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Bone Marrow Changes Induced by Guiera Senegalensis in Acetic Acid-induced Colitis in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC) which are defined as chronic and relapsing inflammations of the gastrointestinal tract caused by variable pathophysiological mechanisms. Synthetic chemical moieties with antioxidant potential are the present treatment regimens, but their high relapse rate and toxicities limit their utility in treatment.
Aims: The aim of this work was to investigate the possible toxic effects that the aqueous extract of *Guiera senegalensis* will elicit on the bone marrow of experimental animals.
Methods: Experimental colitis was induced in animals using acetic acid to mimic human IBD. The effects of oral administration of the extract on the bone marrow were assessed using appropriate tools. A control, colitis control and a treatment control (prednisolone) were used as a guide in the assessment of the findings in this study.
Results: Animals receiving the extract showed essentially normal erythroid nests and granulocytic precursors as well as several lymphocytes. Animals receiving prednisolone, on the other hand,

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showed predominantly granulocytic progenitors with an overall decrease in cellularity of the bone marrow. No toxic effect was observed in bone marrow of animals receiving the extract.
Conclusion: The *G. senegalensis* extract showed no toxicity to the bone marrow and may elicit immunomodulatory properties.

Keywords: Guiera senegalensis; colitis; bone marrow; toxicity.

1. INTRODUCTION

Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC) which are defined as chronic and relapsing inflammations of the gastrointestinal tract caused by variable pathophysiological mechanisms characterized by clinical manifestations including diarrhoea, blood in the stool, abdominal pain, and weight loss [1]. Although the aetiology of IBD still remains poorly understood, complex interactions among genetic, environmental, immunological and reactive oxygen species (ROS) have been implicated in the pathogenesis of IBD [2,3]. IBD occurs throughout the world but is more common in urban areas and presents in the teens and early 20s [4].

Although there are few epidemiologic data from developing countries, epidemiological studies from all over the world have stated that the incidence and prevalence of IBD are increasing with time and in different regions around the world — indicating its emergence as a global disease [5].

Guiera senegalensis (Combretaceae) commonly known as 'Sabara' in Hausa (the most widely spoken language in West Africa especially Northern Nigeria) is a shrub of the savannah region of west and central Africa. *G. senegalensis* has also been shown to positively contain alkaloids, saponins, tannins, flavonoids, amino acids, ascorbic acid, and anthraquinones and also displayed antimicrobial activity. Alkaloids and saponins have received the greatest attention with regards to their possible medicinal potentials [6]. Elemental analysis showed that the values of all the elements analysed compares favourably with values obtained for other plants [7] and thus indicated that *G. senegalensis* leaf contains a significant amount of essential mineral elements. Their quantity is in the order Ca > K > P > Na > Mg > Fe > Zn > Cu. This justified the widespread usage of *G. senegalensis* leaves as medicine traditionally and also showed that the plant has a lot of potentials in traditional and orthodox medicine [7].

Several studies have indicated the presence of alkaloids, flavonoids, quercetin, catechins, saponin, tannins, amino acids, ascorbic acid, anthraquinones and a bitter principle, elastine, in the roots and leaves of *G. senegalensis* with potential anticancer and other forms of biological activities [8]. Their functions and mechanism of actions may include the following among others: antioxidant activity, hormonal action, stimulation of enzymes, interference with DNA replication and antibacterial properties [9].

Bone marrow is the flexible tissue in the interior of bones. In humans, red blood cells are produced by cores of bone marrow in the heads of long bones in a process known as hematopoiesis [10]. Bone marrow is also a key component of the lymphatic system, producing the lymphocytes that support the body's immune system. The bone marrow and thymus constitute the primary lymphoid tissues involved in the production and early selection of lymphocytes. Furthermore, bone marrow performs a valve-like function to prevent the backflow of lymphatic fluid in the lymphatic system [11].

Synthetic chemical moieties like 5-amino salicylate, corticosteroids, antimicrobials and immunosuppressive agents such as azathioprine and mercaptopurine, etc. with antioxidant and anti-inflammatory potential are the present treatment regimens for IBD, prednisolone, among others. But, their disadvantages like high relapse rate, immune suppression and a wide range of side effects limit their utility in the treatment of IBD [12].

