The Effects of Lindane on Erythropoiesis in Rabbits: an Experimental Study

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Lindane is a synthetic insecticidal agent used in the control of insects of agricultural and public health importance. An experimental study was conducted to observe the effects of lindane on hemopoiesis in rabbits. Ninety rabbits were included and divided in nine groups according to dose level. Six rabbits showed suppression of erythropoiesis. This suppression of erythropoiesis was neither related to dose nor to duration of exposure to lindane.

Key words: Lindane, erythropoiesis

Lindane is the commercial name given to the pure gamma isomer of chlorinated hydrocarbon insecticide Benzene Hexachloride. Although insecticidal use of lindane began in 1942, the suppressive effect of lindane on hemopoietic system was first reported in 1953. Further studies revealed that lindane caused inhibition of development of red blood cells in the bone marrow[1]. Later on, in 1975, association between red cell aplasia and exposure to lindane was reported[2]. The time from lindane exposure to the diagnosis of anemia varied from nine days to three months in case of short term exposure and one to three years in case of long term exposure[3]. According to a report published by WHO in 1975, 79 persons exposed to lindane were studied, in which no evidence of suppression of erythropoiesis was found[4]. However, in 1989 cases of anemia due to suppression of erythropoiesis in bone marrow were reported. In all these patients there was a definite history of exposure to lindane[5]. This experimental study was undertaken in order to observe the effects of lindane on erythropoiesis in rabbits.

Materials and Methods

A total of 90 healthy domestic adult rabbits were used as experimental animals. They were divided into nine groups. Ten animals were included in each group. Group I served as normal control. The remaining eight groups were test groups at different dose levels shown in table 1.

Adjustment of highest dose:

LD-50 of lindane for different animals in oral administration is 25 - 200 mg/kg body weight[6]. In order to prevent animal death due to over dosage, highest dose was kept at 120mg/kg body weight.

Preparation of dose:

Lindane is readily absorbed from alimentary canal when dissolved in corn oil[7]. Respective dose for each group was given orally once a day.

Collection of samples. First samples were collected at the end of second week. Sampling was repeated at an interval of two weeks till the end of sixteenth week. Blood samples were collected from medial peripheral vein of rabbit ear by venepuncture. For the collection of bone marrow samples animals were anesthetised by open ether anesthesia. The skin and muscles overlying the femur were incised and upper end of femur was exposed. The femur was resected, and bone marrow taken out to prepare smears on clean glass slides. Animals were sacrificed after collection of samples.

Investigations and procedures:

Following investigations were performed in accordance with the recommended procedures:

1. Hemoglobin estimation
2. Total red blood cell count
3. Packed cell volume
4. Reticulocyte count
5. Bone marrow examination

Results

Observations in animals of test groups were compared with control group animals. In total six test animals showed suppression of erythropoiesis. In this study range of variation of hemoglobin, RBC counts, PCV and reticulocyte counts among control animals is shown in table 2.

Table 1: Dose levels of lindane for different groups of rabbits

Group	Dose Level
I	Control
II	5mg/kg Body weight
III	10mg/kg Body weight
1V	15mg/kg Body weight
V	20mg/kg Body weight
V1	30mg/kg Body weight
V11	60mg/kg Body weight
V111	90mg/kg Body weight
IX	120mg/kg Body weight

Table 3 shows HB, RBC counts, PCV and reticulocyte counts of these animals against their respective dose levels and the time of collection of samples.

Table.2 Range of variation of hemoglobin, RBC counts, PCV and Reticulocyte counts among control rabbits over the period of sixteen weeks.

Parameter	Range of variation
Hemoglobin	8.5 - 13.1 gm/dl
RBC counts	4.2 - 6.2 million/cmm
Packed Cell Volume	30 - 36 %
Reticulocyte counts	1 -3 %

Table .3 Hemoglobin, RBC count, PCV and Reticulocyte counts in rabbits showing suppression of erythropoiesis.

Week	Dose Levels (mg/kg)	Hb (gm/dl)	RBC Count (million/cmm)	PCV (%)	Retics (%)
6th	60	8.7	4.6	27	2.0
6th	90	8.0	3.7	24	0.5
6th	120	9.2	5.0	29	1.5
10th	5	8.0	3.2	26	2.0
10th	20	8.0	3.5	31	2.0
14th	20	8.0	4.1	27	1.0

1. Hemoglobin:
   Table 4 shows the effects of lindane on hemoglobin at various dose levels over the period of 16 weeks. It is evident from the table that mean difference in hemoglobin values, between the groups (different dose levels) and between the time was insignificant.

Table.4 Analysis variance table showing effects of lindane at various doses over the period of sixteen weeks on rabbit hemoglobin

Source of Variation	Sum of Squares	D.F	Variance	P. Value
Between the groups	16.021	8	2.00	2.06 (NS)
Between the time	13.49	7	1.93	1.98 (NS)
Interaction (Time x dose)	54.35	56	0.97	

D.F= Degree of Freedom, NS= Not significant

Table 5 Analysis variance table showing effects of lindane at various doses over the period of sixteen weeks on rabbit red blood cell count.

Source of Variation	Sum of Squares	DF	Variance	P. Value
Between the groups	3.312	8	0.414	1.605** (NS)
Between the time	4.842	7	0.691	2.682 (P< 0.05)
Interaction (Time x dose)	14.439	56	0.257	-

D.F= Degree of Freedom, NS= Not significant

2. RBC Count:
   The effects of lindane on RBC count at various dose levels over the period of sixteen weeks is shown in table 5. Mean difference in RBC counts, between the time was significant ($P < 0.05$). However mean difference in RBC counts between the groups was insignificant.
3. Packed Cell Volume:
   The effects of lindane on PCV is shown in table 6. Mean difference in values of PCV between the groups (different dose levels) and between the time was insignificant.

