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Beryllium-induced Haematological Alterations and their Response to Liv.52

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ABSTRACT

The effect of intravenous injection of beryllium nitrate to adult female Albino rats primed with Liv.52 and to non-primed rats has been studied on various hematological parameters after 2, 10 and 30 days of such injection. There was a significant decrease in RBC count, Hb%, neutrophils, blood sugar, alkaline phosphatase, globulin and total protein values with the administration of beryllium nitrate per se, whereas the levels of acid phosphatase, cholesterol, total leucocytes and lymphocytes showed increased values. All the altered values, except in the case of alkaline phosphatase, tended to be normal after treatment with the Ayurvedic remedy, Liv.52. The study is extensively documented.

INTRODUCTION

Beryllium salts are widely used in industry and possess disease-producing potential in human beings wherever in correct industrial practices prevail[1,2]. Aerospace research with beryllium and beryllium hydride propellants has been a potential source of toxic exposure[3,4]. Beryllium intoxication causes acute chemical pneumonitis[2], chronic pneumonitis with granulomata[4], pulmonary tumours[5], bone sarcoma and rickets[6]. Aldridge and his co-workers have reported that the lowering of blood sugar and liver damage may be a cause of beryllium poisoning. In metal toxicity, various hematological parameters serve as sensitive indexes in clinical diagnosis thus enabling delineation of the disease at a pre-clinical or sub-clinical stage. Till now no specific alteration in blood biochemistry has been reported for chronic beryllium disease. Viewing beryllium's toxic potential, it becomes important to delineate the causes and treatment for its toxic effects. Furthermore, although a large number of compounds like aurintricarboxylic acid (ATA), salicylic acid and many others have been tested against beryllium toxicity[8,9], none of them has met with success. Hence, research to discover an effective therapeutic drug is still required. The present study reveals the haemotoxic potential of beryllium nitrate when injected intravenously. Concomitantly, an attempt has been made to cure Beryllium's over manifestations with an Ayurvedic drug, Liv.52, which has been reported to be effective against toxic chemicals[10,11].

MATERIALS AND METHODS

Adult, healthy, pathogen-free female albino rats (weighing 150 ± 10 g) of Swiss strain were selected from the Defence Research Laboratory, Gwalior. All the animals were maintained under uniform husbandry conditions and were given "Hindustan Lever" rat pelleted diet and water *ad libitum*. All the animals were weighed daily for 20 days to record their normal growth rate.

Beryllium nitrate was dissolved at a concentration of 0.316 mg/ml in pyrogen free distilled water and was injected intravenously. Liv.52 was procured from The Himalaya Drug Company, Bombay. It contains the extracts of a few beneficial plants viz. *Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium* and *Tamarix gallica*.

Animals showing normal growth rates were selected and were divided into four groups of fifteen each. The animals of groups 1 and 3 were kept as such and received the vehicle only. But rats of groups 2 and 4 were primed with Liv.52 for 10 days (1 ml/rat/day orally) prior to the experiment. Animals of group 1 served as control and continued to receive vehicle only. Each rat of groups 3 and 4 was injected with beryllium nitrate intravenously at a dose of 0.316 mg/kg body wt. once only ($1/10^{th}$ of LD_{50} as described earlier). Rats of groups 2 and 4 additionally received Liv.52 syrup daily till the last day of the experiment. All the rats were sacrificed after 2, 10 and 30 days of beryllium administration. At autopsy the cardiac blood was collected for the estimation of blood serum parameters. Heparinized blood was used for quantifying blood sugars. Simultaneously, serum samples were processed for acid and alkaline phosphatases, total and esterified cholesterol, total albumin and globulin proteins. For other haematological tests non-heparinized blood was used. Haemoglobin (%) was estimated using Sahli's apparatus. WBC and RBC counts were enumerated by using Neubaur's chamber. DLC was carried out in blood films stained with Leishman's stain. Results were analyzed using student's 't' test.

RESULTS

Our results revealed that the administration of beryllium nitrate intravenously did not alter the body weights of the animals significantly; however, it evoked severe alterations in blood/serum biochemical parameters (Table 1). Furthermore, these changes were restored to normal values with Liv.52 treatment. Serum acid phosphatase activity and cholesterol levels increased significantly after 2, 10 and 30 days of beryllium nitrate administration. With the supplementary treatment of Liv.52 syrup, elevations in the enzymatic activity of acid phosphatase and cholesterol levels were prevented and the values remained quite low even at the shortest duration. Alkaline phosphatase activity and blood sugar content depicted statistically significant, decreased values at various duration after beryllium administration. The extent of reduction was maximal after 2 days followed by further decreased values. However, with the conjoint treatment with Liv.52 in beryllium exposed rats (group IV) these values remained significantly high when compared with the beryllium nitrate *per se* group (III) at all the consecutive schedules. Serum total and globulin protein contents showed reduced values at longer duration, whereas serum albumin content showed no change with beryllium nitrate *per se*. After Liv.52 and beryllium treatment (group IV) the values of all the parameters recouped to normal, except serum alkaline phosphatase activity.

