Beryllium Induced Biochemical Alterations in Rodent Model and its Treatment with Calcium Salt of Diethylene Triamine Penta Acetic Acid with α -tocopherol

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ABSTRACT

To evaluate therapeutic efficacy of chelating agents tiron (Sodium-4,5- dihydroxy-1,3-benzene di-sulphonate) and CaNa$_3$DTPA (Calcium tri-sodium di-ethylene tri-amine penta acetic acid) in presence of a-tocopherol against beryllium induced toxicity, adult female albino rats were exposed to beryllium nitrate for 28 days followed by therapy with tiron (471 mg/kg, ip) and CaNa$_3$DTPA (35 mg/kg, ip) alone and in combination with a-tocopherol (25 mg/kg, po). Results revealed non-significant fall in hemoglobin and total serum protein content while significant fall in blood sugar level and activity of serum alkaline phosphatase. On the other hand, significant rise in the activity of serum transaminases and LDH was noticed after beryllium administration. Significant increase in total and esterified cholesterol was found in liver and kidney after toxicity. Significant increase in lipid peroxidation and decreased level of reduced glutathione in both the organs showed oxidative stress due to beryllium exposure. CaNa$_3$DTPA showed moderate therapeutic efficacy; however, its effectiveness was enhanced with a-tocopherol to some extent. Tiron in combination with a-tocopherol exerted statistically more beneficial effects in reversal of beryllium induced biochemical alterations.

Keywords: *a-tocopherol, Beryllium toxicity, CaNa$_3$DTPA, Combination therapy, Tiron*

INTRODUCTION

Beryllium induced lesions in experimental animals and industrial workers have been well documented. Beryllium is a naturally occurring element that is present in earth's crust[1]. It was not known to have health hazards until it was used for industrial purposes. It is the lightest, bivalent and low-density hard metal having specific ability to add strength when small amount is added to copper and nickel to make alloys. Due to its unique chemical and physical properties, use of beryllium is increasing almost in every modern industry like aerospace, defense, electronics and ceramics. Because of its ubiquitous nature, it is found in coal, wood, foodstuffs and gemstones such beryllium is increasing almost in every modern industry like aerospace, defense, electronics and ceramics. Because of its ubiquitous nature, it is found in coal, wood, foodstuffs and gemstones such as aquamarine and emerald[2]. The

general population is exposed to naturally occurring beryllium from ambient air, drinking water, diet and smoking on a daily basis. Emissions from burning of fossil fuels i.e. coal and oil also increase beryllium level in atmosphere. Beryllium exposure can cause (i) acute pneumonitis- a currently rare condition caused by inhalation of beryllium salts or low fired beryllium oxide at concentration greater than 100 $\mu g/m^3$; (ii) contact dermatitis from dermal contact with beryllium salts and (iii) chronic beryllium disease (CBD)- a potentially debilitating and fatal respiratory disease3 . Ultimately beryllium gets accumulated in liver, kidney and bones and induces toxicity.

Therapeutic approach of chelating agents in combination with antioxidants for possible metal detoxification is an important aspect against metal poisoning. Tiron (sodium-4,5-dihydroxy-1,3-benzene disulphonate) is a relatively non toxic chelator, which has been tried in treatment of various metal poisonings, including uranium[4] , lead[5] , vanadium[6] and beryllium[7] .

CaNa$_3$ (calcium trisodium 3DTPA diethylene triamine pentaacetic acid) is a chelating agent belonging to polycarboxylate group of chelators, which is used to be applied in persons contaminated with plutonium or americium. Since CaNa$_3$ is partly absorbed following oral 3DTPA administration, it is usually given by injection[8]. α-Tocopherol is one of the most important lipophilic antioxidants, which acts as scavenger of free radicals within the membrane and protects unsaturated fatty acids from lipid peroxidation [9,10] . The present study has been designed to evaluate therapeutic efficacy of chelating agents tiron and CaNa$_3$DTPA alone or in combination with α-tocopherol in amelioration of beryllium induced toxicity in rats.

