ORIGINAL ARTICLE

GENOTOXIC AND CYTOTOXIC EFFECTS OF ORAL VANADYL SULPHATE

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Background: Vanadyl sulphate is available as herbal medicine against diabetes mellitus and body building supplement, over the counter worldwide. The available data on its safety is controversial and inadequate. The objective of this study was to analyse its safety in usual therapeutic dose range. **Methods:** It was an experimental study carried out at the Department of Biochemistry & Molecular Biology, Army Medical College, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan, from Jun 2014 to Oct 2018. The study was carried out on 105 Sprague Dawley rats for duration of 24 weeks. The animals were randomly distributed in three groups of 35 each. The group I rats were marked as control while rats of group II & III were administered vanadyl sulphate 0.06mg/day and 0.3mg/day respectively. Alanine amino transferase (ALT) and Malondialdehyde (MDA) were measured in serum while comet assay was performed on WBCs. **Results**: The plasma levels of ALT and MDA were significantly raised in group II and III subjects. Single cell gel electrophoresis (SCGE) / comet assay showed minimal "tail moment" in control group and increased tail moment in group II and III in a dose dependent manner which indicates dsDNA breaks. **Conclusion**: It was observed that vanadyl sulphate causes hepatocellular toxicity, oxidative stress and damage to the DNA in usual therapeutic/ supplemental doses. Due to hazardous effects, its use in humans as alternate medicine may be reviewed.

Keywords: Vanadyl sulphate; Hepatotoxicity; Oxidative Stress; DNA Damage; Comet Assay

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INTRODUCTION

Vanadium is a transition metal found naturally in shellfish, black pepper, dill seed, mushrooms, grains and parsley.[1] It is thought to be an essential element in the diet of many animals but its essentiality in human diet is not still established.[1] The use of salt in herbal/conventional medicine for treating diabetes,[2] and as a component of body building supplement in athletes demands for evaluation of its safety in long term use. Vanadyl sulphate has been proposed to produce hydrogen per oxide (H_2O_2) which can cause carcinogenesis.[3] Proposal that H_2O_2 production is the gate way to toxic effects can be tested by estimation of malondialdehyde levels. H_2O_2 damages lipids to form malondialdehyde (MDA), a known carcinogenic metabolite.[4] A few vanadium salts are proposed to cause significant oxidative stress in vivo.[5] Some researchers believe that vanadium is toxic to the tissues, causes oxidative stress.[6] leading to altered enzyme functions like glucose 6 phosphate dehydrogenase (G6PDH) and acetyl cholinesterase in brain tissue, which can be countered by ascorbic acid.[7] The oxidative stress caused by vanadium may enhance apoptotic activity in cancer cell lines.[7] The oculotoxicity of vanadium has also been reported.[8]

But on the other hand, some studies suggest that vanadyl sulphate is safe even in higher doses.[9,10] These studies had made the safety of the salt controversial and there was a need for further study in this regard. Most of the studies showing its toxicity, used vanadium in too high doses.[11] and thus these findings cannot predict the behaviour of the salt in therapeutic dose range. Similarly, some of the studies advocating safety of the salt used it in their study for too short span of time.[12] thus safety in long term use again remains a question.

Based on present literature on vanadium it is evident that there may be some oxidative stress and damage to various tissues for example hepatocytes as a result of its use. Vanadyl sulphate is being marketed and used worldwide including Pakistan and the United Kingdom, for control of diabetes mellitus (as herbal medicine) and as body building supplement, usually in a dose of 20mg per day in a normal average adult man.[13] It is available as "over the counter" drug/ supplement not only in Pakistan but also in developed countries including USA.[13]

Vanadium (V^{+4}), being a heavy metal, can induce DNA double stranded breaks (DSBs) but mode of action is not clear.[4] Some chemicals lead to formation of free radicals which can cause DNA

DSBs.[14] DSBs may lead to cellular death.[15] or carcinogenesis.[14] Damage to the DNA can be measured by comet assay.[16] There are few studies, conducted to evaluate vanadyl sulphate for its genotoxic potential and those too are either of maximum about 5 weeks duration.[10,17] or use salt in doses well above therapeutic ranges.[17] In our study salt was administered for 24 weeks to animals excluding recruitment and acclimatization period. The dose range was kept around the usual dose range prescribed in human beings. The genetic effects of vanadium in occupationally exposed humans are controversial.[18] The genotoxic effects of vanadium are reported to result in increased risk for developing cancer, genetic syndromes due to DNA damage, fetal malformations and cancer.[18] The detrimental effects of vanadium to the DNA double stranded macro molecule are exerted mainly through its oxidative properties.[19]

