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THE EFFECTS OF IMIDAZOILE ON BLOOD, REPRODUCTIVE AND HISTOPATHOLOGICAL CHANGES IN ALBINO MICE

ZAINAB A. SHIHAB*
ISRA'A MUHSEIN ESSA MUHSEIN**
SUZAN, A.A.A. AL-AZIZ**
SALEH, K. MAJEED***

*Dept. of Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, Basrah University, Iraq
**Dept. of Microbiology, College of Veterinary Medicine, Basrah University, Iraq
***Dept. of Pathology and Poultry Pathology, College of Veterinary Medicine, Basrah University, Iraq

ABSTRACT

The present study showed the experiment of toxicologic pathology of imidazole which was associated with reproductive toxic effects with dead or absorbed fetuses, also, histochemical and enzymatic effects on blood picture. The enzymatic effects reflecting damage to the liver, also, the study showed liver and kidney as the target toxic organs and indirect haemopoietic effects resulting in extramedullary effect in the spleen.

INTRODUCTION

Imidazole is a colorless yellow solid with an amine-like odour. It has a water solubility and a heterocyclic compound containing two nitrogen with pKa = 7.0 and pKa=14.9. The melting point ranges from 88.3 to 89.9 ^{0}C, the boiling point is at 267.8 ^{0}C at 1013.3 hPa and the vapour pressure is 0.00327 hPa at 25^{0}C (Basf, 1997).

Imidazole is readily absorbed and excreted in humans and in test animals after oral and rectal administration. Peak plasma levels are reached within 15 to 30 minutes in rats and within approx. Three hours in humans (Basf, 2002). Induction of microsomal P450 enzyme in the liver cells of rats and rabbits is restricted to certain isoenzymes such as 7-ethoxycoumarin-Odeethylase and isoenzyme 3a (Ullmann's, 2000). Imidazole is of moderate oral toxicity in a scientifically valid study. LD50 in rats was determined to be 960-970 mg/kg body weight. 80% also, is corrosive to skin under occlusive conditions and irritating to rabbit eye when tested according to OECD TG 405 (Basf, 2002).

Liver and kidney are target organs in subacute and subchronic (OECD TG 408) rat studies at dose levels of 180 mg/kg body weight per day and above (ECETOC, 1998). Slight centrilobular liver cell hypertrophy and relative liver weight increase was noted. Diffuse microglobulin accumulation was noted in the proximal tubules of the renal cortex only in male rats but was considered to a species-specific effect (TRGS, 2001). Red blood cells were additionally affected in 28-d experiments and female rats receiving 125 mg/kg body weight

per day or more and male rats receiving 500 mg/kg body weight per day were affected (Basf,1987).

Imidazole was not mutagenic in bacterial test systems generally meeting OECD TG 471 with the Salmonella typhimurium strains TA 98, TA 100, TA 1535, or TA 1537, with or without the presence of metabolic activation by S-9 mix containing rat liver microsomes, with or without preincubation. Also, did not induce Unscheduled DNA Synthesis in rat primary hepatocytes in a study equivalent to the OCED TG 482. It was not clastogenic in the mouse micronucleus test according to the OECD TG 474 when imidazole hydrochloride was tested in vivo. The salt dissociates into protonated imidazole and chloride in the stomach following oral gavage (Basf, 1992).

The aim of this study was to investigate the pathological and physiological effects of imidazole on labrotary mice at Basrah city/ southern Iraq.

MATERIALS AND METHODS:

- Experimental Design:

The laboratory animals which used in experiment was albino mice and divided into two parts: The first divided into three groups:

1- The first treated group: this group consists of eight mice which were treated via stomach tube with 0.75 mg/ kg. of imidazole per day for 15 days as intermediate dose.

2- The second treated group: this group consists of eight mice which were treated via stomach tube with 1.5 mg/ kg. of imidazole per day for 15 days as high dose.

3- The third control: this group consists of eight mice which were treated via stomach tube with 0.9% of normal saline per day for 15 days as untreated control.

The second part of experiment was related with the reproductive parameters and it was done after the first part, it was consisted of 12 male and 24 female mice and allowed to mate then separated from each other as follows:

1- The first treated group: this group consist of eight female mice which were treated via stomach tube with 0.75 mg/ kg. of imidazole for 12 days from 10-21 day of pregnancy and then let to deliver.

