Original Article

Experimental Study of Antimony Induced Hepato Toxicity in Rabbits

Antimony Induced Hepato Toxicity

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ABSTRACT

Objectives: To demonstrate the effects of antimony on hepatic tissues of Rabbits. To correlate the severity of tissue damage to the dose of antimony and to an immunologically altered state of the animal.
Study Design: Experimental study.
Place and Duration of Study: This study was carried out at PGMI and KEMU, Lahore from January 1988 to March 1988.
Materials and Methods: This study was carried out on 40 healthy rabbits weighing 1.5kg divided into 4 groups each group having 10 animals with one control group. Group I animals were injected with antimony sodium tartrate of ½ MLD 6mg/kg body weight I/V at interval of 2 days for 12 weeks, whereas experimental dose of 1.71mg/kg body weight was injected I/V at interval of 4 days to group II animals. Those of group III were injected 2ml of specific bovine albumin 30%(DADEUSA) followed by schedule of group II animals. Group IV (control group) animals were injected I/V with distilled water.
Results: Hepatic Enzymes, Serum Alanine Aminotransferase and Serum Gamma Glutamyl transpeptidasc (GT) levels were estimated at the end of six weeks and twelve weeks. These were found to be raised gradually from 7th week onwards until the experiment was terminated.
Conclusion: It is concluded from this study that antimony sodium tartrate has toxic effects on liver tissue and can cause hepatocellular damage (if given for prolong period)
Key Words: Antimony, Hepato Toxicity, Hepatic enzymes, S-ALAT, γ GT, H&E, PAS, MSS.

INTRODUCTION

Antimony and its compounds were used medicinally as early as 4000 B.C. Antimony is used in Alloys, lead, tin, and copper. Its compounds are used in textile industry, flame proofing, dies, paints, rubber compounding, ceramic and glass opicifiers [1]. Human beings have always been exposed to antimony but its amount is substantially increased due to industrial production [2]. As regards the therapeutic applications in the 19th century, potassium antimony Tartrate (Tartar emetic) began to be used for the treatment of shistosomiasis and leishmaniasis [3]. The heavy metals have their toxic effects on liver kidneys central nervous system, skin muscles, bone etc. Keeping in mind a wide industrial use of the metals, we have opted to study the effects of antimony on liver tissue.

MATERIALS AND METHODS

This study was carried out on 40 healthy rabbits weighing 1.5kg divided into 4 groups each group having 10 animals with one control group.
Group I animals were injected with antimony sodium tartrate of ½ MLD (minimum lethal dose) 6mg/kg body weight I/V at interval of 2 days for 12 weeks.
Group II where as experimental dose of 1.71mg/kg body weight was injected I/V at interval of 4 days to group II animals.
Group III were injected 2ml of specific bovine albumin 30%(DADEUSA) followed by schedule of group II animals.
Group IV (control group) animals were injected I/V with distilled water.
Blood chemistry was done to see the effects of antimony on liver functions of animals of all groups, to determine the (S-ALAT) activity sclavo diagnostic G.P.T kit was used by calorimetric method and Serum γ GT (Gamma Glutamyl transpeptidase) using kinetic colorimetric method. Blood samples were collected by using disposable syringes from one of peripheral ear veins at the end of six and twelve weeks. Five ml of blood was collected in a test tube, kept for 2 hours to clot and serum was separated after centrifuging the test tubes at 3000 RPM.
Tissue Histology of liver was done at the end of experiment after sacrificing all the animals. Liver tissues were preserved in 10% formalin saline solution, to study the different morphological lesions.
Following staining procedures were carried out:-

1. Haemotoxylin and eosin staining (H&E)
2. Periodic Acid Schiff staining (PAS)
3. Methenamine sliver staining (MSS)
4. Reticulin staining (RS)

RESULTS

Laboratory Results: Liver Chemistry:-. Serum Alanine Aminotranferase (S-ALAT) levels were estimated in group I using the colorimetric method (Reithman-Frankel, 1959, 1970) (modified) at the end of six weeks, and their mean value was found to be 12.3±4.1 IU/L. Their levels rose gradually from 7th week onwards until the experiment was terminated and

their mean value was found to be 25.5±9.09IU/L at the end of experiment (Table 1).

Serum Gamma Glutamyl Transpeptidase (ɣ GT IU/L) levels were estimated using the Kinetic Colorimetric method. The mean value of the ɣ GT was 4.6 ± 1.91 IU/L at the end of six weeks which rose gradually from 7th week onwards until the experiment was terminated and their mean value was found to be 9.3 ± 4.22 IU/L at the end of 12th week (Table 2).

In animal group II and group III, there was gradual increase seen in the levels of S-ALAT and ɣ GT (Gamma Glutamyl Transpeptidasc) after six weeks till the end of experiment (Tables 3, 4, 5, 6).

