusion: Decrease level of vit C and E increase the level uric acids were observed. Therefore, it may be suggested that increase intake of vitamin C may be helpful in lowering uric acid concentration**.**

Key Words: Uric Acid, Vitamin C, Vitamin E, Fructose, Al Bino Rats, Induced Hyperuricemia

INTRODUCTION

Free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital [1].They are capable of triggering chain reactions which can damage the different cell constituents.

In order to check free radicals formation to avoid oxidative stress, body has different anti- oxidant defence systems. An antioxidant can be defined as: "any substance that when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate. The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals.[2] Uric acid now is not considered as merely a metabolic waste. It has been proposed that increase in life span observed in human evolution to some extent might be due to protective action of uric acid [3]**.** Increase uric acid levels have been found in oxidative stress and ischemia which might be compensatory mechanism of protection against free radicals .[4] Ascorbate is a good free radical scavenger due to its chemical properties[5] .In our body Vitamin C is required as electron donor for 8 enzymes which act as monoxygenase or dioxygenase.Three of these enzymes are required for the hydroxylation of lysine and proline in collagen molecule,synthesis of carnitine, norepinephrine synthesis from dopamine and tyrosine metabolism[6].

Ascorbate can reduce lipid peroxidation. Protein also undergo oxidation by different means. [7] DNA are affected indirectly by protein or lipid oxidation or directly by oxidation of DNA[8].The most important mechanisms of DNA damage, however, are believed to involve direct attack of oxidants on individual nucleotides in DNA.[9]. Ascorbate might be able to diminish DNA damage by reducing radical species directly, decreasing formation of reactive species such as lipid hydroperoxides or preventing radical attack on proteins that repair DNA and also can prevent nitrosamine formation so subsequent formation of some reactive nitrogen species is prevented.[6]

The biochemical studies on the mechanism of vitamin E antioxidant potential were initiated by Tappel.[10] Its antioxidant interactions has been demonstrated in vitro long ago but the evidence in humans is now also being established by certain studies.[11].There have been evidence found by scientist in animal studies that multi molecular weight antioxidant systems especially vitamin C and selenium are necessary for the function of Vitamin E[12]

Vitamin E may act as a fat-soluble antioxidant that stops the production of reactive oxygen species formed

when fat undergoes oxidation.[13] In membranes tocopherols react with lipid peroxyl radicals to yield a relatively stable lipid peroxidation and the tocopheroxy radical to interrupt the radical chain reaction. For this reason, vitamin E is the major lipid-soluble antioxidant against lipid peroxidation in plasma and red blood cells .[14] In this way integrity of long-chain polyunsaturated fatty acids which function as important signaling molecules have been maintained. This peroxyl radical scavenger activity of Vitamin E has been described in various studies by scientists.[15]The antioxidant potential of vitamin can be attributed to redox potential of its chromane ring.[16]Vitamin E deficiency in humans may manifest as peripheral neuropathy[17]which is similar to that observed in patients with Friedreich ataxia. Therefore , a specific function vitamin E that might be proposed is in its protection of long chain fatty acids because its deficiency is found to be associated with deficiency of this vitamin.[18]Secondly it is important for preserving membrane qualities such as fluidity, lipid domains., etc.[19] But in addition to this there are certain studies which are in favour that there is no significant antioxidant potential of Vitamin E.[20]

Uric acid now is not considered as merely a metabolic waste. It has been proposed that increase in life span observed in human evolution to some extent might be due to protective action of uric acid.[21]Uric acid along with albumin and ascorbic acid account for more than 85% of total antioxidant activity.[22]Total radical trapping activity (TRAP) includes uric acid as major contributor as it accounts for 38-47% in comparison to vitamin C and vitamin E which account for 13-17% and 2-8% respectively.[23]It has been found at contribute as much as two-thirds of all free radical scavenging activity in plasma therefore it serve as the most abundant aqueous antioxidant in humans .It does so by preventing lipid peroxidation and quenching hydroxyl,superoxide and peroxynitrite radicals.[24] Increase uric acid levels have been found in oxidative stress and ischemia which might be compensatory mechanism of protection against free radicals[25,24]Uric acid cause inactivation of Nitric oxide and peroxynitrite radicals.[26].Along with dopamine ,uric acid also help in repair of oxidative free radical induced damage of DNA in certain brain cells.[27]Another important function of urate is found in its ability to form chelating agents with transition metals ions like iron and copper ,thus scavenging them. This protects ascorbic acid from oxidation by these metals and interesting feature is that uric acid is itself does not get oxidized.[28]

We carried out an animal study to see the effect of uric acid on vitamin C and E in an induced hyperuricemia with 60% fructose. Since there was no previous assumed biologic rationale to support a potential effect of Vit E on serum uric acid.

