Study of the preventive potential of Zingiber officinale, against myelotoxicity and against the development of leukemia, induced by benzene.

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Abstract: This study aims at revealing the preventive potential of Zingiber officinal against leukemia (blood cancer), we were based mainly on the benzene injection subcutaneously in a group of rabbits to cause disease by a chemical method. In parallel we subjected another group of rabbits to the injection of benzene under the same conditions with the feeding ginger to very specific doses, where it was found that the benzene-induced myelotoxicity was significantly counteracted. The histological study also reveals the hepato-protective power of Zingiber officinal.
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Keywords: Zingiber officinale, leukemia, Benzene, Bone Marrow, Myelotoxicity.

1. Introduction

The detection of the anti-cancer activity of ginger has been the subject of many studies, including studies of the anti-leukemic activity of Zingiber officinal. The majority of published works, are based on experience and in-vitro tests, conducted outside the living organism, the studies among which we can mention the following: the work Eunyoung Lee, and Yong Joon (1998) entitled: « Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol». We can also mention the work of Mei-Hua Hsu and others, (2005) entitled: « 1-(3,4-Dimethoxyphenyl) 3,5-dodecenedione (I6) induces G1 arrest and apoptosis in human promyelocytic leukemia HL-60 cells ». and the work of Qing-Yi Wei, and others, (2005) entitled « Cytotoxic and apoptotic activities of diarylheptanoids and gingerol-related compounds from the rhizome of Chinese ginger ». The goal of all these works is to cause apoptosis in carcinogenic cells in the advantage of demonstrating the power of ginger molecules to start intracellular mechanisms, led to the destruction of carcinogenic cells.

On the other side, in this paper we will try to achieve an in vivo experiment on animals, in such a way that the pharmacological response of cells present in the body that is home to a multitude of chemical reactions is quite different, when these cells are outside of the living organism.

2. Materials and methods

Based on many specialized publications, and particularly:

a) The work of Margot Kissling and Bruno Speck, 1972. Who have tried to study the toxicity of benzene on rabbits from hybrid albino race between Vienna and Alaska, which have been submitted to the subcutaneous injection of 0.2 ml / kg/day of pure benzene, 3 times per week, resulting in severe pancytopenia within 1 to 3 months. The number of leukocytes in peripheral blood fell below 1000/mm^3. Therefore this type of pancytopenia is due to the benzene interference with DNA and RNA synthesis not to the failure of the hematopoietic cells. Therefore, the majority of rabbits develop aplastic anemia, which turns later with acute myeloid leukemia (Margot K, Bruno S, 1972).

b) The recent publication showed that benzene causes acute myeloid leukemia (Curtis D Klassen Ph.D, 2008).

At Saidal laboratory of pharmacotoxicology, it was tried to cause leukemia using subcutaneous injection of benzene in a dose of 0.2 ml / kg / day, 3 times per week.

2.1. Benzene biotransformation

The most important adverse effect of the benzene is hematopoietic toxicity. Chronic exposure to benzene can damage the bone marrow, which may manifest initially as anemia, leukopenia, thrombocytopenia, or combination thereof. Depression marrow seems to be dependent on the dose of the benzene. Survivors of aplastic anemia frequently exhibit a paraneoplastic condition called myelodysplasia, which can progress to acute myeloid leukemia (Curtis D Klaassen Ph.D, 2008).

2.2. Animal materials

We used 4 groups of rabbits, each batch contains at least 3 rabbits, a hybrid albino race between New Zealanders and race in California, selected for laboratory analysis. In such a way that they are treated separately in cages.

- Lot (1): control rabbits C1, C2, C3. For these three rabbits we make the administration of the diet used in the laboratory of pharmaco-toxicology without any changes.
- Lot (2): Includes rabbits Z1, Z2, Z3. This group of rabbits was used to study the influence of the consumption of Zingiber officinal by gavage (Fig. 1), during a long period, on the blood tests and body behavior.
- Lot (3): Includes rabbits L1, L2, L3, L4, L8, L9, L10. This is the group of rabbits, targeted to cause leukemia. The principle of which is to inject subcutaneously 0.2cc / kg of pure benzene (98.98%), 3 times a week (Fig. 2).
- Lot (4): Includes rabbits L5, L6, L7. The use of these three rabbits aims at determining the preventive potential of Zingiber officinale against leukemia, in such a way that we do the administration of ginger by gavage (oral), simultaneously with the injection subcutaneous 0.2cc/kg of pure benzene, three times per week.

The biochemical, and physiological characteristics of New Zealand rabbits race are compiled in the following tables: Table 1; Table 2.

2.3. Plant materials

We used the crude extract of ginger taken from fresh rhizome by centrifugation, and the powder of ginger, in such a way that the two forms of the plant are imported from China. The purpose of using these two forms was to gather the pharmacological effect of hydrated molecules (gingerols) present in the fresh rhizome, and the dry molecules (shogaols) present in the powder in a dry state.

The administered doses at Z1, Z2, Z3; L5, L6, L7 during the experiment are compiled in the following table: Table 3.

3. Results and Discussion

3.1. Haematological behavior of the rabbits during 12 months

For this fact, it is the collection of blood from the marginal ear vein of the rabbit (fig. 3), then the blood is put into EDTA tubes, and for the analysis, we use the Conter Coulter which automatically gives CBC (Complete Blood Count), used at the hematology laboratory, hospital of Medea.

Remark

WBC: White Blood Cells.

RBC: Red Blood Cells.

PLA: Platelet.

- Interpretation

Rabbits L2, L3, L8 were killed at the beginning of the experiment, due to a (possibly intramuscular made) injection error. Rabbits L1, L4, L9, L10: have made a leukocyte drop from the initial three months. These values continue to fall until they have a severe leukopenia, within 10 months (Fig. 4, Fig. 5, fig. 6, Fig. 7) On the contrary in the work (Margot K, Bruno S, 1972), leukopenia is made during 1/3 of our time, and since we have respected almost the same conditions of work, we can lead the resistance to the hybrid race between New Zealanders and Californians we used, which may have to be a marrow rich with enzymatic activity of the NQO1 (NAD (P) H: quinone oxidoreductase); and NQO2 (NRH: quinine oxidoreductase).

NQO1 and NQO2 are polymorphic enzymes, and studies suggest that these two enzymes play a key role in protecting the marrow from hematotoxic effects of benzene, or other environmental factors. A study shows that mice lacking NQO1 are more likely than wild-type mice to benzene hematotoxycity. It provides that NQO plays a role in the detoxification of benzoquinone, and the loss of this protective mechanism may be the mechanism by which strengthens the blood-toxicity of benzene (Curtis D Klaassen Ph.D, 2008).

The number of leukocytes in peripheral blood fell below 1500/mm^3 for most rabbits of this lot, except for L10, which reached a value of 2000/mm^3, but it should be noted that all these values have fallen below the range of hygienic rabbits mentioned in Table 1. For platelets only, L4 has a pathogenetic value, which indicates that the benzene has affected the precursor of thrombocyte, such as megakaryocytes, erythrocytes, and precursors such as erythroblasts.

