Evaluation of Anti Hyperuricemic Activity of Vitamin E on Potassim Oxonate Induced Hyperuricemic Rat Model

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Abstract

Background Hyperuricemia is often associated with oxidative stress and vitamin E alters uric acid level in hypertensive rats, consequently, vitamin E may have a significant role in non-hypertensive hyperuricemia. Therefore, we aimed to evaluate anti-hyperuricemic activity of vitamin E on potassium oxonate-induced hyperuricemic rats.

Methods Eighteen adult Wistar Albino rats of similar weights were equally divided into group I (normal control), group II (disease control) and group III (treatment control). Group I received 0.5ml normal saline per oral while group II and group III received potassium oxonate (250 mg/kg-intraperitoneal) on day 1, 3, 6, 10, 13 and 15. Group III also received vitamin E (200 mg/kg-per oral) for 15 days. Blood samples were collected through retro orbital plexus from all the animals on day 15 to evaluate uric acid, creatinine, total cholesterol, triglycerides, albumin and blood urea nitrogen, as well joint diameter, and kidneys' weight was also evaluated.

Result We observed a statistically significant rise ($p<0.05$ or $p<0.001$) in all the parameters and a significant decline in serum albumin level ($p<0.001$) in group II as compared to group I, but no significant difference ($p>0.05$) in all the parameters between group I and III. In contrast to this, a significant reduction in all the parameters ($p<0.05$ or $p<0.001$) and significant increase in serum albumin level ($p<0.001$) were observed in group III as compared to group II.

Conclusion Our findings suggest that vitamin E has an anti-hyperuricemic activity against hyperuricemia induced rats.

Introduction

Hyperuricemia is a common metabolic disorder with an elevated level of serum uric acid[1], more than 7.2 mg/dl in adult males, and 6.0 mg/dl in women and women.[2] Hyperuricemia can subsequently develop into gout, tophi formation, kidney stones and acute kidney failure[3] and is also associated with the risk of cardiovascular events, particularly chronic heart failure[4] and essential hypertension.[5] Uric acid is a potent antioxidant in the extracellular environment whereas intracellularly high level of uric acid can generate reactive oxygen species (ROS) causing oxidative stress in numerous kinds of cells that includes vascular smooth muscle cells, endothelium, renal tubular cells, islet cells and hepatocytes.[6–8] Oxidative stress occurs when cell cannot neutralise such ROS.[9] This stress can subsequently damage cellular components such as membranes, lipids, proteins, lipoproteins and DNA[10–15] as well as alter the intracellular activities including decline in the synthesis of protein,[16–17] increase in apoptotic activity.[18] Consequently, oxidative stress can induce several chronic diseases such as hypertension, congestive heart failure, COPD, renal failure, cancer, Parkinson disease, multiple sclerosis, rheumatoid arthritis and many more.[19]

Serum uric acid level is a consequence of increased purine metabolism or decreased elimination. During purine metabolism, uric acid is formed from the final enzymatic oxidation of xanthine oxidase (XO) on

xanthine, which is generated from the same process on hypoxanthine.

$$\text{Hypoxanthine} + O_2 + H_2O \lozenge \text{Xanthine} + H_2O_2$$

$$\text{Xanthine} + 2O_2 + H_2O \lozenge \text{Uric acid} + 2O_2^- + 2H^+$$

$$\text{Xanthine} + O_2 + H_2O \lozenge \text{Uric acid} + H_2O_2$$

However, in animals uric acid undergoes further oxidation to allantoin by an enzyme absent in human called uricase [20] Hence, rodents can regulate the level of uric acid in their blood and this prevents them from hyperuricemia. Consequently, hyperuricemia and gout are entirely related to humans. Experimentally, inhibition of hepatic uricase can be achieved by potassium oxonate. This inhibition elevates uric acid level in blood and thus, causes hyperuricemia in rodents such as rats, making it possible to develop potassium oxonate-induced rat model.[21] Studies have used uric acid, creatinine, total cholesterol (TC), triglycerides (TG), blood urea nitrogen (BUN) and albumin levels to investigate hyperuricemia in rat models.[23]

Vitamin E is a naturally occurring lipid-soluble antioxidant that possesses potent in-vivo free radical scavenging activity [24] and its protective role against oxidative stress has been reported in both humans and rats.[25] Vitamin E supplementation for 4 weeks has shown to increase urinary uric acid level and decrease serum uric acid level in hypertension-induced rats[26] However, in this study the rat model was not hypertensive free and several other serum biomarkers of hyperuricemia were not explored. Therefore, we aimed to evaluate the anti-hyperuricemic activity of vitamin E on potassium oxonate-induced hyperuricemic rats.

