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Haematopoietic Enhancing Effect of Ethanolic Seed Extract of *Citrullus lanatus* (Watermelon) on Bone Marrow of Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EFB did the study concept and design, wrote first draft of manuscript, literature review and interpretation of results. Authors NJN and ITI participated in experimental work, literature review and final draft of manuscript. Authors MIS and VA designed the study, drafted the manuscript, managed the literature search, interpretation of result, and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the haematopoietic property of citrullus lanatus seed extract by determining the phytochemical, cytological and histological profiles on the bone marrow of wistar rats.
Methodology: Phytochemical properties were determined using a UNICAMM 969 atomic absorption spectrophotometer and functional groups were analyzed using Furrier Transform Infrared ray (FTIR). Fifteen (15) wistar rats average weights of 150 g were divided into three groups A, B, and C of 5 rats each. Group A (controls) received 0.1 ml saline while groups B and C received 100 mg/kg and 200 mg/kg of ethanolic seed extract of *Citrullus lanatus* respectively for 14 days. Thereafter, the animals were sacrificed under anaesthesia using ketamine 50 mg/kg. Bone marrow was collected from the femoral shaft and stained using haematoxylin and eosin (H & E) and its

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smear was also stained cytologically using May-Grünwald-giemsa stain.
Results: Atomic Absorbance Spectrum (AAS) revealed concentrations of 754.20 µg/kg, 53.70 µg/kg, 45.10 µg/kg, 24.20 mg/kg 11.80 µg/kg and 1.10 µg/kg for Iron, Zinc, Lead, Copper, Nickel and Cadmium ions respectively. The FTIR revealed presence of methyl, aldehyde, acetyl, carboxyl and ether groups. Bone marrow histology from groups B and C show greater cellularity compared to the control (group A). The cytology revealed more of myeloid precursors in the control and erythroid lymphoid precursors in the extract groups.
Conclusion: *Citrullus lanatus* seed extract has physiochemical properties which support its hematopoietic effect on the bone marrow and can be use as blood supplement.

Keywords: Citrullus lanatus; bone marrow; histology; cytology; phytochemistry; hematopoietic.

1. INTRODUCTION

Bone marrow is a primary lymphoid organ and the major hematopoietic organ in the body. It is one of the most voluminous and also metabolically active organs in the human body [1]. As a haematopoietic tissue it provides a unique microenvironment for chronological proliferation, differentiation and release of erythrocytes, granulocytes, monocytes, lymphocytes and platelets [2].

Bone marrow is however, sensitive to external influences; it can be suppressed in response to dietary restriction, malnutrition, chronic inflammation, toxicity and proliferative or neoplastic disorder [3,4,5,6]. Nutritional status is an important factor that affects bone marrow cellularity [7] and erythropoiesis [8]. The bone marrow relies on foods that contain specific nutrients in order to produce healthy stem cells and blood cells. These nutrients have targeted action on the bone marrow for example; Iron is required for red blood cells formation. Plant dietary sources of iron include nuts, seeds and vegetables with daily intake of 10 mg to 20 mg.

Watermelon (*Citrullus lanatus*) seeds are among the underutilized fruit products and existing evidence suggest that water melon seeds contain proteins, moderate quantity of minerals and possess good functionality [9]. It also contained high lycopene content which has lots of nutritional and health benefits. Lycopene is a carotenoid phytonutrient that is also important for bone health. Watermelon seed has strong antioxidant and has been recognized to be of immense health benefit [10,11]. It is consumed locally and is traditionally considered to be a haematinic. It is important to access the effect of *Citrullus lanatus* seed on the bone marrow. The aim of this study is to evaluate haematopoietic properties of ethanolic seed extract of *Citrullus lanatus* (watermelon) on the bone marrow of wistar rats by determining the phytochemical properties, cytological and histological effects.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Watermelon pulps were purchased from local market in Enugu State, South-East, Nigeria. The seed was harvested and dried under shade at room temperature. The dried seeds were pulverized into fine powder which was used for the photochemistry and preparation of the extraction.

2.2 Phytochemical Analyses

The presence of elemental constituents and functional groups were analysed using Atomic absorbance spectroscopy (AAS) and Fourier transform infra-red spectroscopy (FTIR) respectively. These were carried out in the Department of Chemistry, School of Sciences, Ahmadu Bello University Zaria, Kaduna State.