Table. 6 Analysis variance table showing effects of lindane at various doses over the period of sixteen weeks on rabbit packed cell volume.

Source of Variation	Sum of Squares	DF	Variance	P. Value
Between the groups	97.109	8	12.138	2.011** (NS)
Between the time	73.875	7	10.553	1.748** (NS)
Interaction (Time x dose)	338	56	6.035	

* D.F = Degree of Freedom, ** NS = Not significant

4. Reticulocyte Count:
   Table 7 shows the effects of lindane on reticulocyte count at various dose levels over the period of sixteen weeks. It is evident form the table that mean difference in reticulocyte counts between the groups (various dose levels) was significant ($P< 0.05$). Mean difference in reticulocyte counts, between the time was also significant ($P < 0.05$).

Table .7 Analysis variance table showing effects of lindane at various doses over the period of sixteen weeks on rabbit reticulocyte count.

Source of Variation	Sum of Squares	DF	Variance	P. Value
Between the groups	21.625	8	2.703	2.319(P< 0.05)
Between the time	37.579	7	5.368	4.606(< 0.05)
Interaction (Time x dose)	65.263	56	1.165	

* D.F= Degree of Freedom

5. Bone Marrow Smear Examination:
   Six out of 90 test animals showed hypoplastic erythropoiesis on bone marrow smear examination. Table 6 shows respective dose levels of these animals and time of sampling alongwith their Hemoglobin levels, RBC counts, PCV and Reticulocyte counts.

Discussion

This study was carried out on 90 healthy rabbits which were divided into nine groups. Each group included ten rabbits. Animals from group I served as normal control. Remaining acted as test groups to see the effects of lindane on rabbit erythropoiesis.

Many workers found low hemoglobin values in a number of cases with a history of repeated exposure to lindane[3,8,9]. In our study low hemoglobin values were observed in four test animals. Test animals at the dose of 90mg/kg body weight showed a hemoglobin value of 8.0 gm/dl at the end of sixth seek of lindane administration. It was below normal range and it showed a decrease of 10.11 percent from the hemoglobin value of control animal. Similarly each of the test animals at the dose levels of 5 mg/kg body weight and 20 mg/kg body weight showed hemoglobin value of 8.0 gm/dl at the end of tenth week of lindane administration. This hemoglobin value was below normal range. It showed a decrease of 9.09 percent from the control animal. Another animal at the dose level of 20 mg/kg body weight showed hemoglobin

value of 8.0 gm/dl at the end of fourteenth week of lindane administration. This animal also showed a decrease of 9.09 percent from the control animal.

Vodopick and Norton described low RBC counts in some patients with a history of exposure to lindane[2,10]. In this experimental study these test animals showed low red cell counts. At the end of sixth week of lindane administration, RBC count of test animal at the dose of 90mg/kg body weight was 3.7 million/cmm. It was below normal limits showing a decrease of 22.91% from the control animal. Similarly at the end of tenth week of lindane administration test animals at dose levels of 5mg/kg body weight and 20mg/kg body weight showed RBC counts of 3.2 million/cmm and 3.5 million/cmm respectively. Test animal at the dose level of 5mg/kg body weight showed a decrease of 33.33% and the animal at the dose level of 20mg/kg body weight showed a decrease of 27.08% from the control animal.

Low packed cell volume was observed by Vodopick and Loge, in a number of patients with a history of repeated exposure to lindane[2,11]. In our study low values of packed cell volume were observed in four test animals. At the end of sixth week animals at the dose levels of 60 mg/kg body weight and 90 mg/kg body weight showed packed cell volumes of 27% and 24% respectively. These animals showed a decrease of 10% and 20% respectively, from control animals. Similarly test animal at the dose level of 5 mg/kg body weight showed packed cell volume of 26% at the end of the tenth week. This was below normal range showing a decrease of 16.12% from the control animal. Another animal at the dose level of 20 mg/kg showed packed cell volume of 27% at the end of fourteenth week. It showed a decrease of 12.90% from the control animal.

Low reticulocyte counts were described in many cases with a history of exposure to lindane[8]. In this experimental study only one test animal receiving the dose of 90 mg/kg body weight showed low reticulocyte count of 0.5 percent at the end of sixth week. It showed a decrease of 83.33% from the control animal.

Many workers have reported hypoplastic erythropoiesis on bone marrow examination in cases with history of exposure to lindane[5,12,13,14]. In our study six test animals showed hypoplastic erythropoiesis. Hypoplastic erythropoiesis was observed at the end of 6th week in case of animals at dose levels of 60 mg/kg, 90 mg/kg and 120 mg/kg while the animals at the dose levels of 5mg/kg and 20mg/kg showed hypoplastic erythropoiesis at the end of tenth week. Another animal receiving a dose of 20 mg/kg showed suppression of erythropoiesis at the end of 14th week.

Conclusions

As a result of this experimental study it can be concluded that lindane caused suppression of erythropoiesis in test animals. This was related to individual susceptibility of the rabbits. No relationship was found either with dose or duration of exposure. No morphological change was observed in either mature or developing red cells.

Experimental studies at best can provide only an indication of the type of damage which may be expected. Intensive studies and reporting of human cases should be undertaken whenever circumstances permit. It is therefore suggested that indiscriminate use of lindane be prevented. Proper preventive measure should be taken by those exposed to lindane and other insecticides either at home or in agricultural practice. The continuous extravagant promotion of certain types of insecticides is also of great importance to those responsible for protection of public health.

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