MATERIALS AND METHODS

Locally bred forty (40) male Albino rats with an average weight of 180±20g were purchased. The rats were grouped and housed in environmentally controlled room(ambient temperature 24±2°C and relative humidity of 55±5%) in the animal house and acclimatized for 07 days**.** The animals were fed standard diet and given tap water ad libitum until treatment. The protocols for experimentation was approved and performed in strict accordance with the Guide for the care and use of laboratory animals (Institute of Laboratory Animal Resources on Life Sciences, US National Research Council, 1996) and the Institutional Animal Ethical Committee (IAEC) of Baqai Medical University, Karachi. Pakistan. The cage size was 8"X18"X10" to keep a group of 05 animals in the cage to prevent from cannibalism.

Sodium Tungstate 10%, 2/3N sulphuric acid,10% sodium bicarbonate,LiCO3, 40%Formaline,Acetic acid, Fructose, Oxonic acid. Spectrosol grade reagents and acids from B.D.H, Poole, UK, were employed. All purified enzymes, coenzymes, substrates, standards and buffers will be purchased from Sigma Chemicals Company, USA. All other chemicals were of analytical grade and was procured from SRL and Qualigens, USA.

All animals housed in standard conditions were initially fed standard diet and allowed adaptation of one (01) week.Albino rats were divided in four(04) groups; A,B,C & D.

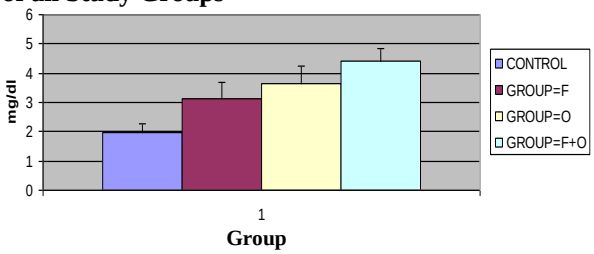
Group A:Ten (10) male albino rats as Control were kept as control and were fed standard diet and water ad libitum for 10 weeks. Group B: Ten (10) male albino rats were fed 60% fructose mixed in standard diet and water ad libitum for 10 weeks. Group C:Ten (10) male albino rats were fed 60% fructose mixed in standard diet and water ad libitum for 10 weeks. They were also injected intraperitonealy oxonic acid 250mg/kg every third day for 10 weeks.Group D:Ten (10) male albino rats were injected intraperitonealy oxonic acid 250mg/kg every third day for 10 weeks. They were fed standard diet and water ad libitum for 10 weeks.Body weights were measured at the commencement and at the end of study. The amount of diet was measured before giving and then subtracted from the amount of food left over daily. At the end of study, rats were dissected in a nearby room separate from experiment area. Approximately 10 mls of blood was drawn from heart using disposable syringe. 8 mls of blood was transferred in heparanized tube, mixed and centrifuged to separate plasma and divided in two epindorf cups for estimation ofvit C ,E and uric acid done by kit methods by randox-manual /Rx monza UA230/UA 233.

RESULTS

Graph 1 shows the comparison of mean plasma uric acid levels of Control with rest of the groups. Mean plasma level of uric acid of Control is found to be 1.97 mg/dl(±0.09) .Group B(fructose) showed mean plasma uric acid of 3.15 mg/dl(±0.17) .This reflects that uric acid was raised to 37% in rats which were exposed to diet comprising 60% Fructose than control. On comparing both groups i.e Control with Group B highly significant statistical correlation (P <0.001) was observed.

The mean plasma uric acid levels of Group C (oxonic acid) was 3.63 mg/dl(±0.22)which is 45% higher than Control. The probability calculated was highly significant (P<0.001) when both groups were evaluated. While comparing Group D (Fructose +Oxonic acid) with Control, highly significant correlation was observed(P<0.001).It was due to high mean plasma serum uric acid level of Group D which was 4.41mg/dl(±0.14) .The combination of fructose with uricase inhibitor ,Oxonic acid raises uric acid to 55% from control and this level is highest of all these groups.