For rabbits L5, L6, L7: we notice that they have overcome the injection of benzene, as cellular parameters of blood were kept in hygienic interval rabbits, and we can save this resistance from the initial 3 months, so ginger foiled myelotoxicity of benzene despite the low dose administered in this period hundredth LD_0. In the following 3 months, during which the rabbits received a high dose of ginger 1/35 LD_0, we notice an increase in the number of white

blood cells in rabbits L5, L6, which represents an immune enhancement induced by Ginger and there was a stable blood parameters in hygienic range for L7 (Fig. 8, Fig. 9, fig. 10).

After that, we tried to reduce the dose of ginger to 1/70 LD_0 for 3 months, in order to highlight the relationship between dose and activity, and therefore was reported decrease in white blood cells, which slightly exceeds the lower limit of the hygienic range.

Thereafter, we brought together the two forms of fresh and dry ginger at a dose of 1/35 LD_0 aimed at having the synergistic effect of the two forms of resistance, where the parameters blood reaches and climb again in the hygienic interval of rabbits for L6, L7, Except for the rabbit L5, but we can say that since the number of leukocytes didn't drop below 2500 in the L5, so there a resistance since leukocytes in rabbits which received only benzene, dropped below 2500.

In the figures that follow, we will elucidate the hematologic parameters in control rabbits and rabbits Z1, Z2, Z3: (Fig. 11 to Fig. 16).

Rabbits for C1, C2, C3, Z1, Z2, Z3 (Fig. 11 to Fig. 16), we see an almost identical leukocyte development, deducing that ginger at different doses, caused no haematological disturbance on a first assumption that ginger occurs specifically in the presence of myelotoxicity.

3.2. The evolution of the Hemogram after bone marrow depression in rabbits subjected to the injection of benzene alone.

- Interpretation

In all rabbits of this lot we saw the total absence of EOS, and BASO (fig. 17) and morphological observation of PNN shows a hyper toxic granulation (see figures. 18, 19) this sign of intoxication confirms that benzene affected the bone marrow by exercising his mutagenic effect and therefore we recorded severe pancytopenia (3 lined falling WBC, RBC, platelet). This cell fall takes values below the lower limit of the hygienic range rabbits.

However rabbits L5, L6, L7 injected with the same dose of benzene, and under the same conditions, except that they have submitted to Zingiber officinale gavage, kept stable hematological parameters, especially cellular morphology intact and non-pathogenic because there is no sign of intoxication (see Fig. 20). It should be highlighted that the 1/35 DL_0 in the ginger crude extract it requires the best resistance regarding benzene-induced cell fall, as well as non-pathogenic leukocyte values.

3.3. The observation of Medullary gramme after bone marrow depression

In order to confirm the bone marrow lesion in rabbits, it is necessary to perform the marrow smear, the sample is taken using a sterile trocar, by penetrating the bone at the iliac crest, then with an ordinary syringe is made the marrow aspiration (Fig. 21). The smear shows the morphology of bone marrow precursor cells (young) of the bone marrow.

- Interpretation

There is a hyper toxic granulation in rabbits marrow smears of the batch subjected to injection of benzene, L1, L9, L10 (Fig. 22, Fig. 24, Fig. 25). As well as signs of myelodysplasia in L10 (Fig. 25) smear reveals:

1) erythroblastic hyperplasia characterized by increased number of erythroblasts, and the increased volume of the erythroid cell (megaloblasts), and the presence of erythroblasts in mitosis, see Fig. 25.

2) The presence of hypo segmented and hyper granular PNN, see Fig. 25.

Knowing these signs of myelodysplasia often present leukemia evolution.

For the L4 the marrow smears is very poor because of severe bone marrow depression (Fig. 23). After a while rabbits L1, L4, L9, have been killed following a severe pancytopenia, even before confirming the achievement of rabbits leukemia, but the diagnosis is the preponderance of acute leukemia, which often manifests as pancytopenia.

4. Pathological Anatomy study of tissue samples (autopsy)

To highlight the influence of benzene and ginger on the livers of rabbits during the experiment, were sacrificed one of each batch to make histological sections of livers observations at the ANAPATH (Anatomy-pathology) laboratory by pathologists doctors (hospital of Medea). The technique which was used is the one proposed by Martoja and Martoja (1967).

4.1. Results and Discussion

- **liver samples**

Microscopic observation reveals a healthy liver parenchyma appearance on control samples (Fig. 26). During the administration of ginger in rabbits designated by Z, the hepatic parenchyma indicates a healthy appearance, without particular lesion (Fig. 27).

In rabbits subjected to injection of benzene, there was a cytoplasmic cell clarification, with toxic granulation a deposit on almost the entire sample, these signs of intoxication confirm the hepatotoxic action of benzene (Fig. 28) and (Fig. 29). It may signal the absence of tumor infiltration.

Remark

All rabbits in this batch have had the same result.

As for the administration of ginger, and injection of benzene in parallel with the L7 we see a heterogeneous appearance due to normal liver cells, healthy in their majority, and some cells with cytoplasmic granulations and clarification (Fig. 30). Therefore, the changes and cellular damage induced

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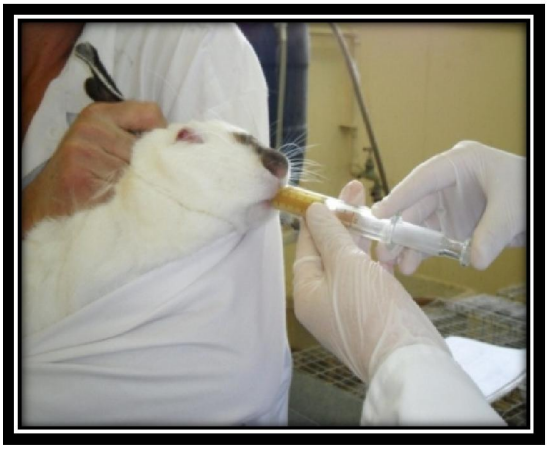
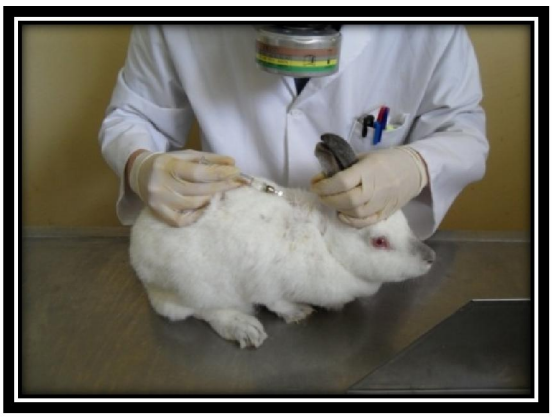




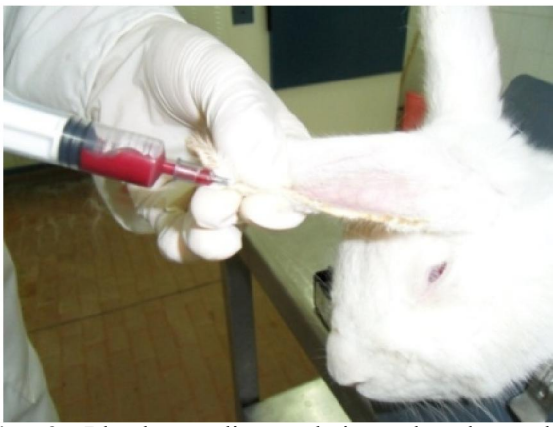


Fig. 3. Blood sampling technique, based on the marginal ear vein of the rabbit.

