DRUG DISCOVERY

Nauclea latifolia (smith) remediates the splenic architecture following paracetamol – induced toxicity in male Wistar rats

Ekanem Akpan Udo[1], **Edem Gabriel Donatus**[1*], **Okon Kingsley Akaninyene**[1], **Nwafor Paul**[2], **Harrison Imekan**[1]

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Author Affiliation:
[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria
[2]Department of Pharmacology, Faculty of Pharmacy, Rivers State University of Science and Technology, Rivers State, Nigeria

[*]**Corresponding author**
Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo,
Nigeria
Email: Profgabe4sure@gmail.com

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ABSTRACT

Nauclea latifolia root extract is used for many therapeutic purposes. This work was carried out to assess the effect of *Nauclea latifolia* root extract on the spleen following paracetamol toxicity in Wistar rats. A total of forty-two male rats were randomly divided into seven groups of six rats each. Group 1 and 2 served as control and received 10ml/kg body weight of normal saline for seven days, respectively. Rats in group 3,4 and 5 were administered with 40, 80 and 120 mg/kg of *Nauclea latifolia* extract respectively. In contrast, rats in group 6 received 100mg/kg of silymarin and rats in group 7 received 80mg/kg of *Nauclea latifolia* extract plus 100mg/kg silymarin, 10 minutes later. On the 8th day, 2000mg/kg of paracetamol was administered to animals in group 2, 3, 4, 5, 6 and 7 respectively. On the 9th day, each animal was sacrificed and the spleen was harvested and preserved. The spleen was processed and stained with hematoxylin and eosin for microscopic examination. Histological sections of the spleen in group 2 showed proliferation of cells in the mantle and marginal zones while group 3, 4 and 5 showed a reduction of lymphoid cells in the mantle and marginal zones respectively. Similarly, tissue sections from animals in group 6 and 7 showed several histopathologies ranging from lymphoid follicle hyperplasia, infiltration of plasma cells, etc. It is concluded from the study that the root extract of *Nuclea latifolia* contain certain substances that can reduce the damaging effect of paracetamol on the spleen but not in the presence of silymarin.

Keywords: *Nauclea latifolia,* Lymphoid cells, Spleen, Silymarin, Paracetamol, Ultrastructure.

1. INTRODUCTION

Herbal treatment is the most ancient form of treatment that has ever existed and is known to humanity. It is as old as human society, especially in developing and underdeveloped countries where many diseases are treated and managed with traditional medicine obtained from plant-based medicines. The term 'medicinal'

as applied to a plant indicates that it contains a substance or substances which modulate beneficially the physiology of sick mammals and that man has used it for that purpose (Fellows, 1991). It is estimated that about 80% of the world population living in vast rural areas of developing and under-developed countries still rely on medicinal plants for their healthcare delivery (Maridas and John-De-Britto, 2008). Plant active ingredients may be extracted through several processes such as concoction, decoction, infusion, maceration and charring depending on the nature of the plant for the purpose of managing several ailments.

Different parts of the medicinal plants are used for therapeutic purposes, including leaves, roots, latex, barks, stems, seeds, and flowers. The spleen is a relatively delicate and considered the most vulnerable abdominal organ. It is the largest of the lymphatic organs and it is known to participate in the body's defense as a site of lymphocyte proliferation and of immune surveillance and response. The spleen serves as a blood tank, storing red blood cells and platelets, and can provide some form of "self-transfusion" to respond to the stress imposed by bleeding. One of the plants that feature prominently from the ethnobotanical survey on medicinal plants is *Nauclea latifolia.* Traditional healers use it in many African countries to treat various sicknesses. The plant has been reported to alleviate ailments such as malaria, stomach and intestinal disorders, sleeping sickness, prolonged menstrual flow, raised blood pressure, and diabetes mellitus (Benoit-vical et al., 1998).

The roots are used as an aphrodisiac, analgesic and remedy for sexual inactivity (Deeni and Hussain, 1991). The anthelmintic activity of the aqueous extract of the stem bark had been evaluated and reported (Onyeyili et al., 2001). The medicinal values of this plant have been linked to its phytochemical constituents, such as indole alkaloids, saponins, glycosides, terpenes, carbohydrates, and anthraquinones. Other known constituents are polyphenols, tannins, flavonoids, phlobatannins, and resins (Lamidi et al., 1995; Trease and Evans, 2002; Shigemori et al., 2003). It is traditionally called a pin-cushion tree or African peach. It is a straggling shrub or tree native to tropical Africa and Asia. It commonly grows in most parts of Nigeria (Odugbemi, 2002). Despite the wide use of this plant *(Nauclea latifolia)* in West Africa, literature concerning its effects on the spleen remains scarce.