MATERIAL AND METHODS:

CHEMICALS:
Beryllium nitrate [Be (NO$_3$)$_2$] was purchased from Fluka (Switzerland), tiron, CaNa$_3$ DTPA from HiMedia Laboratories Ltd. Mumbai, India. All the therapeutic agents were stored refrigerated in desiccators to avoid oxidation and thermal decomposition. All other chemicals used in the study were of pure and analytical grade.

MAINTENANCE OF ANIMALS AND THEIR FEEDING:
Adult female albino rats of *Sprague Dawely* strain (8-10 weeks old having 130 ± 10 g body weight) were randomly selected from animal facility where they were maintained under uniform husbandry conditions of light (14 hr) and dark (10 hr) at 25°C ± 2°C and 60-70% RH. Animals were fed on Standard commercially available pellets of animal diet (Pranav Agro Industries Ltd. New Delhi, India) and drinking water *ad libitum.* Experiments were performed in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Chennai.

PREPARATION OF DOSES:
Beryllium nitrate was dissolved in triple distilled water making up doses of 1 mg/2 ml/kg. Doses of chelating agents CaNa3DTPA (35mg/2ml/kg) and tiron (471 mg/2 ml/kg) were prepared in 0.9% saline and pH was adjusted to 6.4 with sodium bicarbonate before administration. α-Tocopherol was dissolved in olive oil and doses of 25 mg/5ml/kg were administered orally with the help of intragastric rubber catheter. Selection of doses of toxicant, therapeutic agents and duration of treatment was based on earlier studies [7,11,12] .

EXPERIMENTAL DESIGN:
Rats (42) were divided into following 7 groups of 6 animals each:

Group 1 : received sodium nitrate once a day daily for 28 days (1 mg/kg, ip) followed by saline (2 ml/kg, ip) for 5 days and served as normal control;
Group 2 : received beryllium nitrate [Be(NO$_3$)$_2$] once a day daily for 28 days (1 mg/kg, ip) followed by saline (2 ml/kg, ip) for 5 days and served as experimental control;
Group 3 : received toxicant as in group 2 and treated with CaNa$_3$DTPA (35 mg/kg, ip) for 5 consecutive days after toxicant administration;
Group 4: received toxicant as in group 2 and treated with tiron (471 mg/kg, ip) for 5 consecutive days after toxicant administration;
Group 5: received toxicant as in group 2 and treated with α-tocopherol (25 mg/kg, po) for 5 consecutive days after toxicant administration;
Group 6 : received toxicant as in group 2 and concomitantly treated with 3DTPA and α-tocopherol acetate were obtained (25 mg/kg, po) for 5 consecutive days after toxicant administration;
Group 7: received toxicant as in group 2 and concomitantly treated with tiron (471 mg/kg, ip)

and α-tocopherol (25 mg/kg, po) for 5 consecutive days after toxicant administration.

After 24 h of final administration, animals were anesthetized under light ether anesthesia, withdrawing blood in vials by puncturing retro-orbital venous sinus and finally serum was isolated. Liver and kidney were immediately excised, blotted free of adhering fluid and processed for biochemical studies preparations. Standard techniques were applied to assay following blood and tissue biochemical parameters.

Blood biochemical analysis Blood was immediately used for the estimation of hemoglobin[13] and blood sugar[14]. Serum was used for the estimation of aspartate and alamine transaminases (AST and ALT)[15], alkaline phosphatase (SALP)[16], lactate dehydrogenase (LDH)[17] and serum protein contents[18]. Serum albumin was estimated using E-Merck's kit according to the manufacturer's instructions. Estimation of GSH and TBARS in liver and kidney Hepatorenal glutathione (GSH) measurement was performed using dithionitrobenzoic acid and optical density was recorded immediately at 412 nm[19]. The GSH level was calculated using an extinction coefficient of 13600/M/cm and expressed as μmol GSH/g tissue. The TBARS was assayed in liver and kidney for lipid peroxidation (LPO) and the LPO was expressed in terms of nmols TBARS/g tissue using an extinction coefficient of 1.56×10^5 /M/cm[20]. Hepatorenal lipid profile Method of Zlatkis et. al.,[21] was followed for the estimation of total and esterified cholesterol in liver and kidney.