Materials And Methods

Drugs

Potassium oxonate powder (5 gm, 00164, Lot.GN48L-OS, Tokyo chemical industries Co Ltd), vitamin E soft gelatine capsules (Evion 400 mg, batch number: E01BP17056, expiry Date: 06/2019, MERCK LIMITED), saline solution (NS 500 ml, Expiry Date: 06/2019, Himalyan parenteral and formulations Pvt Ltd, Nepal), cholesterol assay kit (REF 10028, LOT 18007, HUMAN Diagnostics Worldwide, Germany), urea kit (REF 10505, LOT 18008, HUMAN Diagnostics Worldwide, Germany), Creatine kit (REF 10051, LOT 17023, HUMAN Diagnostics Worldwide, Germany) and all other assay kits (HUMAN Diagnostics Worldwide, Germany).

Animals

A total of 18, six weeks old adult wistar albino rats, weighing 150 g to 200 g (Department of plant resources, Kathmandu, Nepal) were kept in metabolic cages with a 12/12-hrs day/night cycle under a

maintained temperature of 25±3°C, relative humidity 50-55% and with free access to drinking water and food.[27]

Apparatus

Clinical biochemistry analyzer (RobonikPrietest Biochemistry Analyzer), micropipette, capillary tubes, volumetric flasks, weighing balance, test tube, laboratory thermometer, 3 ml vacuum Centrifuge tubes (Vaccu Lab, LOT 180115, Hunan Liuyang Medical Instrument Factory), Centrifuge Machine.

Drug solution preparation[28-29]

- Normal Saline solution
- Potassium oxonate suspension: 800 mg potassium oxonate powder was suspended in 15 ml normal saline to prepare potassium oxonate suspension for 250 mg/kg intra peritoneal (ip) injection.
- Vitamin E: 200 mg/kg per oral (po)

Experimental Design[28-29]

The rats were randomly divided into three groups consisting of each 6 rats each as follows:

- Group I: treated with normal saline only (Normal Saline po).
- Group II: treated with potassium oxonate (250 mg/kg ip).
- Group III: treated with Vitamin E (200 mg/kg po) and potassium oxonate (250 mg/kg ip).

Groups I and III were orally administered once a day for 15 days. On day 1, 3, 6, 10, 13 and 15, potassium oxonate (250 mg/kg *ip*) was injected one hour before the administration of vitamin E (200 mg/kg po) to all the groups except group I.

Statistical analysis

All the data were presented as mean±SEM (mean±standard error mean), the statistical difference were compared by using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using SPSS (Version 20).

Results

The anti-hyperuricemia activity of vitamin E was determined in hyperuricemia-induced rats using potassium oxonate (250mg/kg ip) and treated with vitamin E (200 mg/kg po). The evaluation of anti-hyperuricemic activity was performed based on uric acid, creatinine, TG, TC, albumin, BUN, body weight, kidneys' weight and joint diameter. The comparison of these parameters across normal control (group I), disease control (group II) and treatment control (group III) has been presented in table 1. This table shows

that the animals in each group have no significant difference in the mean body weight. However, when group I and II were compared, we observed a significant rise ($p<0.05$) in all mean parameters in rats of group I except a significant decline in mean serum albumin (1.72 g/dl, $p<0.001$). There was a significant increase in kidneys' weight by 0.17 g, TC by 7.71 mg/dl and TG by 36.31 mg/dl at $p\leq0.05$ while joint diameter by 0.53 cm, serum uric acid by 2.72 mg/dl, serum creatinine by 2.76 mg/dl and BUN by 11.28 mg/dl at $p<0.001$. No significant difference in all the mean parameters between group I and III was observed statistically. In contrast to this, a significant reduction ($p<0.05$) in all the mean parameters and significant increase in serum albumin level (1.38 g/dl, $p<0.001$) were observed in group III as compared to group II. The decline in kidney weight, TC and TG were 0.23 g, 9.72 mg/dl and 49.79 mg/dl respectively at $p<0.05$ while joint diameter, serum uric acid, serum creatinine and BUN were 0.52 cm, 3.05 mg/dl, 2.76 mg/dl and 11.33 mg/dl respectively at $p<0.001$.