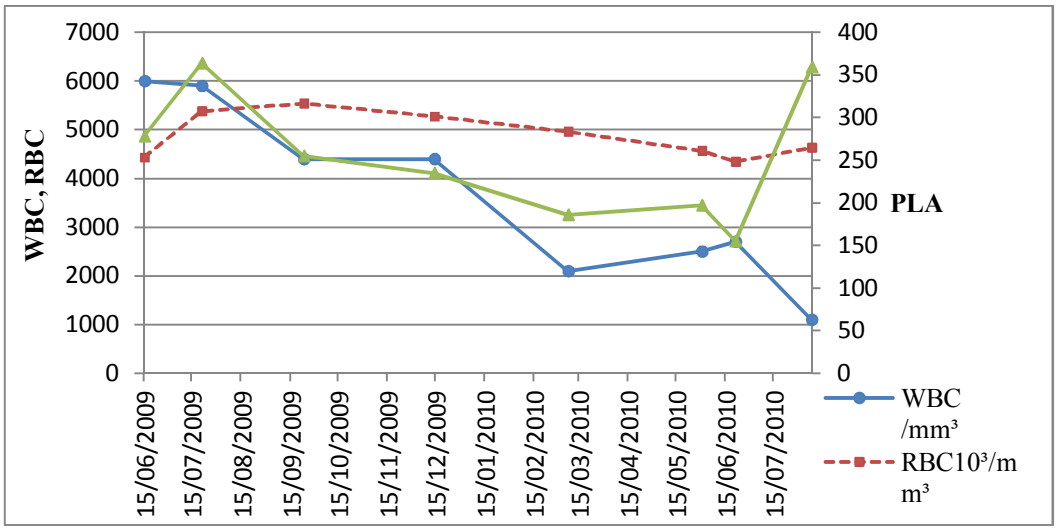


Fig. 4. Blood test of L1 after 12 months of injection the 0.2cc/kg pure benzene.

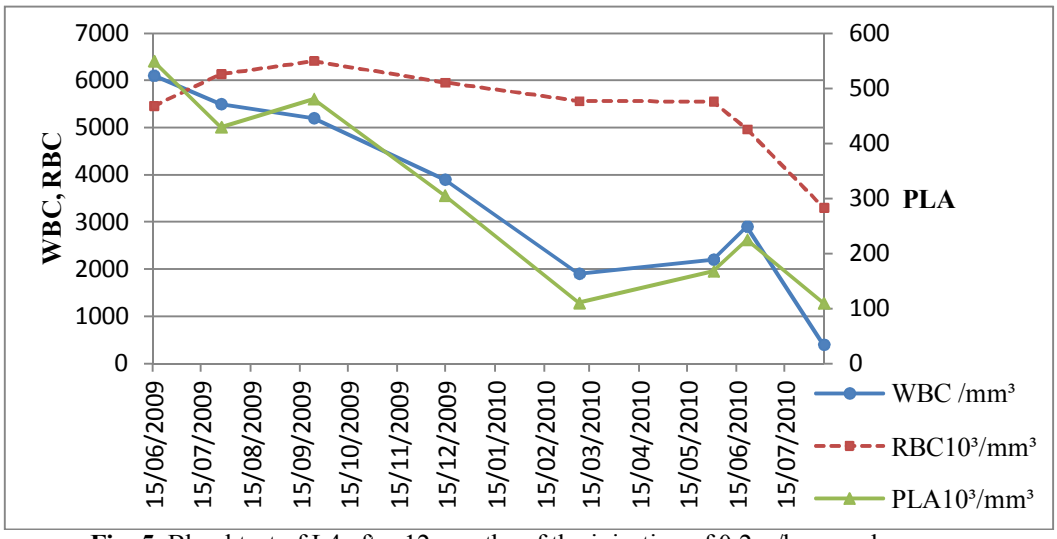


Fig. 5. Blood test of L4 after 12 months of the injection of 0.2cc/kg pure benzene.

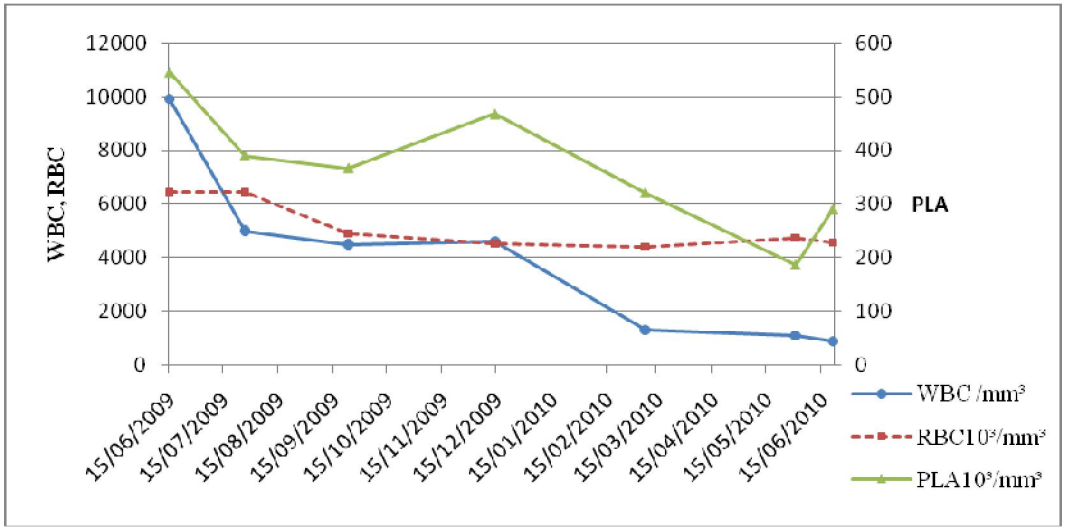


Fig. 6. Blood test of L9 after 12 months of the injection of 0.2cc/kg pure benzene.

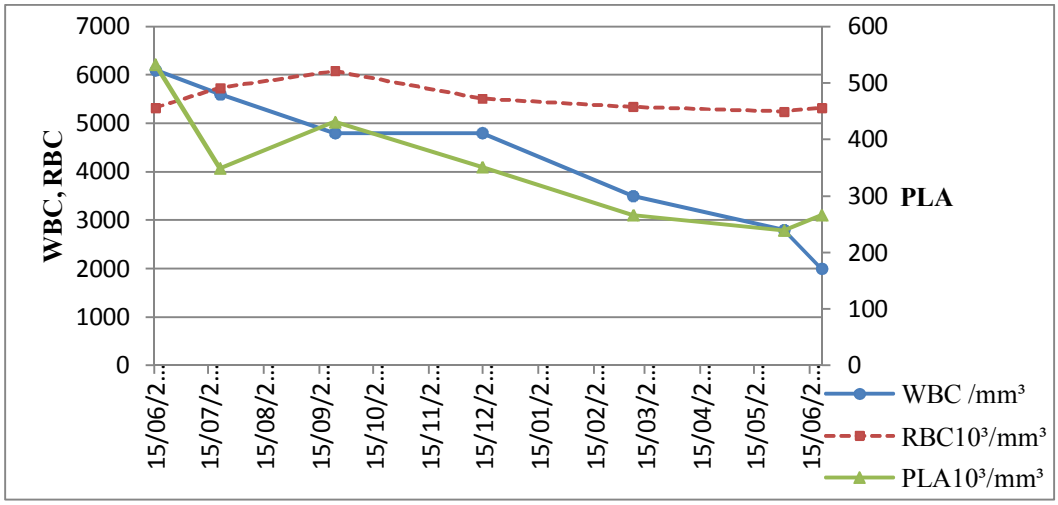


Fig. 7. Blood test of L10 after 12 months of the injection of 0.2cc/kg pure benzene.