The aim of this work is to investigate the possible effects that the extract of the plant will elicit on the bone marrow of experimental animals. A control, colitis control and a treatment control will serve as a guide in the assessment of the findings in this study.

2. METHODOLOGY

2.1 Experimental Animals

Adult Wistar rats (110-150 g) were procured from the animal house of the Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University, Sokoto. They were kept in a well-ventilated room with optimum environmental conditions of temperature, relative humidity, dark/light cycle and were fed standard feed pellets and tap water *ad libitum*. They were acclimatized for two weeks prior to the experiment.

2.2 Plant Collection

The fresh leaves of *G. senegalensis* used for this study were collected from a bush around Arkilla, Wammako Local Government Area of Sokoto State, Nigeria. The plant was authenticated by the Herbarium Officer at the Botany unit of Usmanu Danfodiyo University, Sokoto. It was given a voucher number – UDUH/ANS/0144 and deposited at the herbarium.

2.3 Extract Preparation [13]

The leaves were cleaned and air-dried at room temperature for 7 days and ground to fine powder using mortar and pestle. Three hundred and fifty (350) grams of the powdered material was macerated in 1.5 L of distilled water and left for 24 hours after which it was filtered using Whatman's filter paper Number 4. The filtrate was dried in a hot air oven at 40°C to give 34.5 g of the aqueous leaf extract which was used for the study. The percentage yield was calculated to be 9.86% and the dried extract stored in an airtight container.

2.4 Acute Toxicity Testing

Acute toxicity testing was conducted using Lorke's Method [14]. In Phase I, nine (9) rats were used and randomly assigned into 3 groups of 3 rats each. The 1st group was administered 10 mg/kg body weight of the extract using an oral cannula, the 2nd and 3rd groups received 100 mg/kg and 1000 mg/kg body weight respectively. The animals were then observed for 24 hours to monitor their behaviour for signs of toxicity as well as mortality. In Phase II, three (3) rats were used and randomly placed into 3 groups of an animal each. The animals were administered high doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. They were then observed for 24 hours for signs of toxicity, morbidity and/or mortality.

2.5 Colitis Induction

All animals (except group I) were fasted for 6 hours prior to study, with access to water *ad libitum* and given mild anaesthesia before induction of colitis, 2 ml acetic acid (4% v/v) in 0.9% saline were infused for 30s using a soft flexible paediatric catheter size of 6F 2 mm in diameter, inserted through rectum into the colon up to a distance of 8cm and maintained in a supine Trendelenburg position for 30 seconds to prevent leakage of the intracolonic instil [15].

Table 1. Experimental design

Group	Saline (intrarectally)	4% acetic acid (intrarectally)	A.L.E.G.S. (orally)	Prednisolone (orally)
I (control)	2 ml Day 1	-	-	-
II (Colitis control)	-	2 ml Day 1	-	-
III	-	2 ml Day 1	100 mg/kg Day 1-7	-
IV	-	2ml Day 1	200 mg/kg Day 1-7	-
V	-	2 ml Day 1	400 mg/kg Day 1-7	-
VI (Treatment control)	-	2 ml Day 1	-	2 mg/kg Day 1-7

Note: A.L.E.G.S. stands for Aqueous Leaf Extract of Guiera senegalensis

2.6 Experimental Design

Colonic inflammation was induced in fasted rats using a modification of the method of Jagtap et al. [16]. The study comprised of thirty (30) animals and was divided into six groups (I - VI). Each group consists of five (5) animals. It is worthy to note that the symptoms of IBD may be controlled by immunosuppression using, among others, prednisone [12].

On the 8th day, animals were sacrificed by anaesthetic (propofol) overdose. Bone marrow was obtained by cutting off the femur, aspirating the tissue and smearing it on a clean grease-free glass slide. Air dried slides were stained appropriately with Giemsa stain and commented on.