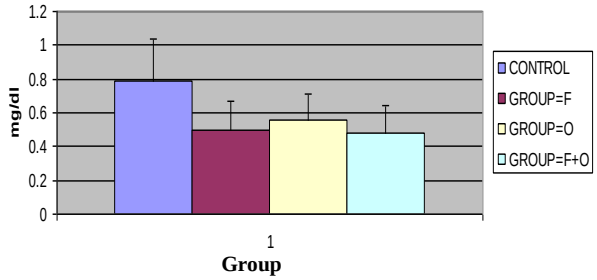
Bar Graph 1: Comparison of Serum Uric Acid levels of all Study Groups



Serum Uric Acid

Groups	Control	Group=F	Group=O	Group=F+O
M.V	1.97	3.15	3.63	4.43
S.D	0.3	**0.55**	**0.63**	**0.43**

Bar Graph 2: Comparison of Serum Vitamin E levels of all Study Groups



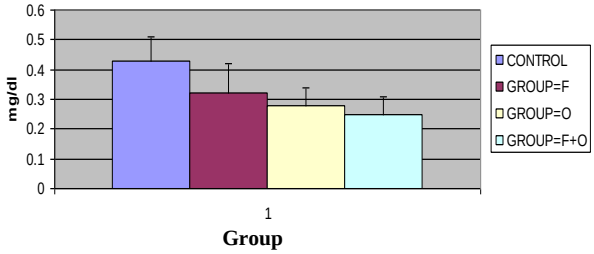
Vitamin E

Groups	Control	Group=F	Group=O	Group=F+O
M.V	0.79	0.5	0.56	0.48
S.D	0.25	**0.17**	**0.15**	**0.16**

Graph 2 shows the comparison of mean plasma vitamin E levels of Control with rest of the groups. Mean plasma vitamin E level of Control was found to be 0.79mg/dl(±0.08) .Group B(Fructose) showed mean plasma vitamin E of 0.5mg/dl(±0.05) reflecting 58% lower vitamin E levels in rats which were exposed to diet comprising 60% Fructose. On comparing both groups i.e Control with Group F significant statistical correlation (P<0.01) was observed. The mean plasma vitamin E levels of Group C (oxonic acid) was 0.56mg/dl(±0.05)which are although lower but somewhat closer to Control. Therefore non-significant (P>0.01) correlation was observed on comparison of both group .While comparing Group D (Fructose +Oxonic acid) with Control, significant correlation was observed(P<0.01).It was due to the fact Group D had considerable low mean plasma vitamin E levels of 0.5 8 mg/dl(±0.06) which are 64% less than rats of group control.

Graph 3 shows the comparison of mean plasma vitamin C levels of Control with rest of the groups. Mean plasma vitamin C level of Control was found to be 0.43mg/dl(±0.03) .Group B (Fructose) showed mean plasma vitamin C of 0.32mg/dl(±0.03) reflecting 34% lower vitamin C levels in rats which were exposed to diet comprising 60% Fructose. On comparing both groups i.e. Control with Group B significant statistical correlation (P<0.01) was observed.The mean plasma vitamin C levels of Group C (oxonic acid) was 0.28mg/dl(±0.02)which which are 53% lower than Control. Therefore highly significant (P<0.001) correlation was observed on comparison of both group .While comparing Group D (Fructose +Oxonic acid) with Control,highly significant correlation was observed(P<0.001).It was due to the fact Group D had considerable low mean plasma vitamin C levels of 0.25 mg/dl(±0.02) which are 72% less than rats of group control.

Bar Graph 3: Comparison of Serum Vitamin C levels of all Study Groups



Vitamin C

Groups	Control	Group=F	Group=O	Group=F+O
M.V	0.43	0.32	0.28	0.25
S.D	0.08	**0.1**	**0.058**	**0.06**

DISCUSSION:

Uric acid has long been described as metabolic waste of purine metabolism with strong relation to number of pathologies involving many organs of body. On the other hand scientific research has also revealed its role as an antioxidant making its pathological status

ambiguous. In present study we elaborated that antioxidant status by incorporating vit C and E as antioxidants and evaluating their relationship with uric acid.