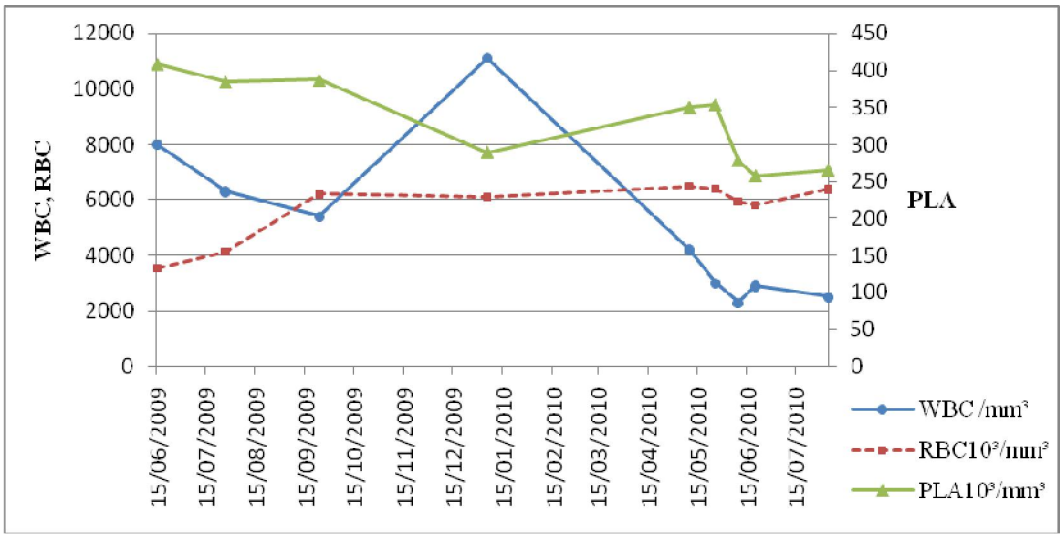


Fig. 8. Blood test of L5 after administration of benzene and Zingiber during 12 months.

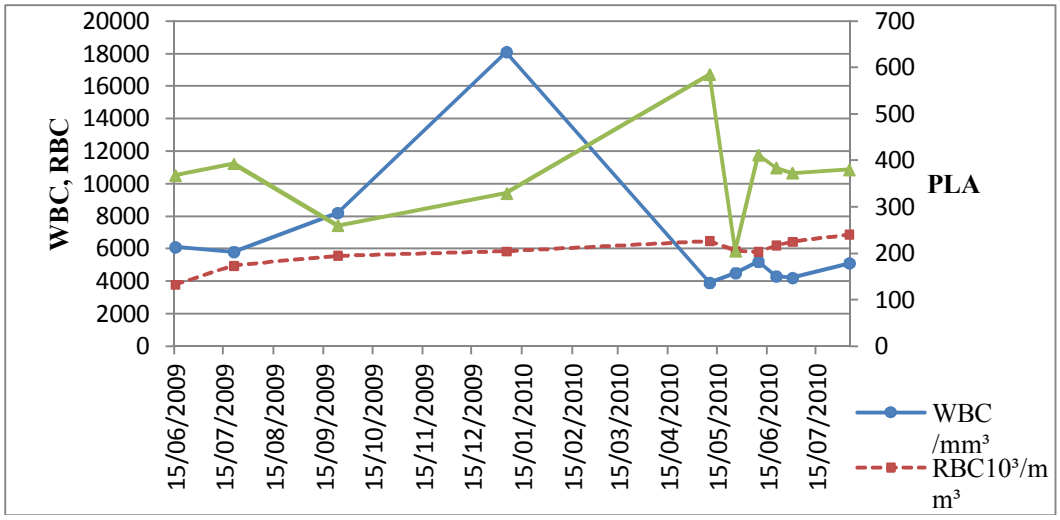


Fig. 9. Blood test of L6 after administration of benzene and Zingiber during 12 months.

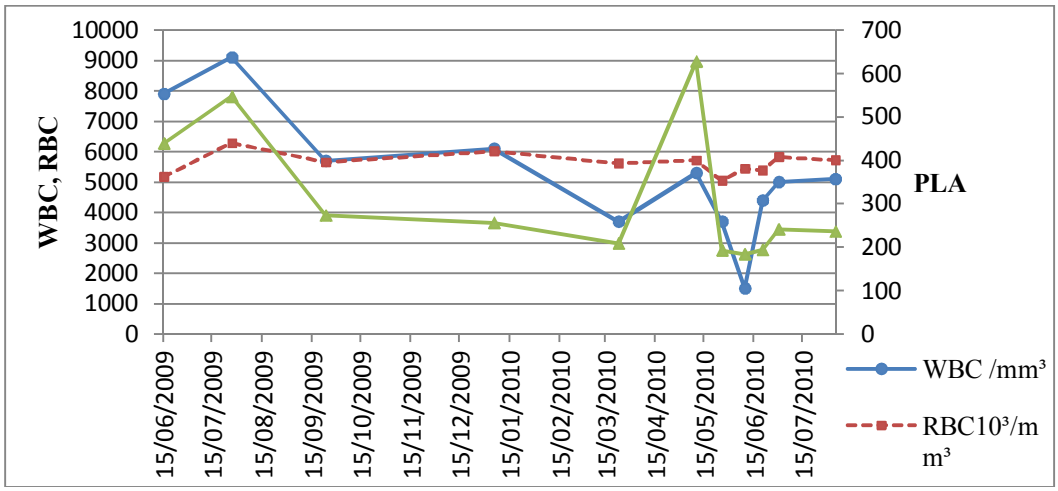


Fig. 10. Blood test of L7 after administration of benzene and Zingiber during 12 months.

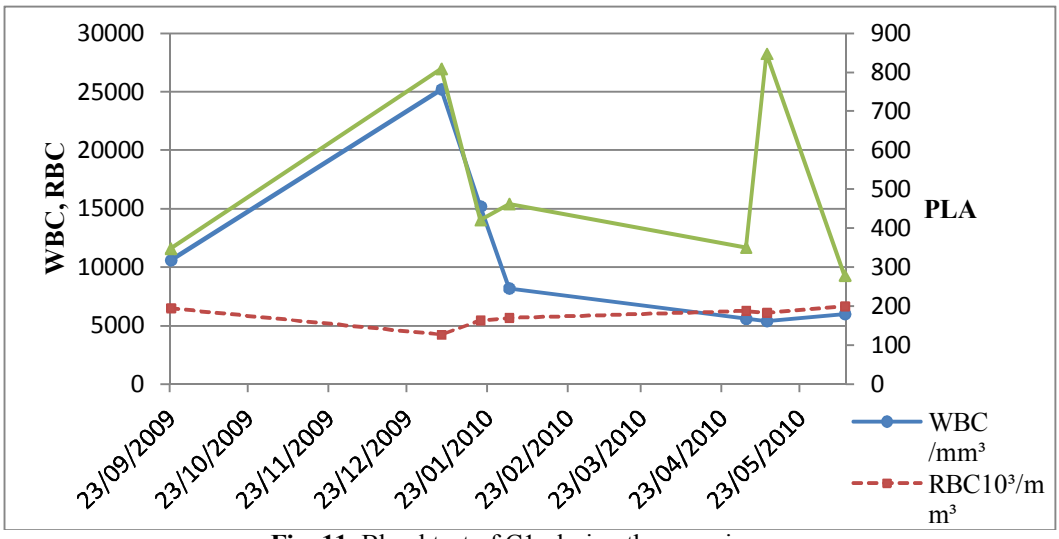


Fig. 11. Blood test of C1, during the experience.