In Table 2 total erythrocyte counts and hemoglobin percentages reveal significant decreased values at early duration with beryllium nitrate *per se* treatment. However, these values recouped to normal after 30 days of exposure. Total leucocyte and lymphocyte counts were increased with beryllium nitrate treatment at all the duration. The percentages of neutrophils and granulocytes decreased with beryllium nitrate *per se* (group III). However, with conjoint treatment of Liv.52 and beryllium nitrate (group IV) erythrocyte counts, hemoglobin percentages, and differential leucocyte counts showed significant improvement when compared with the respective beryllium nitrate *per se* group. The altered blood morphological picture associated with beryllium toxicity revealed marked appearance of crenated erythrocytic cells and pyknotic nuclei. Generally erythrocytes were of microcytic nature with mild to moderate hypochromia, moderate poikilocytes and some sickle-shaped cells. Haemoglobin distribution was disturbed in some ells. An overwhelming preponderance of mature lymphocytes has been observed in differential leucocyte counts.

Lymphocytic cells showed scanty cytoplasm. However, the altered blood picture recouped towards normal with Liv.52 treatment.

Table 1: Effect of beryllium nitrate on blood serum biochemical constituents in adult albino rats primed with Liv.52 syrup (values are mean ± SE. Six rats were used in each set)

Biochemical parameter	Duration after exposure (days)	Group I Control (Vehicle only)	Group II Liv.52 *per se**	Group III $Be(NO_3)_2$ *per se***	Group IV $Be(NO_3)_2$ to Liv.52 primed rats***
Acid phosphatase (units/100 ml)	2	1.21 ± 0.09	1.45 ± 0.01	3.28 ± 0.12[a]	1.54 ± 0.02[a]
	10	1.32 ±0.08	1.48 ± 0.02	3.32 ±0.18[a]	1.47 ± 0.01[a]
	30	1.22 ± 0.07	1.50 ±0.03	2.81 ± 0.15[a]	1.43 ±0.01[a]
Alkaline phosphatase (units/100 ml)	2	48.29 ± 1.85	47.21 ± 1.93	12.79 ±0.28[a]	31.21 ± 1.43[a]
	10	45.29 ± 3.33	48.45 ± 2.36	20.29 ± 0.42[a]	35.30 ± 1.82[a]
	30	48.32 ±2.09	50.01 ± 2.26	30.28 ± 0.09[a]	41.29 ±1.58[b]
Blood sugar (ml/100 ml)	2	80.76 ± 1.90	81.40 ± 1.58	52.08 ± 1.38[a]	71.92 ± 1.63[b]
	10	82.41 ± 2.29	82.67 ± 2.64	70.48 ± 2.60[b]	81.92 ± 2.63[b]
	30	80.96 ± 3.34	83.06 ± 2.85	80.65 ± 2.26	82.18 ± 2.64
Total cholesterol (mg/100 ml)	2	54.44 ± 1.88	52.70 ±2.19	103.50 ± 2.07[a]	60.96 ± 2.36[a]
	10	52.52 ± 1.22	51.40 ± 2.35	99.30 ± 2.17[a]	59.09 ± 3.45[a]
	30	50.47 ± 2.80	49.51 ± 2.75	63.01 ± 2.92[b]	52.47 ± 2.70[b]
Esterified cholesterol (mg/100 ml)	2	25.49 ± 0.88	25.70 ± 1.13	61.42 ± 0.50[a]	30.62 ± 1.45[a]
	10	25.69 ± 0.97	24.69 ± 1.15	55.74 ± 0.89[a]	27.91 ± 1.71[a]
	30	24.79 ± 0.98	25.89 ± 1.72	44.19 ±0.98[a]	24.81 ± 1.57[a]
Total serum proteins (g/100 ml)	2	7.94 ± 0.12	8.00 ± 0.21	7.96 ± 0.11	8.12 ± 0.24
	10	7.72 ± 0.21	8.32 ± 0.31	5.83 ± 0.43[b]	8.25 ± 0.21[a]
	30	7.92 ± 0.49	8.42 ± 0.22	5.65 ± 0.11[b]	8.322 ± 0.22[a]
Albumin	2	1.00 ± 0.08	1.50 ± 0.24	1.03 ± 0.12	1.23 ± 0.25
	10	1.12 ± 0.09	1.78 ± 0.25	1.08 ± 0.08	1.55 ± 0.12
	30	1.12 ± 0.08	1.92 ± 0.21	1.71 ±0.03	1.75 ± 0.12
Globulin	2	5.54 ± 0.92	5.62 ± 0.26	4.96 ±0.11	5.05 ± 0.02
	10	6.55 ± 0.11	5.65 ± 0.24	4.58 ± 0.18[b]	5.28 ± 0.03[b]
	30	5.94 ± 0.13	6.61 ± 0.21	3.83 ± 0.14[b]	5.55 ± 0.04[b]