2.3 Ethanolic Extraction

The powdered watermelon seed was thoroughly sieved and 150 g of the fine powdered watermelon seed was submerged in 500mls of 95% ethanol. The mixture was vigorously stirred and then filtered, thereafter the filtrate was left to evaporate for 48 hours while exposed to air in an open container. The ethanolic extract was then stored at low temperature in the refrigerator at 4ºc for the administration.

2.4 Animal Procurement and Care

Fifteen (15) healthy, adult wistar rats weighing an average of 150 g were obtained from the Pharmacology Department of Federal Teaching Hospital, Abakaliki. The experimental animals

were allowed to acclimatize for 14 days in the animal house of the Department of Anatomy, Enugu State University of science and technology, under optimum conditions (photoperiod: 12 hour natural light and 12 hour dark) with free access to rat pellets and water ad-libitum.

2.5 Ethical Approval

The study was reviewed and approved by the Institutional Ethics Committee. The experimental protocols and techniques used in the study were in accordance with the research guidelines of the Faculty of Basic Medical Sciences, Enugu State University of Science and Technology and acceptable principles for laboratory animal use and care (EU Directive of 1986:86/609/EEC). The animals were handled according to guidelines of National Institute for Health, USA [12].

2.6 Animal Treatment

After the acclimatization period, the animals were randomly grouped into control and extract groups. The control (group A) received 0.1ml saline while the extract groups B and C were administered 100 mg/kg and 200 mg/kg orally of the ethanolic extract respectively for 14 days. On the day 1 post- treatment each animal was anaesthetized by 50 mg/kg of ketamine injection, and then the bone marrow was harvested from the shaft of the femur for histological and cytological studies.

2.7 Histological Study

This was carried out as described by [13]. At the end of the experiment the metaphysis from the various groups of rats were collected for histopathology after which they were fixed in 10% formal saline, decalcified in formic acid and dehydrated in ascending grades of ethanol. Thereafter, the tissues were cleared in chloroform overnight, infiltrated and embedded in molten paraffin wax. The blocks were later trimmed and sectioned at 5 – 6 microns. The sections were deparaffinized in xylene, taken to water and subsequently stained with Haematoxylin and Eosin (H and E) for light microscopy.

2.8 Cytological Study

The study adapted non-invasive method in the collection of the bone marrow sample. The femur as the common site for the collection of bone marrow samples beside the tibia in small rodents like rats was used. The femur allows reasonable quantity to be harvested for microscopic studies. The femurs were removed and trimmed of extraneous fats. The shaft were split using a scissors while a clean sterile stick (non-invasive method) was used to pick the bone marrow which was then spread or smeared over a clean microscope slides. These were allowed to dry before slides were stained using May-Grünwald-Giemsa stain [14] and examined using an Olympus CX-40 microscope (Olympus, Tokyo Japan).

2.9 Statistical Analysis

The calibration curves for each element were prepared and the concentrations of the elements were extrapolated from their respective plots and represented as mean ± standard deviation in Table 1. Total myeloid precursors in proportion to the total erythroid precursors was calculated and reported as the Myeloid: Erythroid (M:E) Ratio and presented as + low, ++ moderate, +++ high in Table 2.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

3.1.1 Elemental analysis (Atomic absorbance spectroscopy)

The Atomic absorbance spectroscopy (AAS) revealed the presence of some heavy elements in the *Citrullus lanatus* seed. The mean concentration of each metal is presented in Table 1.

3.1.2 Fourier trans form infra-red spectroscopy (FTIR) analysis

The fourier transforms infra-red spectroscopy (FTIR) shows the absorption bands of the *Citrullus lanatus* seed and the various functional groups were then deduced from the peaks and frequencies (Table 2).