It is recommended to use descriptive terms to assess bone marrow analysis; this is consistent with the STP position paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System [17]. However, a semi-quantitative method will be used to assess the groups,

2.7 Bone Marrow Cellularity Assessment and Scoring

Point counting method as used by Kerndrup et al. [18] was employed to assess cellularity since the counting method was ideal for aspirated smears [19]. The 4-point scoring method of Gruppo et al. [20] was used which is supported by the European consensus on grading bone marrow cellularity [21].

Severely hypocellular - 0
Moderately hypocellular - 1
Normocellular - 2
Hypercellular - 3

3. RESULTS

Acute toxicity study revealed no morbidity, behavioural changes or mortality in the rats indicating that the lethal dose is above 5000 mg/kg. There was negligible difference in the volume of bone marrow aspirated from the femurs of the experimental animals. However, microscopic changes were evident.

Control animals showed normal bone marrow aspirate revealing erythroid nests and granulocytic progenitors of varying maturity. A few lymphocytes were seen as well as abundant adipocytes of various sizes. Smears from colitis control animals showed an increase in granulocytic progenitors and lymphocytes indicating active inflammation.

Animals fed with the extract showed normal erythroid nests with a good number of granulocytic progenitors and lymphocytes. Treatment control animals, however, had few erythroid and lymphoid progenitors but abundant granulocytic progenitors. They also showed an overall reduction in cellularity of the bone marrow confirming its immune suppression and/or toxicity.

4. DISCUSSION

Acetic acid induced colitis bears close resemblance to human IBD in terms of pathogenesis, histopathological features and inflammatory mediator profile [22] and is, therefore, a reliable animal model that can be useful for evaluation of drugs for IBD [23].

Acute toxicity study revealed no morbidity, behavioural changes or mortality in the rats indicating that the lethal dose is above 5000 mg/kg. This is an indication that the extract is safe for consumption. This result is consistent with the findings of several authors including [7,13,24,25].

Changes in bone marrow cellularity can be an indicator of systemic toxicity and, therefore, bone marrow should be included in the battery of tissues examined because many compounds that target the bone marrow have been associated with profound alterations in immune function [26,27]. In fact, many compounds that target the bone marrow have been associated with profound alterations in immune function [28] which is implicated in IBD progression [2,3].

In any toxicologic study, the bone marrow from the treated animals should be compared with age- and sex-matched control animals due to the variation in normal cytological features that can be seen between sexes, strains and species of animals [17]. By implication and because hematopoietic and lymphopoietic lineages share a common progenitor stem cell, it is reasonable to anticipate that systemic toxicity could affect multiple cell lineages [17,26,27]. Although the best indicator of immunomodulation would be a change in the lymphocyte population, lymphoid lineage cells are difficult to distinguish from many of the other nucleated cells in the bone marrow

and a general description should be made [17,27].

Colitis control animals showed a decrease in erythroid progenitors compared to control animals in their bone marrow aspirate (Fig. 2). This might indicate an active inflammatory process [26] or as a result of reduction in food intake [29].

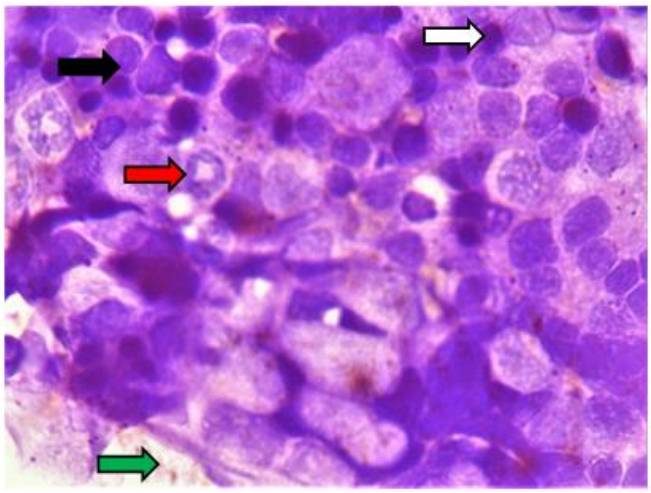

Fig. 1. Photomicrograph of bone marrow aspirate from a control animal. Smear shows an erythroid nest (black arrow), granulocytic progenitors (red arrow), lymphocyte (white arrow) and fat cells (green arrow) (Giemsa stain. Mag. x1000)