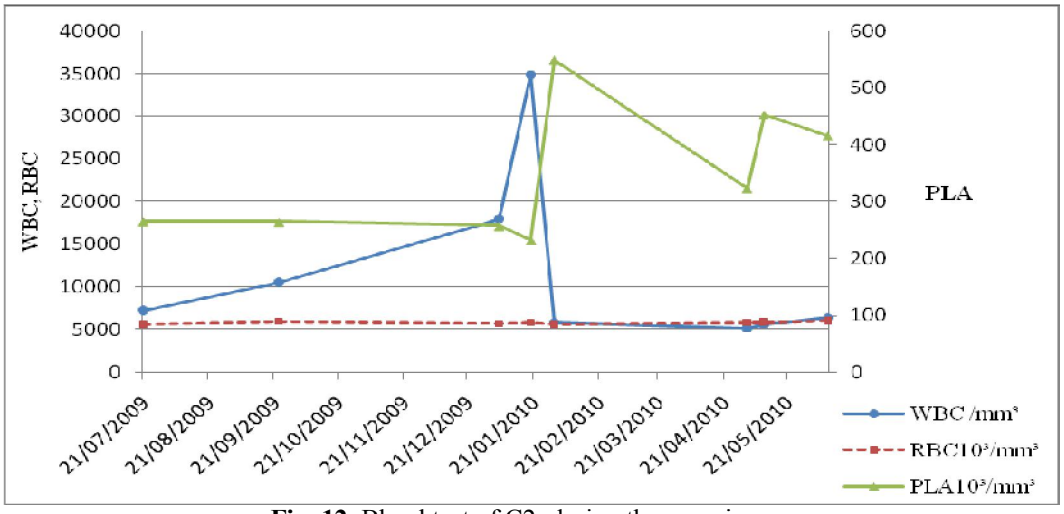


Fig. 12. Blood test of C2, during the experience.

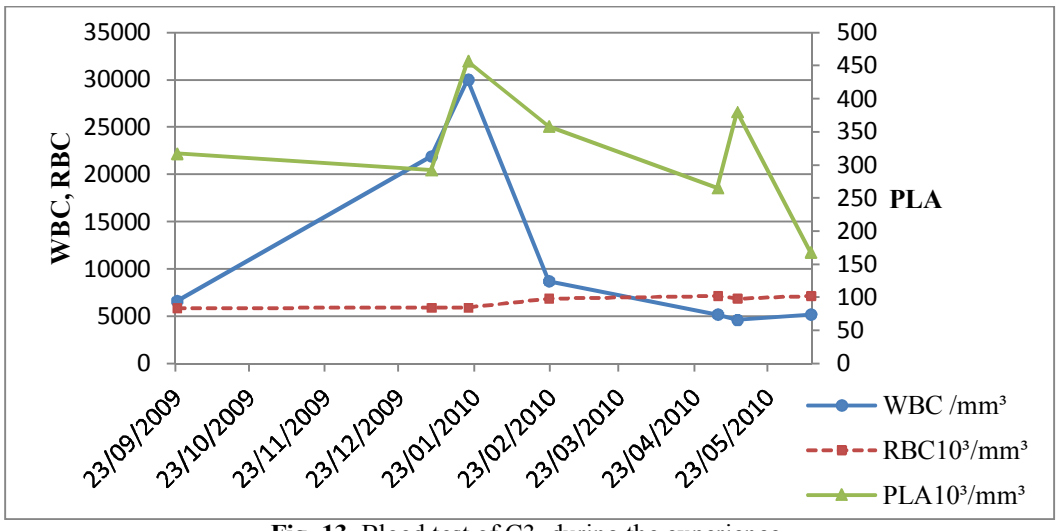


Fig. 13. Blood test of C3, during the experience.

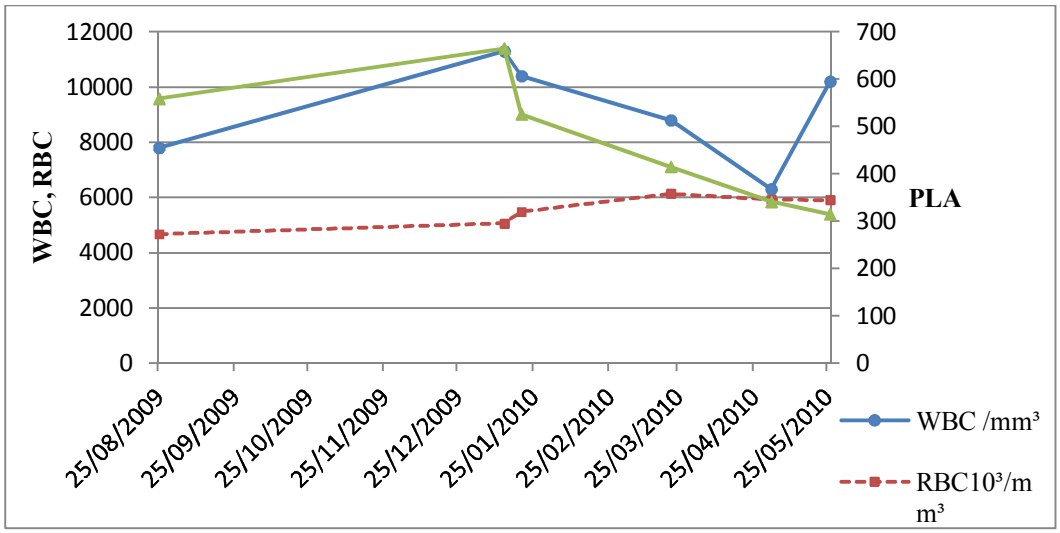


Fig. 14. Blood test of Z1, after 12 months of the administration of ginger.