*p value versus group I >0.05; ** p values versus group I [a]<0.001; [b]<0.005;
***p values versus group III [a]<0.001; [b]<0.005.
The results were analysed by using student's 't' test.

DISCUSSION

Organic and inorganic toxins present a wealth of experimental data on serum acid and alkaline phosphatase anomalies associated with their toxic accumulation[18]. A rise in the activity of serum acid phosphatase due to beryllium poisoning was observed after 2, 10 and 30 days exposure Witschi and Aldridge[19] showed a direct correlation between the rise in acid phosphatase activity and amount of beryllium present in liver. The rise in the serum enzymatic activity and amount of beryllium present in liver. The rise in the serum enzymatic activity depends upon the lysosomal disruption in various tissues[20]. Vacher *et al*.[21] reported a rise in serum β-glucuronidase and transaminase activity after beryllium exposure due to phagocytosis and necrosis. Other factors involve a rise in serum enzymatic activity in hepatotoxicity depending upon alterations in the cell membrane properties,

permitting a rapid leaching of enzymes[22]. With Liv.52 therapy the enzyme level returns to normal as it maintains the lysosomal integrity in tissue[23]. It prevents the rupturing of lysosomes and further release of enzymes in the blood. The decrease in activity of serum alkaline phosphatase encountered during beryllium toxicity is attributed to the displacement of Mg ++ ions intrinsic to this enzyme[24,25]. Pertinent literature reveals that Liv.52 treatment improves serum alkaline phosphatase in various liver disorders[26]. The exact mechanism of action of Liv.52 in beryllium toxicity is yet to be elucidated.

Table 2: Effect of beryllium nitrate on blood cellular components in adult albino rats primed with Liv.52 syrup (values are mean ± SE. Six rats were used in each set)

Haematological parameter		Duration after exposure (days)	Group I Control (Vehicle only)	Group II Liv.52 *per se**	Group III Be $(NO_3)_2$ *per se***	Group IV Be $(NO_3)_2$ to Liv.52 primed rats***
Haemoglobin (g/100 ml)		2	13.92 ± 0.98	13.35 ± 0.16	8.24 ± 0.24[a]	13.84 ± 0.95[a]
		10	13.09 ± 0.92	13.74 ± 1.90	10.25 ± 0.30[b]	13.29 ± 0.88[b]
		30	13.88 ± 0.88	13.48 ± 0.90	13.14 ± 0.21	13.37 ± 0.58
RBC (million/cu mm)		2	8.84 ± 0.02	9.04 ±0.08	4.25 ± 0.48[a]	8.59 ±0.24[a]
		10	9.30 ± 0.21	9.36 ± 0.25	5.50 ± 0.75[a]	8.80 ± 0.14[a]
		30	9.38 ± 0.25	9.34 ± 0.28	8.08 ± 0.85	9.31 ± 0.21
WBC (thousand/cu mm)		2	5.53 ± 0.49	5.45 ± 0.44	10.87 ± 0.35[a]	5.31 ± 0.22[a]
		10	5.60 ± 0.52	5.42 ± 0.42	9.92 ±0.45[a]	5.49 ±0.34[a]
		30	5.50 ± 0.56	5.08 ± 0.53	8.92 ± 0.55	5.62 ± 0.25
Differential counts	Neutrophil (%)	2	25.01 ± 2.51	25.05 ± 2.32	9.05 ± 0.02[a]	28.25 ± 2.52[a]
		10	27.04 ± 3.32	24.02 ± 1.89	8.02 ± 0.01[a]	25.78 ± 2.51[a]
		30	28.25 ± 1.90	22.00 ± 2.32	27.01 ± 2.35	26.55 ± 1.40
	Lymphocyte (%)	2	68.75 ± 3.25	65.95 ± 4.23	88.02 ± 5.21[a]	67.55 ± 1.42[a]
		10	68.02 ± 4.24	66.98 ± 4.55	90.18 ± 5.29[a]	70.22 ± 2.72[a]
		30	67.25 ± 5.26	68.02 ± 4.29	67.90 ± 5.22	71.34 ± 3.71
	Eosinophil (%)	2	3.02 ± 0.04	2.02 ± 0.08	1.50 ± 0.01[a]	2.75 ± 0.04[a]
		10	2.98 ±0.25	2.05 ± 0.03	1.00 ± 0.02[a]	2.00 ± 0.03[a]
		30	2.50 ±0.35	2.98 ± 0.04	2.00 ± 0.03	2.34 ± 0.06
	Monocyte (%)	2	2.21 ± 0.34	1.98 ± 0.24	1.48 ± 0.03[a]	1.45 ± 0.01[a]
		10	2.00 ± 0.28	1.95 ± 0.35	0.00 ± 0.04[a]	2.01 ± 0.02[a]
		30	2.00 ± 0.45	2.02 ± 0.34	1.04 ± 0.02	0.97 ± 0.01
	Basophil (%)	2	1.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
		10	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.60 ± 0.00
		30	0.00 ± 0.00	1.02 ± 0.00	2.00 ± 0.00	0.21 ± 0.00