Table No.1: Serum Alanine Aminotransferase (S-Alat) IU/L after IV Dose of Antimony Sodium Tartrate I in Ten Rabibts of Group I. Duration 12 weeks.

Animal number	Weight of Rabbit K(kg)	End of 6th week IU/L	End of 12th week IU/L
1.	1.5	18	40
2	1.5	13	24
3	1.3	12	20
4	1.5	16	38
5	1.5	7	15
6	1.5	4	10
7	1.5	11	20
8	1.0	15	30
9	1.5	11	28
10	1.5	16	30
Mean X		12.3	25.5
Standard deviation (S.D)		4.1	9.09

Table No.2: Serum Gamma Glutamyl Transpeptidase (ɣ-Gt) IU/L after IV Dose of Antimonmy Sodium Tartrate in Ten Rabbits of Group 1. Duration 12 weeks.

Animal Number	Weight of Rabbit (kg)	End of 6th Week IU/L	End of 12 week. IU/L
1.	1.5	4	7
2.	1.5	7	15
3	1.3	1	2
4	1.5	2	4
5	1.5	4	10
6.	1.5	7	15
7.	1.5	6	12
8.	1.5	5	10
9.	1.5	6	12
10.	1.5	4	6.
Mean X		4.6	9.3
Standard Deviation (S.D)		1.91	4.22

In group IV (Control group) animals, S-ALAT and ɣ GT levels were estimated. The S-ALAT was 20.0, ± 6.32 IU/L and ɣ -GT was -3.16 ± 0.90 at the start, which remained the same at the end of the experiment. (Table 7 & 8)

Histological and Microscopic Study:

Group I

Gross Examination: Livers of all the animals appeared normal in size, shape and color, except two animals in which surface of liver shows slight nodularity. They were triangular in shape and were covered with thin capsule.

Microscopic Examination: Eight animals revealed normal Lobular architecture. Three animals showed mild dilatation of central veins and sinusoids but no significant congestion observed.

Microscopic examination of livers revealed severe degree of lymphocytes infiltration around portal tracts which were widened . Single cell necrosis along with focal fatty change.

A moderate amount of fibrosis around the portal tracts extending to the adjacent portal tracts.

Group II

Gross Examination: Most of the animals revealed congested spots diffusely scattered on surface of liver.

Microscopic Examination: Liver showed mild to moderate degree of congestion of central veins and sinusoids.

Mild to moderate degree of lymphocytic infiltration, singe cell necrosis seen in six animals. Few animals revealed fibrosis around the portal tracts..

Group III

Gross Examination: Livers were rectangular in shape covered with thin capsule.

Microscopic Examination: Revealed maintained lobular architecture with mild dilatation, congestion of central vein and sinusoids. Moderate degree of focal lymphocytic infiltration in portal tracts in few animals . Single cell necrosis , and focal fatty changes seen in few animals..

Table No.3: Serum Alanine Aminotransferase (S-Alat) IU/L after IV Dose of Antimony Sodium Tartrate I in Ten Rabibts of Group II. Duration 12 weeks.

Animal Number	Weight of Rabbit (kg)	End of 6th Week IU/L	End of 12 week. IU/L
1.	1.5	15	30
2.	1.5	12	15
3	1.3	6	16
4	1.5	5	26
5	1.5	16	32
6.	1.5	15	30
7.	1.5	8	36
8.	1.0	10	28
9.	1.5	10	24
10.	1.0	6	15
Mean X		10.3	25.2
Standard Deviation (S.D)		3.87	7.15

Group IV (Control Group)

Gross Examination: The examination of the livers of all the animals normal in size, shape and color; covered

by glisson capsule.

Microscopic Examination: It revealed normal architecture and morphology.

Table No.4: Serum Gamma Glutamyl Transpeptidase (ɣ-Gt) IU/L after IV Dose of Antimonmy Sodium Tartrate in Ten Rabbits of Group II. Duration 12 weeks.

Animal Number	Weight of Rabbit (kg)	End of 6th Week	End of 12 week.
		IU/L	IU/L
1.	1.5	10	20
2.	1.5	7	15
3	1.3	6	15
4	1.5	8	16
5	1.5	5	12
6.	1.5	13	25
7.	1.5	16	33
8.	1.5	3	7
9.	1.5	6	10
10.	1.5	10	20
Mean X		8.4	17.3
Standard Deviation (S.D)		3.72	7.21

Table No.5: Serum Alanine Aminotransferase (S-Alat) IU/L after IV Dose of Antimony Sodium Tartrate I in Ten Rabibts of Group III. Duration 12 weeks.