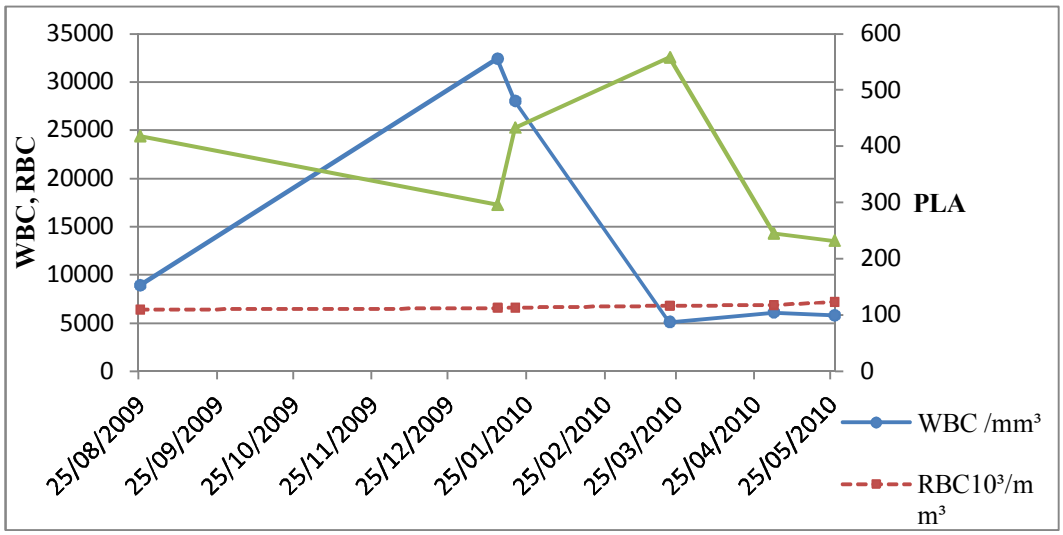


Fig. 15. Blood test of Z2, after 12 months of the administration of ginger

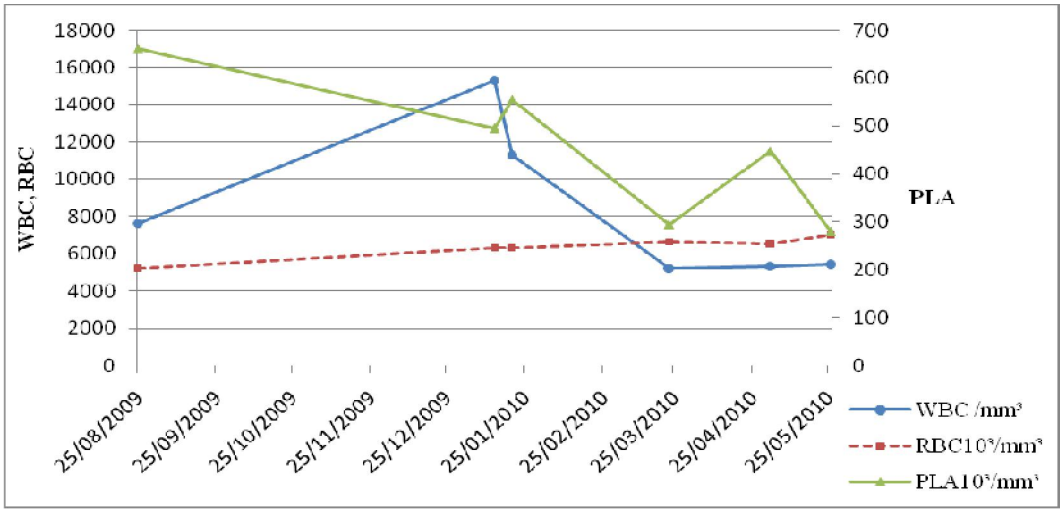


Fig. 16. Blood test of Z3, after 12 months of the administration of ginger.

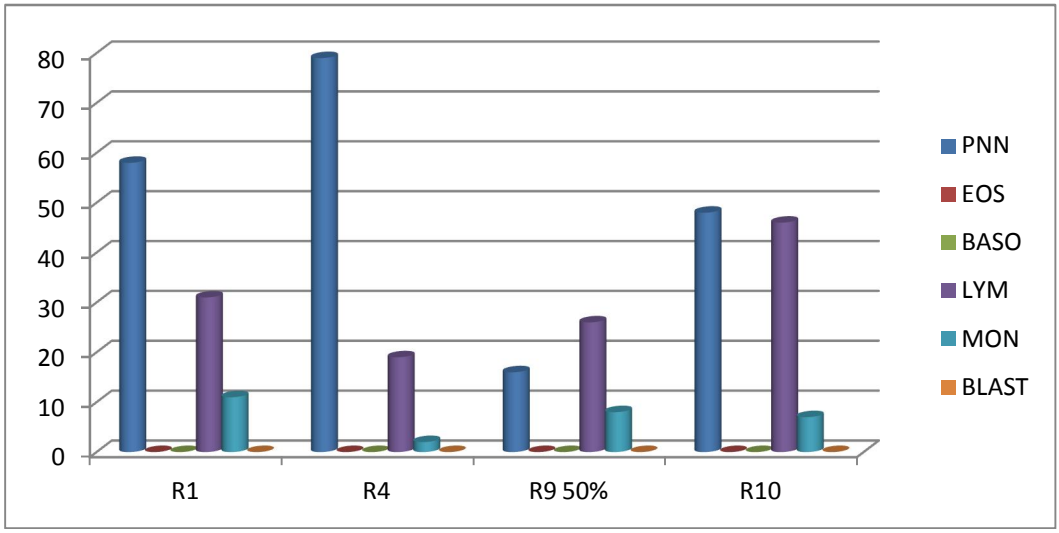


Fig. 17. Leukocyte percentage of rabbits having had a bone marrow depression.

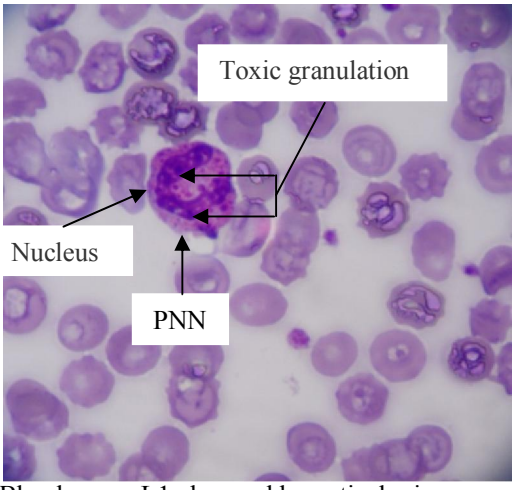


Fig. 18. Blood smear L1 observed by optical microscopy (x100).

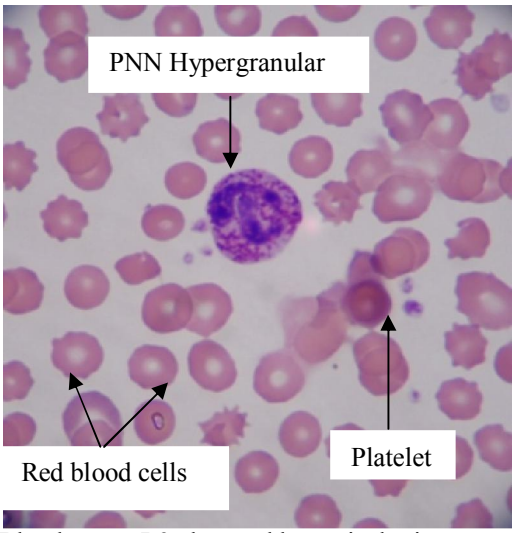
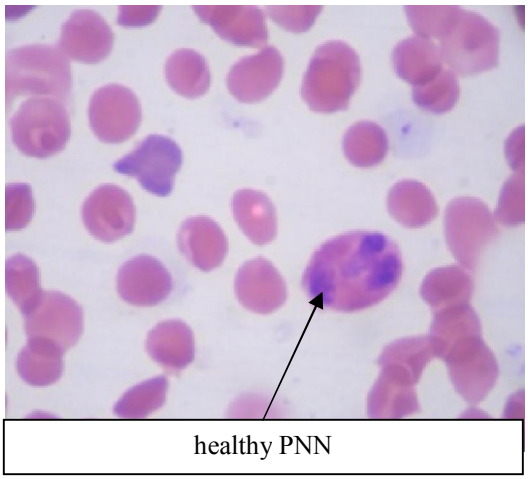


Fig. 19. Blood smear L9 observed by optical microscopy (x100).