2- The second treated group: this group consists of eight mice which were treated via stomach tube with 1.5 mg/ kg. of imidazole for 12 days from 10-21 day of pregnancy and then let to deliver.

3- The third control: this group consists of eight mice which were treated via stomach tube with 0.9% of normal saline for 12 days from 10-21 day of pregnancy and then let to deliver as untreated control.

Specimens Collection:

Each mice after experiment were anaesthetized and the blood samples were collected directly from the heart, then, the blood divided into two parts, one put in vial with EDTA the other put with vial without EDTA according to the method (Sood, 1996; Dacie and Lewis, 1984) for hematological study, and Reitman and Frankel (1975) for biochemical study .

The organs were fixed by formalin 10% and a method of luna, (1968) were done for histopathological sections.

RESULTS:

Hematological Results:

1- Red blood cells count (R. B. C.):

Table (1) showed that mice which treated with imidazole for 15 days with both doses (0.75 and 1.5 mg/kg) deceased in red blood cells with significant differences under $p \leq 0.05$ as compared with control group.

2- Hemoglobin Concentration (Hb):

A significant decrease in hemoglobin concentration was found in mice which treated with (0.75 and 1.5 mg/kg) after 15 days as compared with control group (table, 1).

3- Packed Cells Volume P. C. V. The same results were found with P. C. V.

Table (1): The effect of imidazole on blood parameters of male mice (n= 8) for 15 days post treatment.

Parameters / Group	Red Blood Cells (cell/ mm^3)X10^6	Hemoglobin Con. (mg/dl)	Packed cells volume (%)
T1 (0.75 mg/kg)	4.27 (b) + 0.23	6.11 (b) + 0.09	10.1 (b) + 0.06
T2 (1.5 mg/kg)	3.39 (c) + 0.35	5.20 (c) + 0.31	8.6 (c) + 0.67
Control (0.9 % normal saline)	7.52 (a) + 0.34	14.38 (a) + 1.05	27.3 (a) + 1.38
LSD	0.16	0.515	1.110

- The numbers represent the mean + standard deviation.
- The different letters refer to significant differences under ($P \leq 0.05$).

4- Total and differential white blood cells W. B. C.:

As shown in table (2) the imidazole cause an increase of total W.B.C. with significant differences under $P \leq 0.05$ as compared with control group. Furthermore, increase in lymphocytes, acidophil, monocytes. But there was no significant differences in neutrophil count.

Table (2): The effect of imidazole on total and differential white blood cells of male mice (n= 8) for 15 days.

Parameters / Group	White Blood Cells (cell/ mm^3)X10^3	Neutrophiles (%)	Acidophil (%)	Lymphocyte (%)	Monocytes (%)	Basophile (%)
T1 (0.75 mg/kg)	17.38 (b) + 0.18	27.73 (b) +0.88	3.22 (b) +0.43	48.35 (b) + 2.13	14.85 (a) + 0.64	0
T2 (1.5 mg/kg)	17.56 (a) + 0.10	25.50 (c) + 0.75	4.26 (a) + 0.70	51.86 (a) + 2.58	15.00 (a) + 1.06	0
Control (0.9 % normal saline)	3.65 © + 0.29	57.00 (a) + 1.69	1.25 © + 0.46	37.00 © + 1.69	4.00 (b) + 0.75	0
LSD	0.18	1.06	0.49	1.94	0.75	0.00

- The numbers represent the mean + standard deviation.
- The different letters refer to significant differences among groups ($P \leq 0.05$).

- **Biochemical Results:**

1- Alanine amino transferase (ALT):

In table (3) it can be shown that imidazole with (0.75 mg/kg) and (1.5 mg/kg) lead to increase the ALT significantly under probability ($P \leq 0.05$) after 15 days of treated period T1 (28.43) and T2 (30.82) as compared with the control group and it's seems also a significant different between T1 and T2 groups.

2- Aspartate amino transferase (AST):

The treated animals with imidazole with (0.75 mg/kg) and (1.5 mg/kg) for 15 days cause an increasing the AST significantly under probability ($P \leq 0.05$) (76.95; 87.89) in T1 and T2 respectively (Table 3).

3- Total serum cholesterol (TSCH):

The present result showed that the effect of imidazole on TSCH was increasing significantly under ($P \leq 0.05$) which was 100.57; 119.68 for T1 and T2 respectively as compared with control 37.46, furthermore, an significant differences between T1 and T2 9 table, 3).