Table 2. Observed effect on various bone marrow cell lineages

	Control animals	Colitis control	*A. L. E. G. S.	Treatment control
Erythroid progenitors	1.8±0.45	0.8±0.45	2.2±0.45	1.4±0.55
Granulocytic progenitors	2.2±0.45	2.2±0.84	2.2±0.45	2.8±0.45
Lymphoid progenitors	2.0±0.00	0.6±0.55	2.8±0.45	0.4±0.55
Adipocytes	1.8±0.45	2.4±0.55	1.6±0.45	2.4±0.55

P value = 0.8306 considered not statistically significant. Kruskal-Wallis statistics = 0.9839
** Ranked highest according to Kruskal-Wallis statistics*

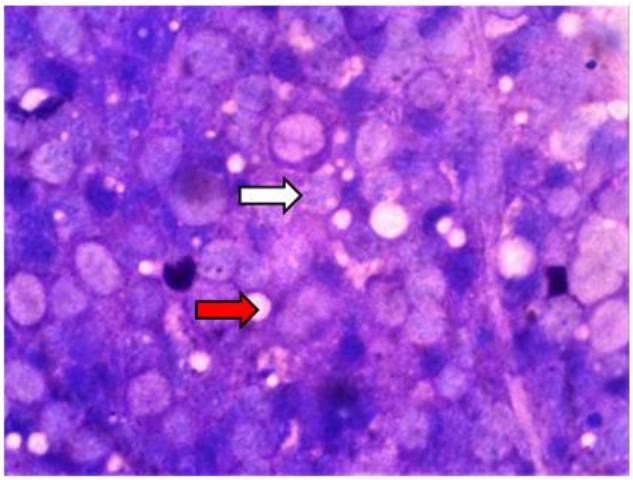

Fig. 2. Photomicrograph of bone marrow aspirate from colitis control animal. Smear shows increased number of granulocytic progenitors (white arrow). A few adipocytes are visible (red arrow). (Giemsa stain. Mag. x1000)

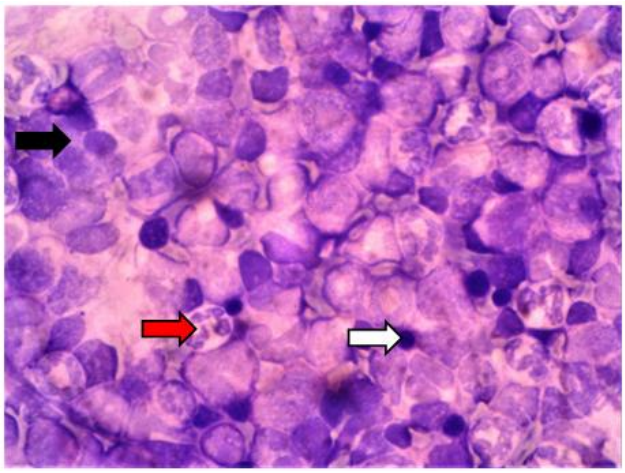

Fig. 3. Photomicrograph of bone marrow aspirate from animal that received 400 mg/kg of the extract. Smear shows erythroid nest (black arrow), granulocytic precursors (red arrow) and lymphocytes (white arrow). (Giemsa stain. Mag. x1000)

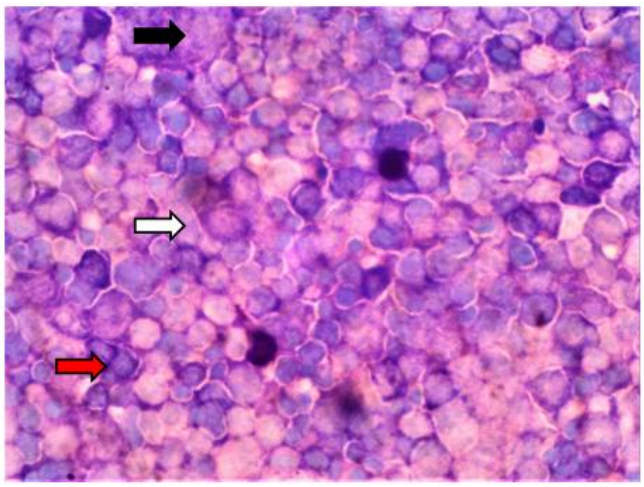

Fig. 4. Photomicrograph of bone marrow aspirate from treatment control animal. Smear shows a megakaryocyte (black arrow) and scanty erythroid progenitors (red arrow) with abundant granulocytic precursors (white arrow). (Giemsa stain. Mag. x1000)