One of the important features of this study was the method by which hyperuricemia have been induced in animal model. Group B (Fructose) was given fructose, group C (fructose +Oxonic acid) was treated with "oxonic acid" and group D (Oxonic acid). The principle hyperuricemic factor in this study was fructose as it is extensively used in beverages and food .Its a rather controversial factor as number of studies both animals and human, are in the favour that fructose can induce hyperuricemia [29] but many studies have opposed this hypothesis[30] and even mixed response has been shown.[31]Present investigation tried to verify this theory. Very few studies have used this combined model of fructose plus oxonic acid. In order to make conditions similar to human, uricase inhibitor oxonic acid was incorporated to abolish the effect of this enzyme in rats . Also these different regimens were used to establish the extent of hyperuricemia caused by fructose.

It was noted that serum levels of ascorbate in all four groups, were found to be decreased in all three hyperuricemic models in comparison with control. Especially the lowest levels were observed in the both groups which were treated with 60% fructose only group B (Fructose) and 60% fructose and 2% oxonic acid group C (Fructose+Oxonic acid) respectively .The levels of ascorbate dropped to 34% and 72% of the levels of Control group. These findings are in harmony with MM Hamalainen and KK Makinen (1982)[33] which have demonstrated that dietary carbohydrates including fructose exhibit selective effects on ascorbic acid metabolism in liver. This might be due to influence on UDP-glucuronic acid pathway or glucuronate-xylose cycle both of which are responsible for producing ascorbic acid in liver.banhegyi et.,al. [33] have also shown the similar findings that fructose has inhibiting effect on ascorbic acid synthesis in animals .Randamized trials have demonstrated the inverse relation between serum levels of urate and ascorbate by using supplements of vitamin C.[34]The proposed underlying mechanisms involved kidneys showing that vitamin C can increase glomerular filtration and/or compete with urate for reabsorption as both depend upon anion exchange transport at proximal tubules.[35] The possible means by which ascorbate favour urate filtration at glomeruli may be due to antioxidant effect which reduces the microvascular ischemia in glomeruli leading to increase blood flow at the site. It has been demonstrated in one study that 500 mg/d vitamin C supplementation for 8 wk reduced uric acid levels by 0.5 mg/dl.[36] While citing this literature about vitamin C it could be taking into mind that present study was only measuring the levels of vitamin c in response to fructose and oxonic acid , the instrumental to the uric acid increase. This was in accordance with the study done in johns hopkin university.[37] Therefore, it can be speculated that the decreased levels of ascorbate may be due to increased levels of uric acid. In fact there is evidence that urate alone when preventing oxidation of lipids, may itself become proxidant. This might be prevented by adding ascorbate to oxidizing LDL.[38]This may lead to utilization of ascorbate. On the contrary, there are number of studies demonstrating ability of uric acid in supplementing antioxidant property of ascorbate by preventing oxidation of ascorbate by forming stable compounds with transition metals such as iron and copper[28]

The levels of vitamin C were found to be lower than normal in all four groups. This might be due to the reason that ascorbate is strong reducing agent and may even get oxidized by when exposed to atmosphere and as we have used the method to estimate ascorbate levels based on virtue of its property of reduction it might be the possible factor in relevant decrease in levels.

In ongoing study, the levels of α tocopherol were found to be decreased in all groups when compared with the control and lowest levels of 0.4 mg/dl observed in the group of highest urate levels that is group D .In comparison to rats of group Control,tocopherols of the hyperuricemic rats of rest of study group is observed to be fallen to 58% and 64% respectively. These findings are in agreement with Marco Bagnati et, al.,[39] who have pointed out in their experimental study the interrelationship between urate and tocopherol by demonstrating that in some selected circumstances, uric acid even at physiological pH is capable of stimulating oxidation of LDL . The synergistic action described in this research by uric acid is that it may reduced tocopheroxyl radical back to its parent compound .This is made possible by the fact that α-tocopherol is present at the LDL water interfaced as proposed by number of studies, therefore it is in access to water soluble uric acid. However it was also revealed in an experimental study that irrespective of the levels of endogenous tocopherol, uric acid may still enhance LDL oxidation by reducing copper so vitamin E is rather consumed in the process. However, some studies including Ruggiero C, et al.[40] demonstrated that no correlation exists between the urate and tocopherols.

CONCLUSION

Increased level of vit C and E decreases the level uric acid. Therefore, it may be suggested that increase intake of vitamin C may be helpful in lowering uric acid concentration especially in gout patient. It is further suggested that future trials are needed to determine the role and relationship of vitamin E and C with uric acid concentration in human.

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