Table (3): The effect of Imidazole on biochemical parameters of male mice (n= 8) for 15 days.

Parameters / Group	(ALT) IU	(AST) IU	(TSCH) mg/dl.
T1 (0.75 mg/kg)	28.43 (b) + 2.51	76.95 (b) + 4.92	100.57 (b) + 0.63
T2 (1.5 mg/kg)	30.82 (b) + 2.14	87.89 (a) + 4.61	114.68 (a) + 0.27
Control (0.9 % normal saline)	15.31 © + 1.78	31.62 © + 3.65	37.46 © + 0.02
LSD	2.438	4.837	0.35

- The numbers represent the mean + standard deviation.
- The different letters refer to significant differences among groups ($P \leq 0.05$.

Reproductive Parameters:

The oral administration of 0.75 mg/kg. of imidazole to female mice during pregnancy period for 12 days since 10 to 21 day led to absorption of many fetuses and few remained dead fetuses were obtained. While, the large dose 1.5 mg/kg. caused dead fetuses beside the inability of the pregnant females to deliver naturally as compared with those of the control group which reach a 26 litters naturally.

Group	No. of pregnant females	No. of live litters	No. of dead litters
T1 (0.75 mg/kg)	8	zero	9
T2 (1.5 mg/kg)	8	zero	3
Control (0.9 % normal saline)	8	26	zero

- **Gross Finding**

The treated animals were dull, loss of appetite, sunk an eyes, cephalotremor and torticolus, viscous saliva dripping , grinding teeth and smi-paralysed hind quarters. The P. M there was hepatic paling, spleenomegaly specially in the higher dose treated animals, furthermore, organs congestion skin prolapse, also, the pregnant females with dead fetuses and large number of absorbed one and delayed delivery upon the normal 19 days as compared with those of the control group.

- **Pathological Changes**

There were many pathological changes found in different organs of treated mice as below:

1-The low dose of imidazole:

A vacculation of cortical tubules were noticed in kidney, some dilated (Figs. 1, 2).

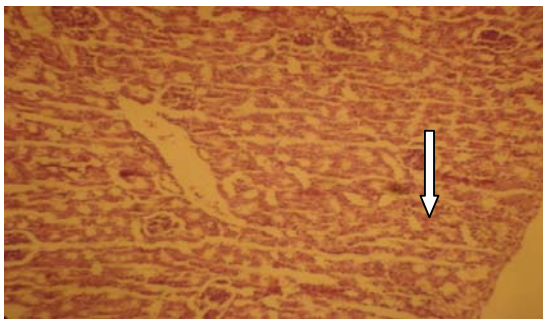

Fig: (1) kidney of mice with low dose of imidazole, with vacculation in cortical tubules. E & H.

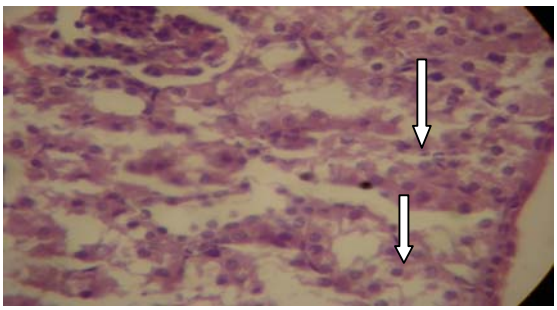

Fig: (2) kidney of mice with low dose of imidazole, with dilated cortical tubules. E & H.

Heart showed vacculation of myocardial muscles cells (Fig. 3), while, an area of myocardial fibrosis and infiltration of adipose tissue and vacculation of myocardial muscles cells were noticed (Fig. 4).

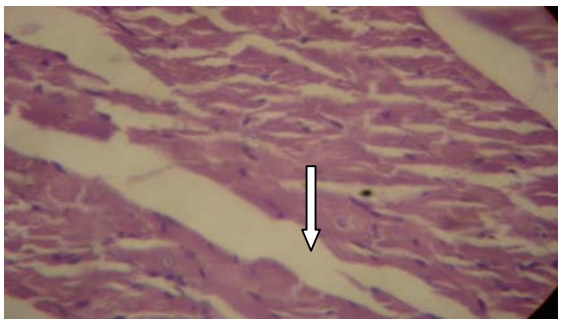

Fig: (3) Heart of mice with low dose of imidazole, with vacculation of myocardial muscles. E & H.