Table 1. Mean concentration of some heavy metals in *Citrullus lanatus* seed

Sample ID	Cu (µg/kg)±SD	Pb (µg/kg)±SD)	Ni (µg/kg)±SD	Zn (µg/kg)±SD	Fe (µg/kg)±SD	Cd (µg/kg)±SD
Seed	24.20 ± 0.0004	45.10 ± 0. 0001	11.80 ± 0.0007	53.70 ± 0.0015	754.20 ± 0.0020	1.10 ± 0.0008

Table 2. Peak, frequency and assignment of FTIR absorption bands of *Citrullus lanatus* seed

S/N	Peak	Intensity	Assignment/Functional group
1	419.01	52.584	C-C bending
2	663.70	49.526	CO_2 bending
3	1102.11	40..500	C-O stretch
4	1339.84	39.989	CH_2 twisting
5	1419.25	36.212	O-H bending
6	1457.90	33.293	O-H stretch
7	1508.18	34.088	N-O asymmetric stretch
8	1648.01	25.305	C-O stretch
8	1710.51	23.549	C=O stretch due to acetyl group
9	2925.90	13.745	C-H stretch
10	3393.53	20.108	O-H stretch

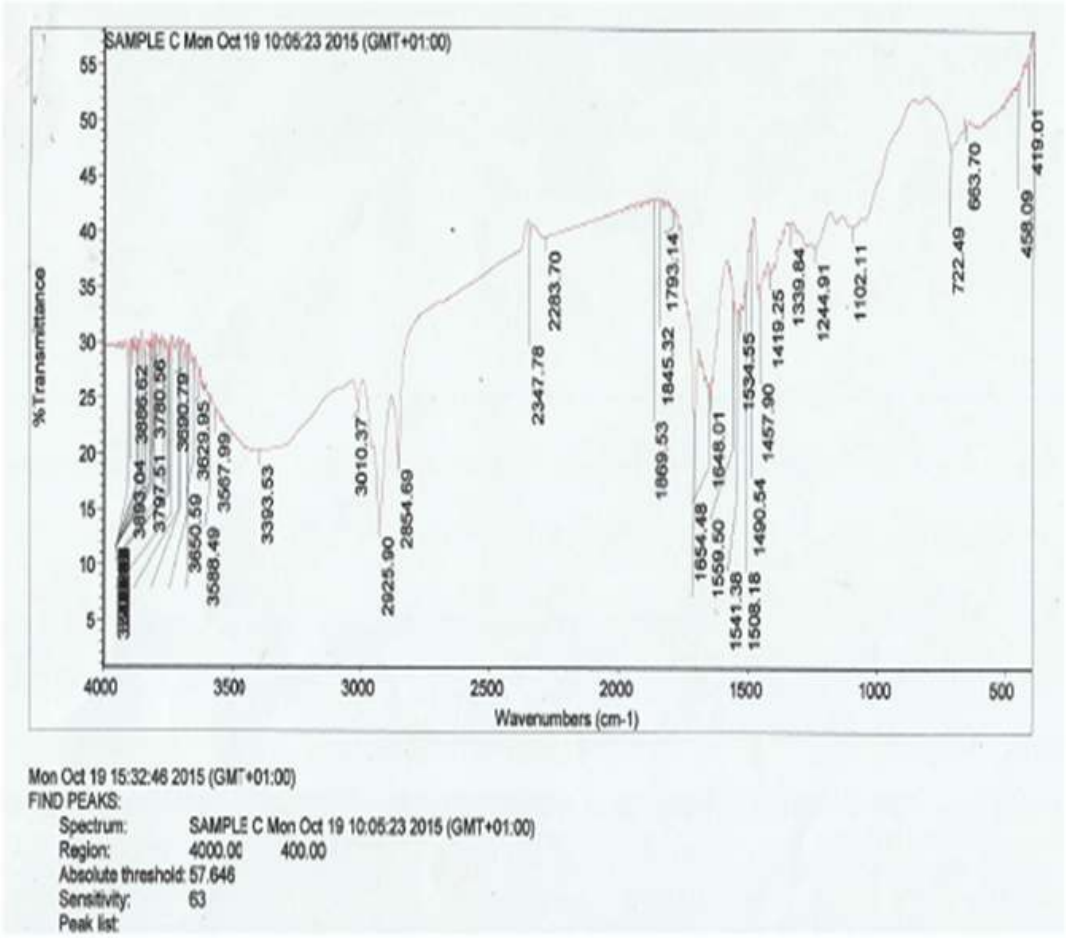

Fig. 1. FTIR spectrum of *Citrullus lanatus* seed

3.2 Histological Findings

These shows the proportion of hematological cells to fats cell (adipocytes) in the sections obtained from the control and treated groups. The extract groups showed more hematological cells (greater cellularity) compared to the control (Fig. 2).