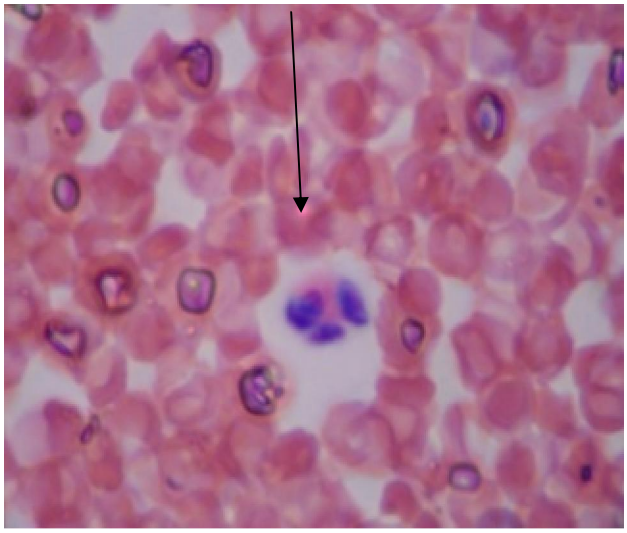


Fig. 20. Blood smear shows healthy cells in rabbits L5, L6, L7(x100).

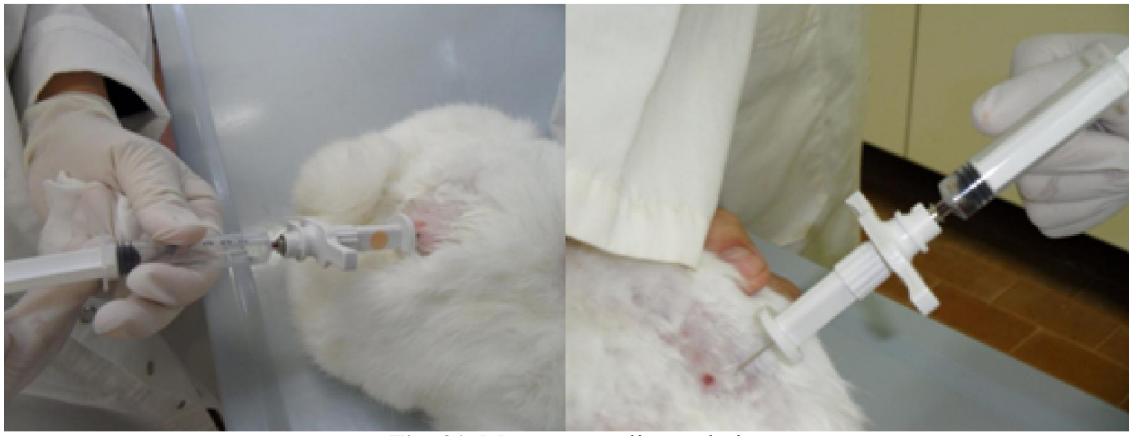


Fig. 21. Marrow sampling technique.

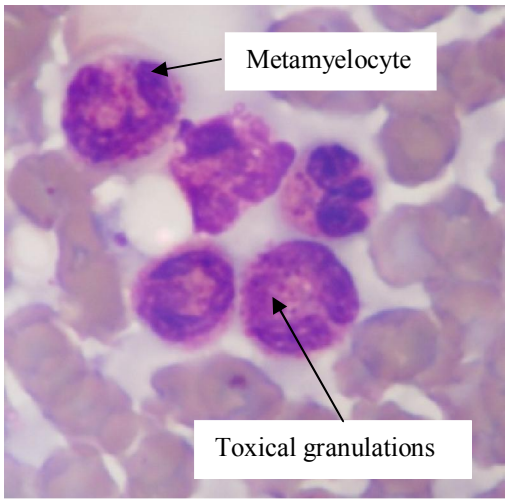


Fig. 22. Marrow smear: hyper toxic granulation in the rabbit L1.

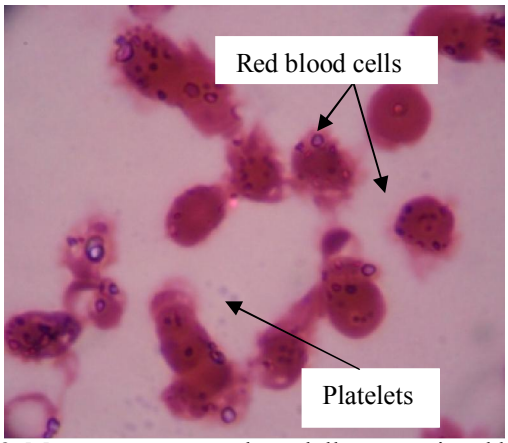


Fig. 23. Marrow smear : weak medullar smear in rabbit L4.

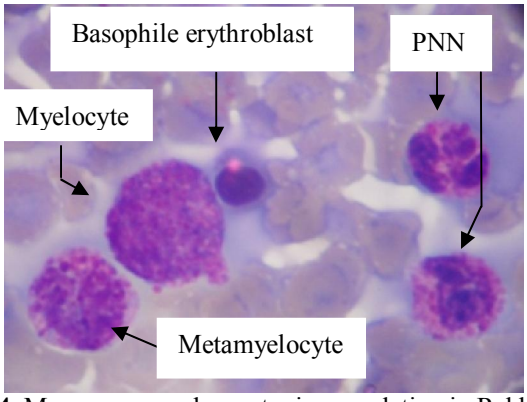


Fig. 24. Marrow smear: hyper toxic granulation in Rabbit L9.

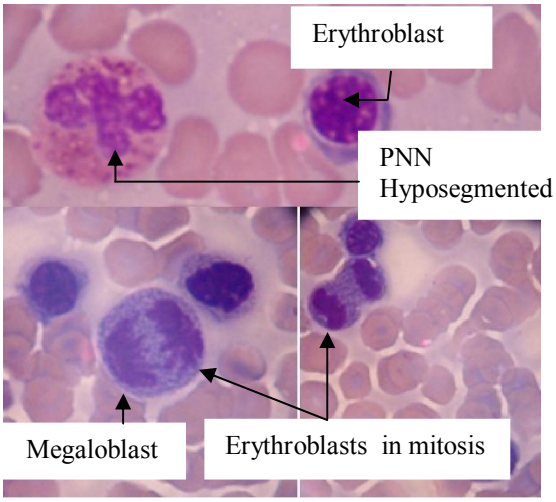


Fig. 25. Marrow smear: sign of myelodisplasia in rabbit L10.