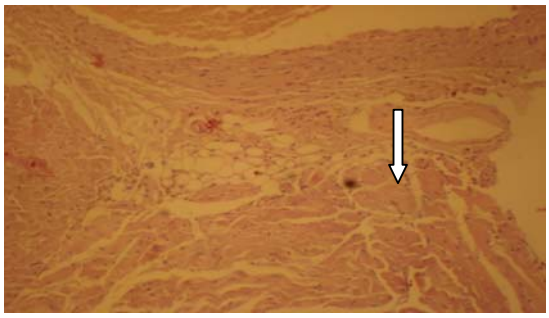

Fig: (4) Heart of mice with low dose of imidazole, with fibrosis and vacculation of myocardial muscles. E & H.

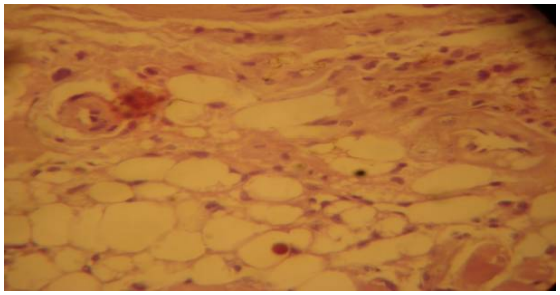

Fig: (5) Heart of mice with low dose of imidazole, with fibrosis and vacculation of myocardial muscles. E & H.

The liver showed a minimal diffuse vacculation of hepatocytes with periportal fibrosis and inflammatory cells and congested portal vein (Fig. 6), some with moderate diffuse vacculation of hepatocytes and periportal aggregate of lymphocytes and mononuclear cells with fibrosis (Fig. 7).

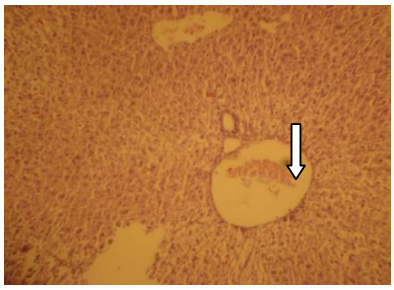

Fig: (6) Liver of mice with intermediate dose of imidazole, with minimal diffuse vacculation of hepatocytes, fibrosis and inflammatory cells. E & H.

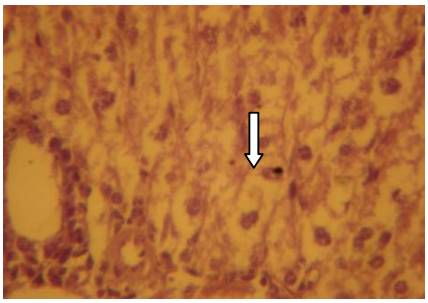

Fig: (7) Liver of mice with intermediate dose of imidazole, with moderate diffuse vacculation of hepatocytes, fibrosis and aggregate of lymphocytes and mononuclear cells. E & H.

An extramedullary haemopoesis were found in spleen of mice with low dose treated (Fig. 8), other were found with megakaryocytes (Fig.9).

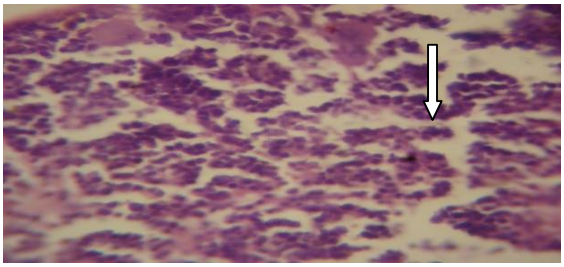

Fig: (8) Spleen of mice with low dose of imidazole, with extramedullary haemopoesis. E & H.

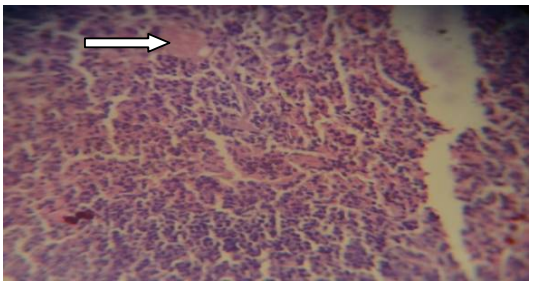

Fig: (9) Spleen of mice with low dose of imidazole, with extramedullary haemopoesis with megakaryocytes. E & H.