3.3 Cytological Findings

The cytology result showed the various hematopoietic stem cells (myeloid and erythroid) in sections of the bone marrow of control and extract groups (Fig. 3). The myeloid, erythroid and other cells population are shown in Table 3.

Table 3. Cytological evaluation of bone marrow for haematopoietic cells

Cell precursors	Control(A)	Group(B)	Group (C)
Myeloid precursors	+++	++	++
Erythroid precursors	+	++	++
Mature granulocytes	+	+	+
Lymphocytes	+	++	++

Keys: -absent, + low, ++ moderate, +++ high

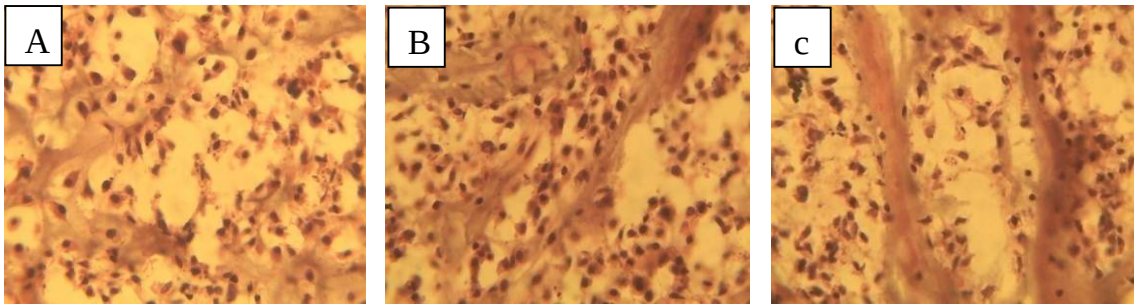

Fig. 2. Bone marrow histology of groups B (100 mg/kg) and C (200 mg/kg) of extract showing greater cellularity compared to the control (group A.) H and E Stain. x400

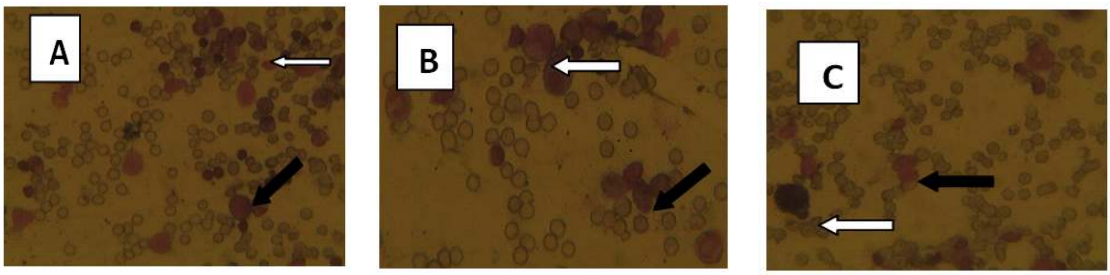

Fig. 3. Photomicrograph of bone marrow cytology: Control group shows more of myeloid precursors (black arrows) than erythroid precursors (white arrows) compared with extract groups (B and C). May-Grünwald-Giemsa Stain. x400

This study revealed that *Citrullus lanatus* seeds contain vital amounts of iron, zinc, lead, cadmium, copper and nickel ions. Iron had the highest mean concentration value compared to other metals. Iron is an essential element needed by humans in protein synthesis and in the formation of some blood cells. Most haematinics contain iron as a major ingredient for example the Ranferon 12 which is often recommended for anaemia and pregnant women. Relative high amount of zinc was found in the watermelon seeds next to iron. Zinc is an essential mineral that stimulates the activity of about 100 enzymes in the body. It supports healthy immune system, it is necessary in the synthesis of DNA, essential for wound healing and supports the healthy growth and development of the body during adolescence, childhood and pregnancy [9]. documented that iron and zinc in watermelon seeds is 85-90% bioavialable, despite the oxalates and phytates in the seed. Bioavailability of a nutrient is the percentage of the consumed nutrient that is accessible for use in normal physiologic functions and for storage [9].