STATISTICAL ANALYSIS:
Data were subjected to statistical analysis through one-way analysis of variance (ANOVA) followed by Student's t-test [22]. Results were considered to be statistically significant at $P \leq 0.05$. % Protection was calculated by the following formula and the values are expressed as mean± SE:

RESULTS:

Blood biochemical analysis-Blood biochemistry **Table 1**- revealed significant fall in blood sugar level, serum albumin and activity of serum alkaline phosphatase, whereas significant rise was noticed in the activities of serum transaminases (AST and ALT) and LDH after beryllium administration; however, hemoglobin and total serum protein contents were declined non-significantly ($P \leq 0.05$). Tiron with and without α-tocopherol was found to be significant in maintaining blood sugar and serum albumin level towards normal ($P \leq 0.05$). Combination and single treatments significantly recovered altered activities of AST, ALT, LDH and SALP at 5% level while more than 80% protection was seen only with the combination of tiron and α-tocopherol. Estimation of GSH and TBARS in liver and kidney. Significant decrease in reduced glutathione and increase in lipid peroxidation in liver and kidney indicated beryllium induced oxidative stress ($P \leq 0.05$; 3DTPA and tiron along with α-tocopherol against beryllium toxicity [Values are mean ± SE of 6 rats in each group]

Combination of tiron and α-tocopherol was found to be most effective that reversed these variables towards normal significantly ($P \leq 0.05$) and showed more than 80% protection. Hepato-renal lipid profile. Significant raise was found in total and esterified cholesterol in liver and kidney, which was lowered towards normal significantly by combined administration of tiron and α-tocopherol ($P \leq 0.05$).

Table 1:Beryllium induced biochemical alterations and it's treatment in rodent model

Groups	Hb (g/100 ml)	B. sugar (mg/100 ml)	S. protein (mg/100 ml)	S. albumin (g/dl)	AST (IU/μ moles/min /L)	AL (IU/L) T	LDH	S (mg Pi/100 ml/h) ALP
Control	15.2±0.84	108±5.97	37.2±2.05	5.63±0.31	68.3±3.77	43.2±2.38	42.2±2.33	206±11.3
Beryllium	13.2±0.72	71.3±3.94*	31.0±1.71	3.30±0.18*	120±6.63*	80.0±4.42*	137±7.57*	119±6.57*
Be+DTPA	13.8±0.76	80.2±4.43	33.4±1.84	3.60±0.19	100±5.52**	72.4±4.00	121±6.68	140±7.73
Protection (%)	30.0±3.86	24.2±4.18	38.7±3.38	12.8±4.81	38.6±3.38	20.6±4.38	16.8±4.59	24.1±4.19
Be+tiron	14.0±0.77	98.2±5.42**	35.0±1.93	4.10±0.22**	80.0±4.42**	54.4±3.00**	98.0±5.41**	167±9.23**
Protection (%)	40.0±3.31	73.2±1.47	64.5±1.96	34.3±3.63	77.3±1.25	69.5±1.68	41.1±3.25	55.1±2.47
Be+α-toco	13.5±0.74	77.2±4.26	34.8±1.92	3.76±0.20	92.1±5.09**	60.4±3.33**	108±5.98**	172±9.50**
Protection (%)	15.0±4.69	16.0±4.63	61.2±2.13	19.7±4.43	53.9±2.54	53.2±2.58	30.5±3.83	60.9±2.16
Be+DTPA+ α-	14.0±0.77	82.7±4.57	35.4±1.95	3.90±0.21	91.3±5.04**	55.1±3.04**	106±5.85**	168±9.28**

toco								
Protection (%)	40.0±3.31	31.6±3.81	70.9±1.60	25.7±4.10	55.5±2.45	67.6±1.78	32.7±3.72	56.3±2.41
Be+tiron+α-toco	14.6±0.80	103±5.69**	36.0±1.99	4.60±0.25**	76.0±4.20**	48.2±2.66**	78.4±4.33**	197±10.8**
Protection (%)	70.0±1.65	86.3±0.75	80.6±1.06	55.7±2.44	85.1±0.82	86.4±0.77	61.8±2.11	89.6±0.57

 F Variance 0.91 9.97 # 1.30 13.5# 13.9# 18.4 # 5.4# 12.5#

*Significant difference at $P \leq 0.05$ compared with *control group; beryllium administered group.