2-The High dose of imidazole

- Kidney

The kidney showed a cortical tubules basophile (regenerating cortical tubules) and atrophic Glomeruli and vacculation of cortical tubules (Fig. 10), other with vacuolated dilated cortical tubules (Fig. 11).

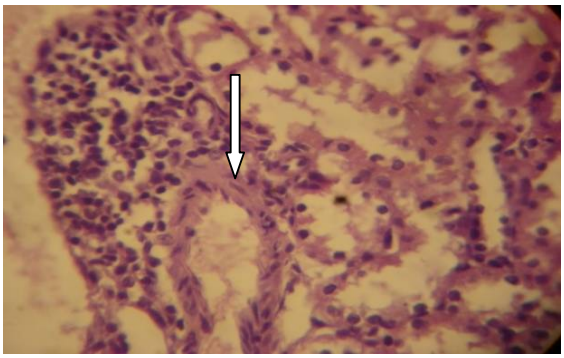

Fig: (10) Kidney of mice with high dose of imidazole, with cortical tubules basophile. E & H.

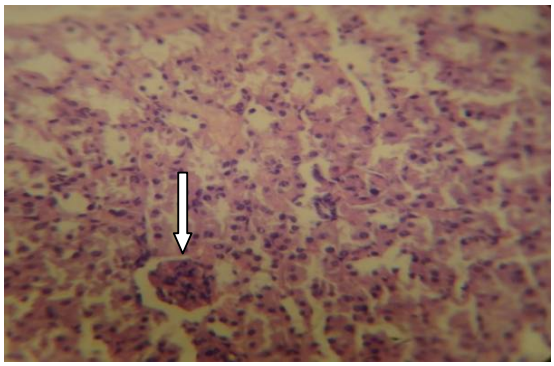

Fig: (11) Kidney of mice with high dose of imidazole, with vacuolated dilated cortical tubules. E & H.

Heart showed a pericardial fibrosis and mononuclear cells and vacculation in myocardial muscles cells (Figs.12, 13).

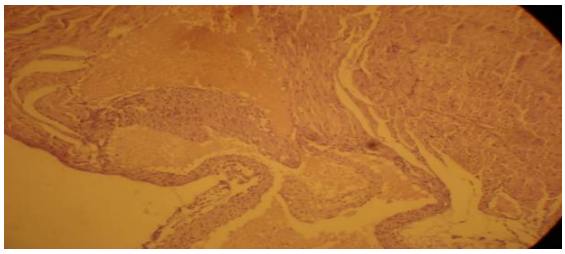

Fig: (12) Heart of mice with high dose of imidazole, with pericardial fibrosis. E & H

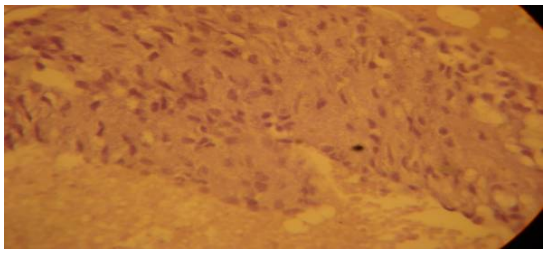

Fig: (13) Heart of mice with high dose of imidazole, with pericardial fibrosis. E & H.

The liver showed a vacuolation of hepatocytes and congestion (Fig. 14), other with minimal diffuse vacuolation and periportal mononuclear cells, fibrosis and congestion with parenchyma foci of mononuclear cells in pericentral vein (Fig. 15), other with few foci of mixed inflammatory cells in parenchyma (Figs. 16, 17).

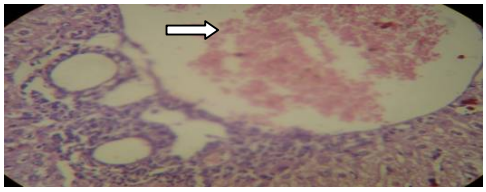

Fig: (14) Liver of mice with high dose of imidazole, with vacuolation and congestion. E & H.