The seeds also contain considerably low concentration of copper compared to other essential metals. Copper is a trace element needed by the body in minute concentration. It is required in the formation of haemoglobin, red blood cells as well as bones. Copper helps in the formation of elastin as well as collagen and is therefore necessary for wound healing. Deficiency of copper is a cause of increased blood fat levels. Copper is essential for the formation of haemoglobin in red blood cells, iron and copper salts are important for actions of haematinics. Significant concentration of lead was also found in *Citrullus lanatus* seed. The concentrations of some heavy metals such as nickel, lead and cadmium in this study were higher and above permissible limit set by World Health Organization and Food and Agricultural Organization of the United Nations 2012.

The FTIR evaluated the functional compounds present in watermelon seed. The FTIR spectrum was measured, as KBr pellet, in the range 400-4000 cm^{-1}. Hydroxyl group band width centred at a wavelength ~3393 cm^{-1}. This is comparable to an earlier report which documented a wavelength ~3309 cm^{-1} [11]. The spectrum shows presence of methyl, hydroxyl and ether moieties which are main functional groups often seen in the chemical structures of most haematinics. These chemical moieties would interact with specific cellular components in the responsive tissues to bring about beneficial structural and functional changes.

In this study such influence on the functionality of the bone marrow was histologically and cytologically examined. Notably, all cellular blood components are derived from haematopoietic stem cells (HSC) in the medulla of the bone marrows of adult animals [15,16]. It provides a unique microenvironment for chronological proliferation, differentiation and subsequent release of matured hematological cells [16,17,18]. Hematological cells in the bone marrow are a class of three lineages which include: erythroid lineage which is the oxygen carrying red blood cells, lymphoid lineage that served as the cornerstone of adaptive immune system and the myeloid lineage with diverse roles for innate and adaptive immunities, blood clotting and ability to differentiate into other blood cells types [19]. In addition to the hematopoietic stem cells, bone marrow contains stromal cells mostly adipocytes or fat cells. These cells give the bone marrow its variable cellularity in different animals [1]. The volume ratio of hematopoietic and fat cells defines the marrow cellularity. Cellularity is age dependent. It is known that as rodents and other species age, normal bone marrow cellularity decreases and is accompanied by a relative increase in adipocytes [1,2].

Histological examination of the bone marrow provides important information regarding the cellularity of the hematopoietic system and effects related to drugs [2]. Our histological finding indicates greater cellularity in the extract treated groups than the control which was also observed to be dose dependant. Hypercellularity of the bone marrow has been recorded in treated animals when there is an increase in hematopoietic cells relative to adipocytes compared with concurrent controls [1]. Although, other factors could be responsible for increasing cellularity of the bone marrow resulting in an increase production in either the myeloid or erythroid cell lines which include, response to cell loss, destruction, or consumption of platelet [2].

Likewise, hypercellularity have been observed in nonspecific or direct response to compound administration but more commonly is due to a regenerative response to decreases in peripheral blood cells, recovery from a xenobiotic-induced bone marrow injury, or inflammation [14]. However, any form of stimulation that produces more of one cell line can cause increased production of other cell lines, causing an overall increase in bone marrow cellularity. The increase cellularity observed was attributed to the consumption of the seed extract of citrullus lanatus where by the 200 mg/kg provided a more effective treatment.

When changes in cellularity warrant further explanation or are treatment related, in context with histologic findings, bone marrow cytologic (e.g., M:E ratio) can be used. This can be expressed as total myeloid precursors in proportion to the total erythroid precursors. (normal myeloid to erythroid (M:E) ratios) which differ with species, but in general, range from 0.5:1 to 3:1 [2]. This study revealed more myeloid precursors in the control group than the extract treated groups. On the contrary, erythroid and lymphoid precursors were higher in the extract treated groups than the control. But, mature granulocytes remained unchanged in both the controls and treated groups, these is due to the fact that this cells have already been committed to their fate of becoming granulocytes.

Conversely, decreased or increased production of either cell line often results in a shift in M: E ratio [2], for example: a decreased M:E ratio is indicative of an increase in red cell production, as is seen with a regenerative anemia (erythroid hyperplasia), or may indicate a decrease in myeloid (myeloid hypoplasia), or a combination of the two. Meanwhile, an increased M:E ratio may indicate an increase in granulocyte production (myeloid hyperplasia), a decrease in red cell production (erythroid hypoplasia), or both [2,20].