On the contrary, it is believed by some others that vanadium supplementation significantly reduces oxidative stress produced by diabetes mellitus. A study on alloxan induced diabetic mice showed that vanadium treatment reduced the oxidative stress caused by DM.[20] Vanadium was shown to reduce serum antioxidant enzyme levels which were raised in DM.[21] on the basis of which vanadium is thought to be an antioxidant element.[22] As shown by Tunali S *et al,* after 60 days of induction of DM in rats, in blood, stomach and spleen lipid peroxidation (LPO) was increased and GSH levels were decreased and these effects were reversed with vanadium administration.[22] So if this is the case then DNA damage is less likely to be significant in vanadium treatment cases.

MATERIAL AND METHODS

This randomized control trial was carried out in the Department of Biochemistry and Molecular Biology at Centre for Research in Experimental & Applied Medicine (CREAM), Army Medical College. The ethical review committee of CREAM evaluated the ethical aspects of the research proposal and granted approval before the start of work. Total duration of study was 24 weeks and 105 healthy Sprague Dawley Rats of average 220 g weight and six to eight weeks age were recruited by nonprobability convenience sampling. Standard conditions, i.e., a photo period of 12 hours dark and 12 hours light and temperature 23±5 °C were provided to animals at animal house.

Each rat was randomly assigned one out of three groups making 35 rats in each group. Animals in this group-1 were given normal pellet diet (NPD) and water *ad libitum* for a period of 24 weeks. The salt (vanadyl sulphate) was not administered to animals of group-I (control group). A usual dose of vanadyl sulphate used in humans is 20–100 mg/day in a 70 kg man which is 0.25–1.2 mg/kg/day. Group II was given 0.25 mg/kg/day while group III was given 1.2 mg/kg/day in aqueous solution[14] through oral gavage for 24 weeks.

At 24 weeks blood samples were taken and preserved for appropriate analysis. Serum ALT was measured Spectro-photometrically (BioTek elx 808 Absorbance microplate reader). The oxidative stress was measured by estimation of MDA in serum by ELISA kit method (Abbkine rat MDA Eliza kit 96 well).

The single cell gel electrophoresis (SCGE) technique was used to detect DNA double stranded (ds) breaks[16] in individual cells. The procedure was carried out at alkaline pH instead of neutral pH, which can show single stranded breaks as well. The value of pH in neutral assay is kept below 13 around 12.1 while in alkaline the pH must be kept more than 13 which causes unwinding and denaturation of DNA thus allowing single stranded broken segments to freely move in an electric field. The reagents were prepared fresh and slides were made in duplicate for each sample. After keeping dipped in lysing solution overnight sample slides were run electrophoresis and stained with ethidium bromide to visualize comets under fluorescent microscope. Comets were photographed and analysed using comet 15 and tail moment was noted which is a product of multiplication of tail length and tail thickness. The data obtained was further analysed by SPSS version 21 software using post hoc Tukey's test along with ANOVA. There was significant difference between results of treated and control groups (p-value less than 0.05).

RESULTS

Alanine amino transferase (ALT) was significantly increased in group II and III (using one-way ANOVA). The marker of oxidative stress, malondialdehyde (MDA) was also raised in serum in the animals of treated groups. The increase was significant in both treated groups and was directly proportional to the dose administered.

The comet assay showed minimal tail moment in controls while this index increased in group II. The increase was significant as compared to that of control group animals. The tail moment was seen even more in cells of group III animals and this increase was significantly higher than the values obtained in cases of group II. This indicates a dose dependent toxicity of the salt to DNA integrity even in the therapeutic dose range.

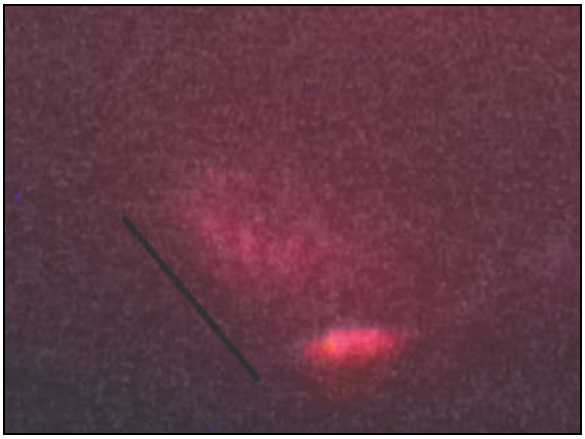

Figure-1: Comet tail moment

Table-1: The mean values and standard deviations regarding ALT, MDA levels and comet tail moment are given in table below