Paracetamol is a potent analgesic and non-opioid drug used to treat fever, headache and, pains. Paracetamol has been implicated in hepatotoxicity, and splenotoxicity. These toxicities resulted in the elevation of CD11b (+), infiltrating Ly6G (+), granulocytic and Ly6G (-) monocytic cells in the spleen and the liver (Mandal et al., 2016). Findings from Abbasi et al., (2018) reported white pulp degradation, depopulation and activation of follicles and cellular disruption in the spleen with overall disorganized stature of the organ. It is assumed that paracetamol influences the physiology of some organs, including the spleen. This work comes as a contribution to ascertaining the effect of paracetamol on the spleen and the possible ways of alleviating this burden with *Nauclea latifolia.*

2. MATERIALS AND METHODS

Experimental Animals

Forty-two (42) adult male albino rats 220-280grams were used for this study. Rats were obtained from the College of Health Sciences Animal House, University of Uyo, Nigeria. The rats were housed in clean cages, fed *ad libitum* with feed (growers mash), and clean drinking water. The rats were maintained at twelve hours (12 hours) light and dark cycles and room temperature of 27-300C. All the rats were handled according to standard guidelines for the care and use of laboratory animals (American Physiological Society, 2002).

Collection of *Nauclea latifolia* roots

Fresh roots of *Nauclea latifolia* were collected from the Medicinal Plant Farm of the Department of Pharmacology and Toxicology, University of Uyo, Nigeria. The plant was identified by a plant taxonomist, in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The fresh roots were washed with water, air dried for seven days and stored in a polythene bag.

Preparation of Extract

Dried roots *of Nauclea latifolia* were turned into powder using an electric blender. The powder was macerated in two-liters of methanol in a three-liter capacity glassware and was made air-tight and left for 72 hours with intermittent shaking. The mixture was filtered using Whatman's filter paper to obtain a solution devoid of solids. The methanol extract was evaporated using a rotary evaporator to get dry concentrate and stored in a refrigerator at about 40C till use.

Experimental Design

Forty-two adults male Wistar rats weighing between 220–280grams were used for this research. The animals were divided into seven groups of six rats per group. Group 1 and 2 served as control, and was treated with normal saline (10ml/kg body weight)

orally. Group 3, 4 and 5 rats were administered with 40mg/kg, 80mg/kg and 120mg/kg of *Nauclea latifolia* extract, respectively. Group 6 were administered with 100mg/kg of silymarin. Group 7 rats were initially administered with 80mg/kg of *Nauclea latifolia* extract solution, then 10 minutes later, 100mg/kg of silymarin was administered. The administration lasted for seven days. On the 8th day, the animals in group 2, 3, 4, 5, 6 and 7 were administered with 2000mg/kg of paracetamol. On the 9th day, the animals were sacrificed, and the spleen was harvested, cleaned and, stored in 10% formalin solution.

Tissue Processing

The preserved spleen was cut into smaller, 5mm thickness, then taken into ascending grades of alcohol from 70% to 95% and, then absolute alcohol for one hour each with occasional agitation to remove water from the tissues. The tissue was cleared in two changes of xylene, infiltrated in molten paraffin at 56oC and embedded in paraffin using molds. After that, the paraffin-embedded spleen was serially sectioned at 5 microns using a rotary microtome. Hematoxylin and eosin (H and E) technique was used in staining the sections. The sections were viewed under the light microscope and photomicrographs of tissue sections were taken.

3. RESULTS

The sections of spleen from control animals that were administered 10ml/kg of normal saline showed typical/standard histological presentation with the central arterioles, mantle zone, white and red pulps clearly demonstrated (Figure 1). The spleen from the group administered with 2000mg/kg of paracetamol showed marked increase in the concentration of lymphoid cells in the mantle and marginal zones, hypertrophic arteriole with fibrosis and smooth muscle cell proliferation as well as disorganization of splenic compartments (Figure 2). Animals that received 40mg/kg of the extract plus 2000mg/kg of paracetamol on the 8th day, showed reduction of lymphocytic cells and improvement of white pulp architecture (Figure 3). Group 4 rats given 80mg/kg of the root extract and 2000mg/kg of paracetamol on the 8th day showed reduced concentration of lymphoid cells in the mantle and marginal zones, reduced smooth muscle hypertrophy in the arteriole and improvement in splenic architecture (Figure 4).