In a nutshell, our finding suggests that the seed extract of citrullus lanatus enhances the activity of the HSC cells particularly the erythroid and lymphoid stem cell lineages in the bone marrow of the treated groups. Invariably, the seed extract of citrullus lanatus can stimulate erythropoiesis and defense mechanism by stimulating the proliferation of immune system of the cells if used as a supplement.

4. CONCLUSION

The extract enhanced bone morrow activity. Administration or use of this extract as a dietary supplement is recommended especially to individuals in low income areas.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Griffith J. Age related physiologic change of the bone marrow and immune system. Geriatric Imaging. Springer Berlin Heidelberg. 2013;891-904. DOI: 10.1007/978-3-642-35579_0_ 34
2. Travlos GS. Normal structure, function, and histology of bone marrow. Toxicol Pathol. 2006;34(5):548-565.
3. Jain NC. Depression or hypoproliferative anaemias in lea and febiger, schalm's veterinary hematology. Philadelphia, PA; 1986.
4. Weirda D. Benzene toxicity to bone marrow stromal cells. Society of toxicologic Pathologist Great Lakes Region Discussion Group: Bone Marrow Toxicity. Toxicl Pathol.1990;18:710-711.
5. Reagan WJ. A Review of myelfibrosis in dogs. Toxicol Pathol. 1993;21:164-9
6. Lund JE. Toxicologic effects on blood and bone marrow, in Feldman BF, Zinki JG, Jain NC 5th eds. Schalm's Veterinary Haematology. Lippincott, Williams and Wilkins, Philadelphia, PA; 2000.
7. Brown JA. Quantitative study of cellular changes in the bone marrow following protein deficiency in the rat. Anat Rec. 1954;120(5):15-33.
8. Bethard WF, Wissier RW, Thompson JS, Schroeder MA, Robson MJ, The effect of acute protein deprivation upon erythropoiesis in rats. Blood. 1958;13:216-225.
9. Lakshmi AJ, Kaul P. Nutritional potential, bioaccessibility of minerals and functionality of watermelon (*Citrullus vulgari*) seeds. Food Sci and Tech. 2011; 44:1821-26.
10. Lim TK. Edible medicinal and non-medicinal plants. Fruits. 2002;2.
11. Rai A, Mohanty B, Bhargava R. Modelling response surface of supercritical extraction of watermelon seed oil using carbondioxide. Seperation and Purification Technology. 2015;141:354-65.
12. National Institutes of Health. Consensus conference statement. Impotence. NIH Consensus Statement. 1992;10(4):1.
13. Bancroft JD, Stevens A. Theory and practice of histological techniques. Churchill Livingstone Edinburgh. 1977;16-64.
14. Freeman K. Bone marrow evaluation. In: Feldman BF, ZinklJG, Jain NC, eds. Schalms Veterinary Hematology 5th ed. Philadelphia, Pa: Lea & Febiger; 1999.
15. Cline MJ, Maronpot RR. Variations in the histological distributions of rat bone marrow cells with respect to age and anatomic site. Toxicol Pathol. 1985; 13:349–355.
16. Diptiman C, Sanjay K, Selvarangan P. Therapeutic potential of adult bone marrow-derived mesenchymal stem cells in diseases of the skeleton. J. Cell. Biochem. 2010;111(2):249-257.
17. Liu ZJ, Zhuge Y, Velazquez OC. Trafficking and differentiation of mesenchymal stem cells. J Cell. Biochem. 2009;106(6):984-991.
18. Kuroda Y, Kitada M, Wakao S, Dezawa M. Bone marrow mesenchymal cells: How do they contribute to tissue repair and are they really stem cells? ,Arch. Immunol. Ther. Exp (Warsz). 2011;59(5):369-378.
19. Connie JE Hematopoietic stem cells: concepts, definitions, and the new reality. Blood. 2015; 125:2605-2613.
20. Provencher-Bolliger A. Cytological evaluation of bone marrow in rats: Indications, methods and normal morphology. Vet. Clin. Pathol. 2004; 33:58–67.

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