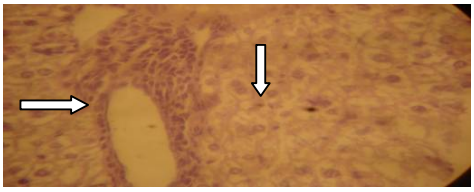

Fig: (15) Liver of mice with high dose of imidazole, with fibrosis and congestion. E & H.

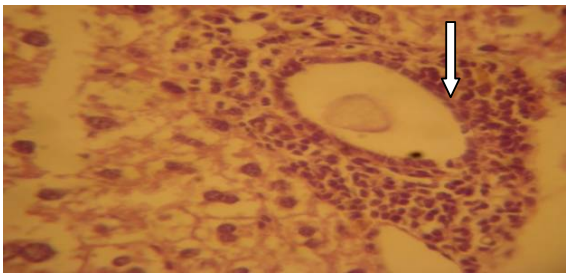

Fig: (16) Liver of mice with high dose of imidazole, with area of mixed inflammatory cells. E & H.

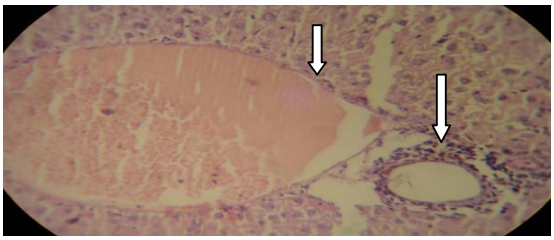

Fig: (17) Liver of mice with high dose of imidazole, with area of mixed inflammatory cells and congestion. E & H.

Spleen were found with extramedullary haemopoesis with megakaryocytes and atrophy of white palp lymphoid tissue (Fig. 18).

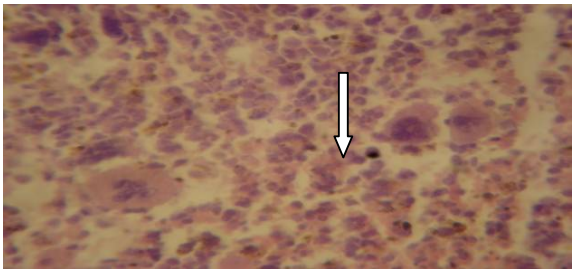

Fig: (18) Spleen of mice with high dose of imidazole, with extramedullary haemopoesis and megakaryocytes and atrophy or white palp lymphoid tissue. E & H.

Discussion

The treatment with imidazole detected a hematological effect as reduced or decreased in number of red blood cells and significant decrease in hemoglobin concentration and reduced packed cell volume. The above treatment related effects was supported by (Basf, 1987) who found an effect on red blood cells in female rats receiving 125 mg/kg body weight in 28 day experiment and in male rats receiving 500 mg/kg body weight. The biochemical results showed a significant increase in (ALT) and (AST) this could be due to evidence of the degenerative toxic hepatic effects of imidazole, as those enzymes only increased when there

will be damage to hepatocytes and this add additional support for being the liver as a target organ for the toxologic pathology effect of imidazole as it was also reported by (TRGS, 2001).

The treated groups also showed significant increase in total serum cholesterol (TSCH), while, the reproductive study showed absorption of many fetuses and few remained dead one.

The present study indicated clearly the hepatotoxic effect of imidazole on liver by presence of vacuolation of hepatocytes associated with varying degree of focal mixed inflammatory cells, mostly in periportal areas and fibrosis that was in agreement with (TRGS, 2001), which reported that liver is a target organ for toxicity of imidazole. Furthermore, the renal changes which was found in the present study as vacuolated and/or dilated cortical tubules which indicate the nephrotoxic effects of imidazole and that was in support of (TRGS, 2001) which found that kidney as a liver a target organ for toxicity of imidazole.

The present research found an effect on myocardial muscles cells as vaculation, which could be a new toxic change which was not reported and was accompanied by fibrosis in the myocardium and intercellular infiltration of adiposities. The extramedullary haemopoesis found as treatment related changes could be related to the haemopoesis toxic effect of the tested compound, which was mentioned by (Basf, 1987). Macroscopically, the enlarged spleen could reflect the extramedullary haemopoesis which was found microscopically. In conclusion, it appeared that toxicologic pathology of imidazole found at many organs as liver and kidney associated with secondary effect on spleen, heart with hematological blood picture related to the treatment with the tested compound. Especially, as there were increase in number of neutrophiles, acidophilic and monocytes.

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