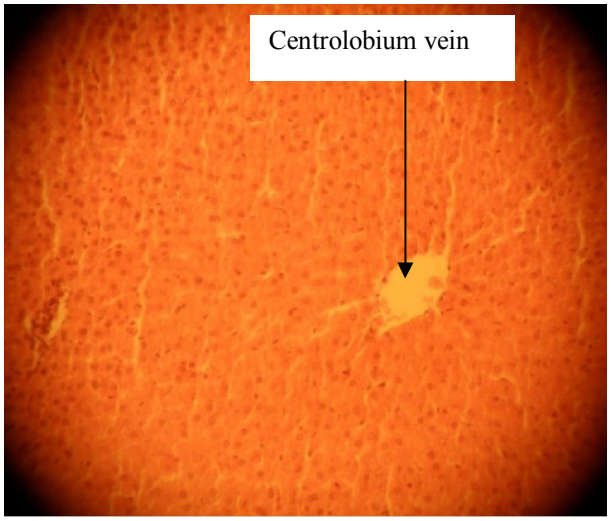


Fig. 26. Hepatic Parenchyma of a healthy histological aspect in rabbit C2 (x10).

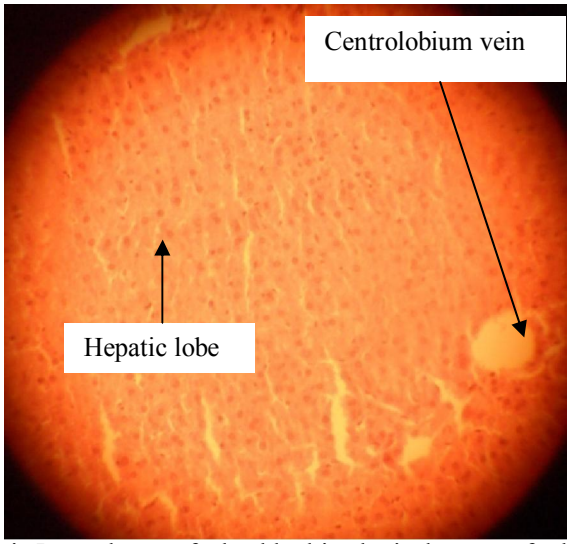


Fig. 27. Hepatic Parenchyma of a healthy histological aspect of rabbit Z3 (x10).

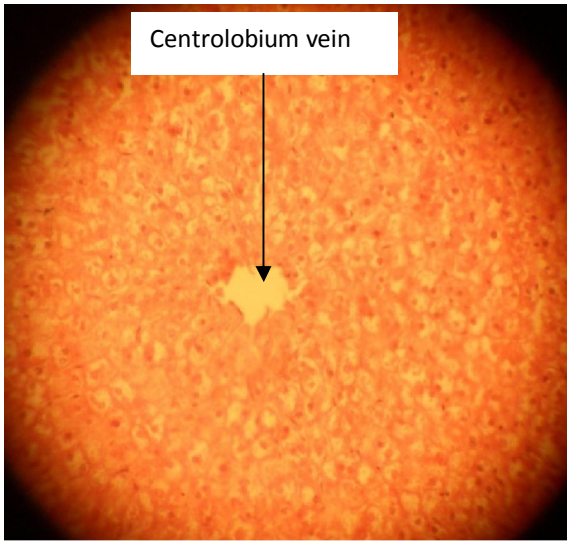


Fig. 28. Hepatic parenchyma cell showing an interesting clarification in almost all tissues of the L10 (x10).

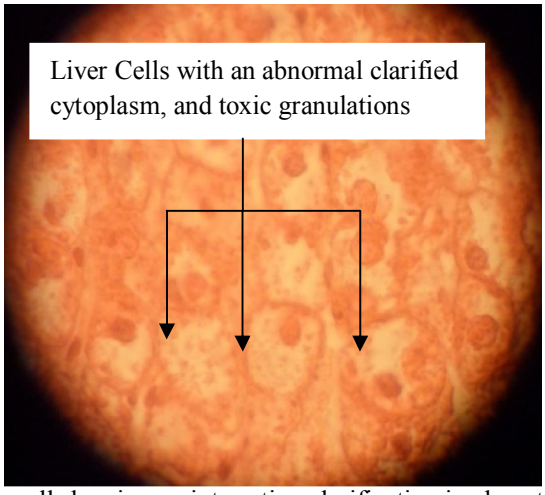


Fig. 29. Hepatic parenchyma cell showing an interesting clarification in almost all tissues of the L10 (x40).

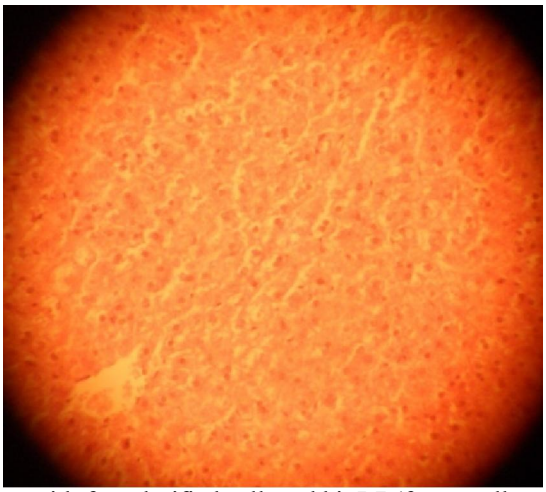


Fig. 30. Hepatic parenchyma with few clarified cells, rabbit L7 (fewer cells with clear cytoplasm) (x10).

Table 1. Physiological parameters of rabbits (Derelanko Michael, 2008).

Parameters	Measurments
RBC	4.5-7.0 10^6/mm^3
WBC	5-12 10^3/mm^3
Patelets	250-750 10^3/mm^3
Recta temperatures	39.5 °C

Table 2. Overall leukocyte percentage of rabbits (Harcourt B, Frances, 2002).

Leukocytes	Percentages %
PNN	30-50
EOS	0-5
BASO	0-8
LYM	30-60
MONO	2-10

Table 3. Different forms and doses administered during the experiment

Doses g/kg	Form	Duration (month)	Goals
1/100 LD_0	Crude extract	3 months	Adaptation of rabbits
1/35 LD_0	Crude extract	3 months	Study of the preventive effect
1/70 LD_0	Crude extract	3 months	Influence of the diminution of the dose on the preventive effect
1/35 LD_0	Crude extract + the powder of ginger	3 months	Study of the preventive effect of the associated forms.

LD_0 (NOAEL) No Observed Adverse Effect Level is the highest dose that causes no toxicity and no mortality (Derelanko Michael, 2008). It is 75 g / kg of fresh rhizome, and 7.5 g / kg of powdered ginger.

5. Conclusion

We demonstrated in this study that ginger contains an effective preventive potential against the blood-toxicity and hepatotoxicity induced by benzene, and as benzene targets primarily the bone marrow by causing genetic mutations that damage hematopoietic cells, which will become the source of proliferation of carcinogenic cells and the onset of leukemia, so the ginger-induced bone marrow protection is a barrier against leukemia, and against marrow damage induced by chemical factor. It is preponderant; therefore, that ginger is effective protector against all natural damage factors (genetic, and congenital).

It is preferable for this work, to be completed and deepened by further studies, to highlight the action mechanisms of ginger components against myelotoxicity, and hepatotoxicity. And to study the therapeutic activity of ginger against leukemia as curative one.

- Ginger is a protective factor against the blood-toxicity induced by benzene.
- The ginger-induced bone marrow protection is a barrier against leukemia.
- Ginger is a protective factor against the hepatotoxicity induced by benzene.

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Conflict of interest

There is no conflict at all.

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