Parameters	Group I	Group II	Group II
	190.50±1.26	188.50 ± 1.48^{ns}	189.67 ± 2.70^{ns}
Kidney weight(g)	0.99±0.367	$1.16\pm0.201^{\#\#}$	$0.93\pm0.157^{**,ns}$
Joint Diameter(cm)	2.14± 0.140	$2.67\pm0.544^{\#}$	$2.15\pm0.193^{*,ns}$
Uric Acid(mg/dl)	2.66±0.457	$5.38\pm0.641^{\#}$	$2.33\pm0.177^{*,ns}$
Creatinine(mg/dl)	0.77±0.457	$3.53\pm0.239^{\#}$	$0.64\pm0.480^{*,ns}$
TC(mg/dl)	33.97±0.79	$41.68\pm1.78^{\#\#}$	$31.96\pm1.34^{**,ns}$
TG(mg/dl)	63.16±8.29	$99.47\pm0.40^{\#\#}$	$49.68\pm8.47^{**,ns}$
Albumin(g/dl)	4.07±0.538	$2.35\pm0.193^{\#}$	$3.73\pm0.157^{*,ns}$
BUN(mg/dl)	17.79±0.90	$29.07\pm1.38^{\#}$	$17.74\pm0.74^{*,ns}$

All values are expressed as the mean ± SEM. #$p< 0.001$ when compared to group I, *$p<0.001$ when compared to group II, ##$p<0.05$ when compared to group-I, **$p<0.05$ when compared to group II, ns: non-significant when compared to group I. Each group consists of 6 rats.

Discussion

Our study evaluated the anti-hyperuricemic activity of vitamin E in potassium oxonate-induced hyperuricemic rats. The evaluation of physical and biochemical parameters suggested that administration of oral vitamin E at a dose of 200 mg/kg in potassium oxonate-induced hyperuricemic rat model show a significant anti-hyperuricemic activity. The significant rise in serum uric acid, kidneys' weight, knee joint diameter, serum creatinine, TG, TC, BUN and the significant decline in serum albumin in rats that were administered with ip potassium oxonate (disease control group II) as compared to rats that

were administered with normal saline (normal control group I) signify the induction of hyperuricemic activity of potassium oxonate. However, potassium oxonate-induced hyperuricemic rats that were simultaneously treated with 200 mg/kg oral vitamin E (treatment control group III) significantly reduced all the parameters but increased albumin level.

Hyperuricemia is a common metabolic disorder characterized by high levels of uric acid in the blood and one of the most important causative factors for gout.[30] It has also been associated with chronic kidney disease,[31] coronary heart disease,[32] and obesity with remission of non-alcoholic fatty liver disease and hypertension.[33-34] The high concentration of uric acid is associated with an increase in xanthine oxidase activity.[35] Extracellularly uric acid is a potent antioxidant but high concentration of uric acid intracellularly can generate ROS because of increased xanthine oxidase activity. ROS are produced by the transfer of electrons to oxygen from hypoxanthine and xanthine to generate hydrogen peroxide in presence of xanthine oxidase. The rise in ROS and subsequent failure of the cell to neutralise them can lead to oxidative stress in the cells[6-9] which can potentially lead to numerous chronic diseases.[19] Antioxidants such as vitamin E possesses potent in-vivo free-radical scavenging activity[24] which might be responsible for reducing ROS induced oxidative stress in our hyperuricemic rat models. There is an evidence that suggest vitamin E has a protective role against oxidative stress in rats and humans.[25] The other reason for hyperuricemic activity might be due to possibility of increase in uric acid level in urine. Our study protocol did not take into account the measurement of urinary uric acid to support this mechanism, but evidence suggest that supplementation of vitamin E for 4 weeks in hypertension-induced rat models increases urinary uric acid level and decrease serum uric acid level.[26]

We did not observe any apparent toxicity in the rats in the dose that was administered for the experiment, which suggests that the dose to be safer but its extrapolation in humans need further evidence and explanation. Vitamin E may be used in humans for the treatment and management of hyperuricemia and gout. However, the mechanism of action of vitamin E on hyperuricemia and its antioxidant property is yet to be thoroughly investigated.

Conclusions

Our study suggests that a repeated oral administration of 200mg/kg of *vitamin E for 15 days has* an anti-hyperuricemic effect in potassium oxonate-induced hyperuricemic rats.

Declarations

Ethical approval and consent to participate

We obtained the ethical approval from Institutional Review Board of Nepal Health Research Council.

Consent for publication

I understand that the text and table for published in the article will be freely available on the internet and may be seen by the general public.

Availability of supporting data

The data used to support the finding of this study are included within the article.

Competing interests:- Not applicable

Funding:-

No special funding were required as this experiment was conducted in college laboratory

Author contribution

Hari Prasad Sapkota:- Study designed, draft preparation, addressing the comments of reviewer, processing for ethical approval, review and submission of final version of manuscript.

Hemraj Sharm:- Support in literature review and correction, designing of a draft to the manuscript.

Shakti shrestha:- Support in literature review and data analysis, finalizing the draft to the manuscript.

Sandhya Shrestha:- Work in lab to complete the experiments and submitted a draft

Sumana Adhikari:- performed all experiments and helped in literature searches

Sudipa Adhikari:- Performed all experiments, helped in literature searches.

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