*p value versus group I >0.05; ** p values versus group I [a]<0.001; [b]<0.005;
***p values versus group III [a]<0.001; [b]<0.005.
The results were analysed by using student's 't' test.

A rise in serum cholesterol level is a frequent finding established with human poisoning and with prolonged occupational exposure without showing any overt signs of atherosclerosis[27]. Consistently with the *i.v.* administration of beryllium nitrate (present data), the total and esterified cholesterol level has elevated substantially. The hypercholesterolaemia is regarded to be due to an increase in cholesterol biosynthesis probably induced by stimulation of hepatic endoplasmic reticular

enzymes[27]. Although the authors have not measured cholesterogenesis in beryllium-exposed and Liv.52 treated rats, it may be assumed that a disturbance in liver function causes alteration in cholesterol metabolism. The indigenous remedy Liv.52 acts as a liver tonic and helps in regeneration of hepatic cells. It prevents fatty changes in the liver[28], and reduces raised levels of cholesterol, lipoproteins and phospholipids in ischaemic heart disease and hypercholesterolaemia[29]. The beneficial effect of Liv.52 on lipid metabolism in beryllium poisoning may be due to its hepatoprotective nature.

Substantial decreases in the levels of blood sugar have been observed after 2 and 10 days of beryllium administration. Hypoglycemia from toxic dosage of beyllium salts has been reported, due to the reduced glycogenesis through in activation of phosphoglucomutase[30], hexokinase[31] and many other key enzymes involved in carbohydrate metabolism[32]. As liver damage is implicated as a factor of importance in beryllium poisoning, delayed removal of exogenous glucose due to liver insufficiency is the way by which toxic doses of beryllium affect blood glucose levels causing a concomitant rise of blood lactic acid[7]. It is interesting to note that the administration of Liv.52 to beryllium-exposed animals maintains the blood sugar levels at high concentration and thus prevents the initiation of toxic action of beryllium. Although the exact mode of the protective action of Liv.52 against beryllium toxicity is not known it can be safely said that Liv.52 accelerates the cellular metabolic activity as it is known to correct liver dysfunction against hepatotoxins[10,11].

Owing to protein-binding properties beryllium binds with the blood $\propto$-globulin proteins and is distributed to various organs[33]. A large amount of beryllium is accumulated in the liver and causes its damage. Most of the proteins originate in the liver where hepatotoxic conditions induce diminished synthetic ability, with a consequently reduced supply of proteins. Synergistically, serum total protein and globulin contents showed reduced values at various durations in beryllium-exposed animals (Table 1). However, Liv.52 has been impressive in the hypoproteinaemia state and the time required for initial improvement was decisively shorter[34]. Similarly, Liv.52 restored the protein contents to normal in beryllium-exposed animals.

Present findings on blood cellular study reveal the occurrence of hypochromic microcytic anaemia and chronic lymphocytic leukaemia due to beryllium's toxic action. Stokinger *et al.,*[35] had reported that there was not much significant difference in ^{14}C activity ratios of globin to haemin in beryllium-poisoned and normal rabbits. This further envisaged that the biosynthesis of haemin or globin was not checked in beryllium toxicity. The other possible explanation for deficient intraerythrocytic haemoglobin percentages and erythrocytes may be due to an abundance of immature red blood cells. Leucocytosis inflicted by beryllium administration may be due to the stimulation of leucocytic cells of the bone marrow resulting in an increased number of lymphocytes. On the other hand, the blood cellular morphology and enumeration was quite normal in the Liv.52 treated rats. Although the erythropoietic action of Liv.52 is well established in some radiation experiments[36], studies are in progress to ascertain the exact mechanism of action of Liv.52 in beryllium toxicity.

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