[Values are mean ± SE of 6 rats in each group]

**# Significant F Variance at 5% level.

Abbreviations: Hb (Haemoglobin); B. Sugar (Blood sugar); S. Protein (Serum protein); S. Albumin (Serum albumin); AST (Aspartate aminotransferase); ALT (Alanine aminotransferase); LDH (Lactate dehydrogenase); SALP (Serum alkaline phosphatase

DISSCUSSION:

Present investigation had been carried out to compare efficacy of chelating agents individually and in combination with α-tocopherol. Results revealed severe alterations in histopathological, blood and tissue biochemical variables after intraperitoneal administration of beryllium. Reduced synthesis of heme and globulin proteins due to beryllium poisoning resulted in a consequent decrease in hemoglobin content of erythrocytes [23,7]. Significant fall in blood sugar level after beryllium intoxication may be due to liver damage and increase in blood lactic acid, which resulted in hypoglycemia. Significant raise in the serum transaminases and LDH may not only be due to phagocytosis and necrosis in tissues of liver and kidney [24,25] but also due to alterations in membrane permeability, which permitted rapid out flow of these enzymes [26,27]. Increased level of AST probably elevated the oxidation of aspartic acid that gets transformed into cholesterol and thus, increased level of cholesterol was noticed in liver and kidney. Inhibition in the activity of serum alkaline phosphatase during beryllium toxicity may be due to the displacement of magnesium ion (Mg^{2+}) by beryllium ions (Be^{2+})[28]. In vivo and in vitro studies have suggested that Be^{2+} ions always compete with Mg^{2+} ions [29], which results in the lowering of activity of alkaline phosphatase [30]. Inhibition may also be due to the formation of insoluble phosphate which further interferes with the absorption of phosphate in gastrointestinal tract. Administration of chelating agents may reduce beryllium ion concentration in liver and kidney, whereas concomitant administration of α-tocopherol inhibited oxidative phenomenon during toxicity, which helped in reversing aforesaid parameters towards normal.

Bulk of the circulating beryllium binds with plasma globulin because of its apparent protein binding property and forms stable beryllium protein complex. In this way, a large amount of beryllium gets transported to various organs and causes damage [27,23]. Thus, impaired functioning of liver declined protein synthesis and so its level in tissues. Hypoalbuminemia may result from increased catabolism during beryllium toxicity[31]. Lipid peroxidation is regarded as one of the basic mechanism of tissue damage caused by free radicals [32]. In the present study, enhanced level of TBARS in liver and kidney was strongly reduced by co-treatment of tiron and α-tocopherol rather than $CaNa_3DTPA$ or its combination. Since, tiron is SOD mimetic, it may have dual effects; one is quenching the adverse manifestation of beryllium ions through chelation[33] and other is scavenging superoxide anions[34], whereas α-tocopherol is able to quench the LPO chain and protect membrane from attack of free radicals. The GSH, a non-protein thiol is involved in many cellular processes, including detoxification of endogenous and exogenous compound[35].

In the present studies decreased glutathione level after beryllium administration may be due to its increased utilization by hepatocytes [36,37] because GSH acts as a scavenger for toxic chemical agents[38] besides as natural antioxidant. Combined administration of chelating agents and α-tocopherol counteracted free radical mediated cell injury during severe hepatorenal damage conditions and have elevated GSH level, which in turn helped in mitigating tissue damage. It can be hypothesized that chelators may reduce beryllium body burden and simultaneously exogenous antioxidant α-tocopherol reduced oxidative stress leading to fast recovery in damaged tissues. In the present study $CaNa_3DTPA$ showed moderate therapeutic potential, whereas α-tocopherol

enhanced its effectiveness to some extent. Tiron in combination with α-tocopherol exerted more beneficial effects over a combination of CaNa$_3$ and α-tocopherol DTPA treatment against beryllium induced biochemical, histopathological and ultrastructural alterations suggesting that combination therapy of tiron and α-tocopherol could be preferred as a better choice in treatment of beryllium induced toxicity. However, chelator especially tiron should be further investigated at relatively lower doses as potent therapeutic agent against beryllium intoxication.

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