Animals that received the extract showed essentially normal erythroid nests and granulocytic precursors as well as several lymphocytes (Fig. 3). It was more comparable to the control animals indicating an improved immune process in the animals [29]. This is in line with the work of Azza et al. [24] who found no haematological abnormality in animals administered an aqueous extract of the plant. The increase in lymphocytic progenitors may point to immunomodulation given the role of lymphocytes in general immunity [26]. This may be due to its phytochemical properties [6,7] especially alkaloids and saponins.

Animals that received prednisolone, on the other hand, showed predominantly granulocytic progenitors with an overall decrease in cellularity of the bone marrow (Fig. 4). This further confirms the toxic effect of the drug on the bone marrow [30].

5. CONCLUSION

The extract does not bear the side effects of immune suppression and lymphoid tissue toxicity that prednisolone have as evidenced by physical and microscopic evaluation of the bone marrow. The similarities with the cytology of the bone

marrow of control animals as well as lymphoid populations indicate the safety of the extract as well as its potential immunomodulatory properties.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the National Health Research Ethics Committee, Nigeria (NHREC) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shrikant VJ, Bhavin AV, Payal DS, Dinesh RS, Shailesh AS, Tejal RG. Protective effect of aqueous extract of *Oroxylum indicum* Linn. (Root bark) against DNBS-induced colitis in rats. Indian Journal of Pharmacology. 2011;43(6):656-661.
2. Fiocchi C. Inflammatory bowel disease: Etiology and pathogenesis. Gastroenterology. 1998;115:182-205.
3. Papadakis KA, Targan SR. Current theories on the cause of inflammatory bowel disease. Gastroenterology Clinics of North America. 1999;28:283-96.
4. Kumar V, Abbas AK, Aster JC. Robbins and Cotran's pathologic basis of disease. 9th Edition. Elsevier-Saunders E-book. Chapter 17 – The Gastrointestinal Tract; 2015.
5. Bernstein CN, Fried M, Krabshuis JH, Cohen H, Eliakim R, Fedail S, Gearry R, Goh KL, Hamid S, Khan AG, LeMair AW. World Gastroenterology Organization Practice Guidelines for the diagnosis and management of IBD in 2015. Inflammatory Bowel Diseases. 2015;16(1): 112-124.
6. Sule MS, Bichi LA, Atiku MK. Antimicrobial and preliminary phytochemical screening of *Guiera senegalensis*, *Euphobia lateriflora and Mitracapus scaber*. West African Journal of Pharmacology and Drug Research. 2002;18:12-13.
7. Mohammed SY. Quantitative phyto-chemical and elemental analysis of *Guiera senegalensis* leaf extract. Journal of Pharmacognosy and Phytotherapy. 2013; 5(12):204-207.
8. Jigam AA, Akanya HO, Dauda BE, Ogbadoyi EO. Antiplasmodial, analgesic and anti-inflammatory effects of crude *Guiera senegalensis* Gmel (Combretaceae) leaf extracts in mice infected with *Plasmodium berghei*. Journal of Pharmacognosy and Phytotherapy. 2011;3(10):150-154.
9. Sule MS, Mohammed SY. Potency of partially purified Anthocyanin from leaf extract of *Guiera senegalensis* against carbon tetrachloride – induced lipoperoxidation in rats. Bayero Journal of Pure and Applied Science. 2009;2(2):155-158.
10. Birbrair A, Frenette PS. Niche heterogeneity in the bone marrow. Annals of the New York Academy of Sciences. 2016;1370:82–96.
11. Vunjak-Novakovic G, Tandon N, Godier A, Maidhof R, Marsano A, Martens TP, Radisic M. Challenges in cardiac tissue engineering. Tissue Engineering Part B: Reviews. 2010;16(2):169–187.
12. Joshi R, Kumar S, Unnikrishnan M, Mukherjee T. Free radical scavenging reactions of sulfasalazine, 5-aminosalicylic acid and sulfapyridine: Mechanistic aspects and antioxidant activity. Free Radical Research. 2005;39:1163-1172.
13. Akuodor GC, Essien AD, David-Oku E, Chilaka KC, Akpan JL, Ezeokpo B, Ezeonwumelu JOC. Gastroprotective effect of the aqueous leaf extract of *Guiera senegalensis* in albino rats. Asian Pacific Journal of Tropical Medicine. 2013;6(10): 771-775.
14. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54:275-287.
15. Fabia R, Willén R, Ar'Rajab A, Andersson R, Ahrén B, Bengmark S. Acetic acid-induced colitis in the rat: A reproducible experimental model for acute ulcerative colitis. European Surgical Research. 1992;24:211-225.
16. Jagtap AE, Shirke SS, Phadke AS. Effect of polyherbal formulation on experimental models of inflammatory bowel disease. Journal of Ethnopharmacology. 2004; 90(2):195-204.
17. Haley P, Perry R, Ennulat D, Frame S, Johnson C, Lapointe JM, Nyska A, Snyder P, Walker D, Walter G. STP position paper: Best practice guideline for the