Mean ±SD				
Variables	**Group 1 (n = 33)**	**Group 2 (n = 32)**	**Group 3 (n = 31)**	***p*-value**
ALT (U/L)	33.91±5.62	64.13±11.40	121.17±24.59	0.01
MDA (nmol/mL)	0.39±0.05	0.50±0.06	0.61±0.07	0.01
Tail Moment (μ)	1.41±0.17	1.77±0.14	2.08±0.14	0.01

Table-1: Results of Toxicity and Geno-toxicity profile of group I, 2 and 3 rats

DISCUSSION

Many processes in the cell generate free radicals [23] and being neutralized constantly by another set of antioxidant mechanisms. When the balance goes towards more free radicals, it leads to DNA damage and lipid peroxidation.[23] Malondialdehyde a metabolite of lipid peroxidation was measured and found increased in treated rats in a dose dependent manner.

There have been various types of result outcomes in vanadium administered animal subjects previously. Vanadium is proposed to have oxidant effect as shown in a study conducted by Scibior A and colleagues in which MDA was found increased in hepatic tissue of treated rats.[24] Another study showed increased circulating plasma MDA levels and reduced plasma L-ascorbate levels in rats.[25] Our results are similar to those of these studies and we also suggest an oxidative role of vanadium in therapeutic dose range especially when administered for longer durations.

In experiments conducted by Areum Daseul Kim in year 2011 showed that Jeju ground water containing vanadyl sulphate significantly increased glutathione levels and scavenged free radicals exerting a protective effect on liver cell line.[26] These findings are contrary to that of our study. The study by Kim *et al* has a limitation that glutathione level and ROS were measured at a plasma level of 26 μg/L which is much lower than that achieved after usual therapeutic dose range. Therapeutic dose range is 20–100 mg daily orally in humans, 5% of which is absorbed from intestine and diluted in almost 5L of blood in circulation. That means 0.8 mg/L and 4mg/L are the concentrations achieved in plasma after an oral intake of 20 mg and 100 mg respectively which are much more than the dose used by Kim *et al.* This means that VOSO4 may induce glutathione and scavenge free radicals at minute naturally occurring concentrations in water and food but it is doing the other way around at therapeutic/ higher doses conventionally used.

With vanadium treatment, exacerbation of cyclosporine induced hepatotoxicity was reported in 2005.[27] The nephrotoxicity and lipid parameters derangement by the salt has also been reported recently.[28,29] Oxovanadium compounds have useful insulin mimetic effects but also show hepatic toxicity.[30,31] Our study for a considerably longer duration also endorses hepatotoxic effects of use of vanadyl sulphate even in usual dose range.

SCGE showed significant proportional DNA damage in vanadium treated animals in this study. Previously there are two distinct schools of thought. One suggests vanadium to be Geno toxic while other thinks otherwise. It has been seen in this experiment that vanadium causes significant oxidative stress and generates increased amount of ROS. Base modifications have been thought to play a role in DNA damage by free radicals through lipid peroxidation.[23]

Recent studies have demonstrated through comet assay that vanadium compounds cause DNA damage and ROS generation in a dose dependent manner.[31] Moreover, vanadium compounds also decrease the expression of DNA repair genes and promote apoptosis.[32] Newer compounds of vanadium are thus being studied for DNA nuclease activity in plasmids.[33] The ROS generation underlies the main mechanisms for genotoxicity.[34] So, there was a need to ascertain whether these changes are happening at usual therapeutic doses or not. The results obtained are consistent with the results of abovementioned studies. In this study highly, significant DNA damage was observed at both doses within therapeutic range.

The controversial views of researchers on efficacy and safety of vanadium was the main reason that vanadium salts could not get approval as anti-diabetic drug.[35] Findings of this study suggest that these salts should rather be investigated for anti-tumour activity where controlled toxicity to malignant cells is required.

CONCLUSION

Vanadyl sulphate in therapeutic dose range when administered for longer duration causes hepatocellular damage, oxidative stress and double stranded DNA breaks. The salt is thus not safe for human consumption even in usual doses and its use must be reconsidered.

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AUTHORS' CONTRIBUTION

SZH: Literature search, study design, data collection, data analysis, data interpretation, proof reading, lab work, provision of resources. AR: Study design, data analysis, data interpretation, proof reading and provision of resources. AKN: Data analysis, data interpretation, provision of resources. SAK: Data analysis, data interpretation, proof reading, provision of resources. SJ: Data analysis, data interpretation, proof reading, provision of resources.

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