Animal Number	Weight of Rabbit (kg)	End of 6th Week	End of 12 week.
		IU/L	IU/L
1.	1.5	13	30
2.	1.5	14	28
3	1.3	18	34
4	1.5	15	30
5	1.5	10	25
6.	1.5	12	30
7.	1.5	22	40
8.	1.5	17	36
9.	1.5	16	34
10.	1.5	21	40
Mean X		15.8	32.7
Standard Deviation (S.D)		3.63	4.73

Table No.6: Serum Gamma Glutamyl Transpeptidase (ɣ-Gt) IU/L after IV Dose of Antimonmy Sodium Tartrate in Ten Rabbits of Group 1II. Duration 12 weeks.

Animal Number	Weight of Rabbit (kg)	End of 6th Week	End of 12 week.
		IU/L	IU/L
1.	1.5	5	10
2.	1.5	6	12
3	1.3	2	4
4	1.5	7	15
5	1.5	3	5
6.	1.5	6	11
7.	1.5	4	10
8.	1.5	2	4
9.	1.5	3	5
10.	1.5	8	15
Mean X		4.6	9.1
Standard Deviation (S.D)		2.01	4.11

Table No.7: Serum Alanine Aminotransferase (S-Alat) IU/L after IV Dose of Antimony Sodium Tartrate I in Ten Rabibts of Group IV (Control). Duration 12 weeks.

Animal Number	Weight of Rabbit (kg)	End of 6th Week	End of 12 week.
		IU/L	IU/L
1.	1.5	15	15
2.	1.5	25	25
3	1.3	20	20
4	1.5	10	10
5	1.5	30	30
6.	1.5	10	10
7.	1.5	25	25
8.	1.5	25	25
9.	1.5	20	20
10.	1.5	20	20
Mean X		20.0	20.0
Standard Deviation (S.D)		6.32	6.32

Table No.8: Serum Gamma Glutamyl Transpeptidase (ɣ-Gt) IU/L after IV Dose of Antimonmy Sodium Tartrate in Ten Rabbits of Group 1V (Control). Duration 12 weeks.

Animal Number	Weight of Rabbit (kg)	End of 6th Week	End of 12 week.
		IU/L	IU/L
1.	1.5	2.4	2.4
2.	1.5	4.1	4.1
3	1.3	5.2	5.2
4	1.5	2.5	2.5
5	1.5	2.8	2.8
6.	1.5	3.33	3.33
7.	1.5	2.4	2.4
8.	1.5	3.1	3.1
9.	1.5	3.3	3.3
10.	1.5	2.5	2.5
Mean X		3.16	3.16
Standard Deviation (S.D)		0.90	0.90

DISCUSSION

In present study, the levels of serum-Alanine Amino transferase and serum Gamma-glutamyl transpeptidase varied in different groups. The levels were estimated at the end of 6th and 12th week. In animals of all groups, there was gradual increase in the level of SALAT and Serum ɣ -GT. (Gamma Glutamyl Transpeptidasc) at the end of 12 weeks where as in control group liver enzyme levels were remained the same as estimated at the start of experiment

The patients of schistomomiasis treated with antimonials showed elevation of SGOT and SGPT values which indicate hepatic damage [4]. Simillarly rise in hepatic enzymes observed in patients treated with antimonial suggesting hepato cellular damage [5] .

Simillarly transient rise in alanine amino transferase activity observed in a patient of cutaneous leishmaniasis treated with antimonials [6]. However hepatotoxicity is observed more typically during prolong therapy with antimonial compounds.

Parenteral treatment with antimony compounds has caused hepatic necrosis although reversible elevation of liver enzymes activities are more typical [7].

The above mentioned studies coincides with the observations made in the present study.

As regards morphological appearance of liver tissue, trivalent antimony compounds were found in abnormally high concentration in liver and thyroid [8]. Where as slight to moderate parenchymatous degeneration of liver was observed by workers [9].

In the present study, most of animals from various groups revealed a maintained lobular architecture except few animals showed disorganized architecture. Lymphocytic infiltration of moderate (++) to severe degree (+++) was common feature in most of the animals of various groups. A fatty change was observed in 30% of the animals from group I and III where as no change was seen in group II animals.

Single cell necrosis was observed in most of animals of all groups. A few animals of group I and II, revealed a moderate amount of fibrosis around the portal tracts.

Fatty degeneration of liver reported in chronically exposed guinea pigs to antimonial compounds [10]. Similar fatty degeneration was observed in another experimental study [11]. These observations coincides with the findings seen in the present study.

Centrilobular necrosis was reported in occupationally exposed workers to antimony [12] which is not observed in the present study. On the other hand a moderate amount of fibrosis around the portal tract, a morphological change that has not been demonstrated in the literature.

CONCLUSION

It is concluded that the morphological lesions in the liver appear to be dose and time dependent. As regards biochemical evidence hepatic injury, serum enzyme (S-ALAT, ɣ - GT. (Gamma Glutamyl Transpeptidasc), levels were found to be raised significantly.

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