routine pathology evaluation of the immune system. Toxicologic Pathology. 2005;33: 404–7.
18. Kerndrup G, Pallesen G, Melsen F, Mosekilde L. Histomorphometrical determination of bone marrow cellularity in iliac crest biopsies. Scand J Haematol. 1980;24:110–114.
19. Fong TP, Okafor LA, Schmitz TH, Thomas W, Westerman MP. An evaluation of cellularity in various types of bone marrow specimens. American Journal of Clinical Pathology. 1979;72(5):812-6.
20. Gruppo RA, Lampkin BC, Granger S. Bone marrow cellularity determination: Comparison of the biopsy, aspirate, and buffy coat. Blood. 1977;49(1):29-31.
21. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica. 2005;90(8): 1128-32.
22. Hartmann RM, Morgan-Martins MI, Tieppo J, Fillmann HS, Marroni NP. Effect of *Boswellia serrata* on antioxidant status in an experimental model of colitis rats induced by acetic acid. Digestive Diseases and Sciences. 2012;57:2038-2044.
23. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. Korean Journal of Physiology and Pharmacology. 2014;18:279-288.
24. Azza OFE, Afaf IA, Galal M. Toxicopathological effects of *Guiera senegalensis* extracts in wistar albino rats. Journal of Medicinal Plants Research. 2008;2(1):1–4.
25. Saidu TB, Galadima M, Jigam AA, Abalaka EM. Antibacterial and potential toxicological evaluation of leaf extracts of *Guiera senegalensis* against gram negative bacteria. Journal of Advances in Medical and Pharmaceutical Sciences. 2016;9(1):1-13.
26. Elmore SA. Enhanced histopathology of the bone marrow. Toxicologic Pathology. 2006;34:666-686.
27. Travlos GS. Normal structure, function, and histology of the bone marrow. Toxicologic Pathology. 2006;34(5):548-565.
28. Irons RD, Ed. Toxicology of the blood and bone marrow. Target Organ Toxicology and Serology. Raven Press, New York; 1985.
29. Reagan WJ, Irizarry-Rovira A, Poitout-Belissent F, Bolliger AP, Ramaiah SK, Travlos G, Walker D, Bounous D, Walter G. Best practices for evaluation of bone marrow in nonclinical toxicity studies. Veterinary Clinical Pathology. 2011;40(2): 119-134.
30. Yamamoto T, Irisa T, Sugioka Y, Sueishi K. Effects of pulse methylprednisolone on bone and marrow tissues. Corticosteroid-induced osteonecrosis in rabbits. Arthritis & Rheumatism. 1997;40(11):2055-2064.

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