The section of spleen from group 5 rats that received 120mg/kg of the root extract plus 2000mg/kg of paracetamol on the 8th day demonstrated marked reduction of lymphoid cells in the mantle zones of the white pulp, reduced smooth muscle hypertrophy in the arteriole and red blood cells in the marginal zone of red pulp (Figure 5). The sections of spleen from group 6 rats administered 100mg/kg of silymarin and 2000mg/kg of paracetamol on the 8th day showed hypertrophied arteriole with fibrosis, reduction in the lymphoid cells of the mantle and marginal zones of the white pulps (Figure 6). The spleen from group 7 rats that received 80mg/kg of the root extract, 100mg/kg of silymarin and 2000mg/kg of paracetamol showed complete depletion of lymphoid cells from the mantle and marginal zones of the white pulps, diffused red pulp, fibrous infiltration and congested arteriole (Figure 7).

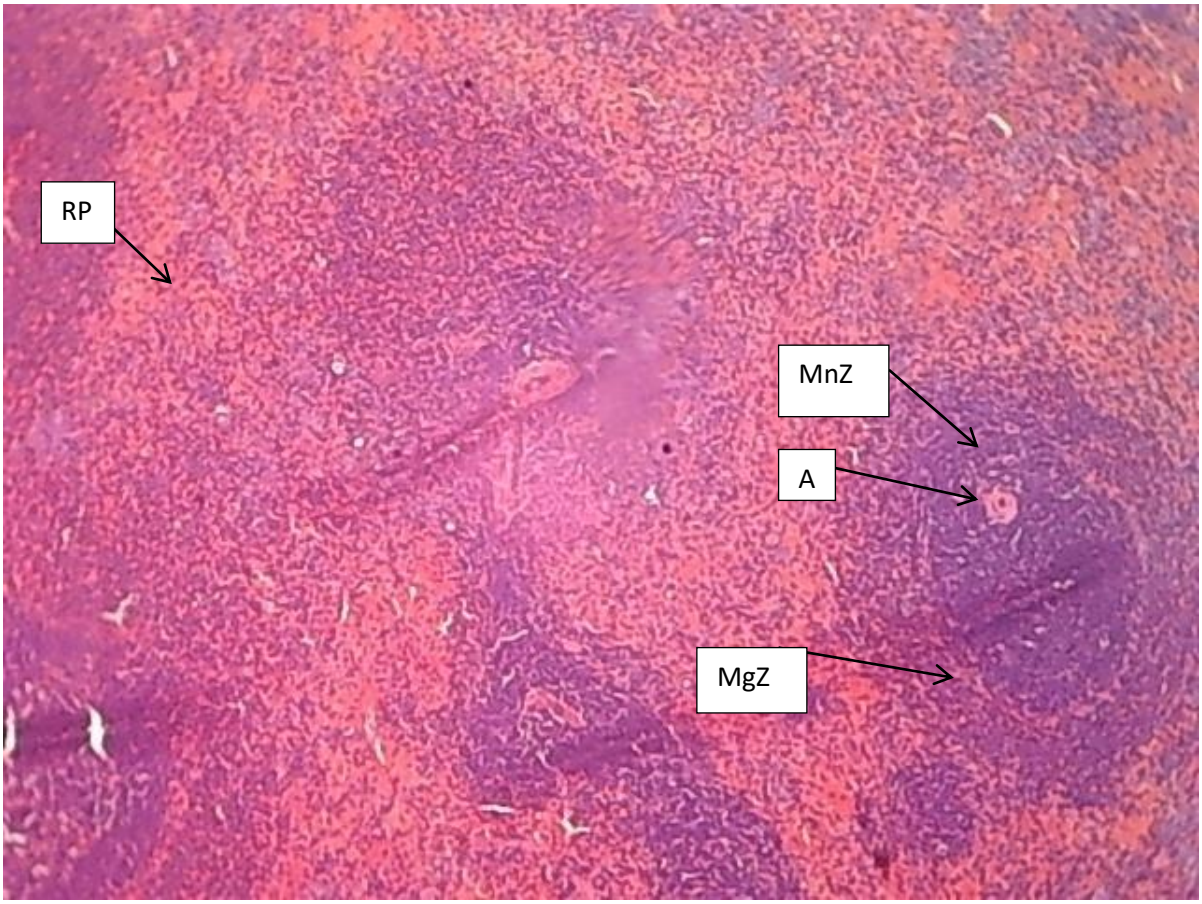

Figure 1 Micrograph (T/S) of spleen of control rat showing, typical splenic histology, central arteriole (A), mantle zone (MnZ), and marginal zone (MgZ) of the white pulp, and red pulp (RP) X100. H & E stains.

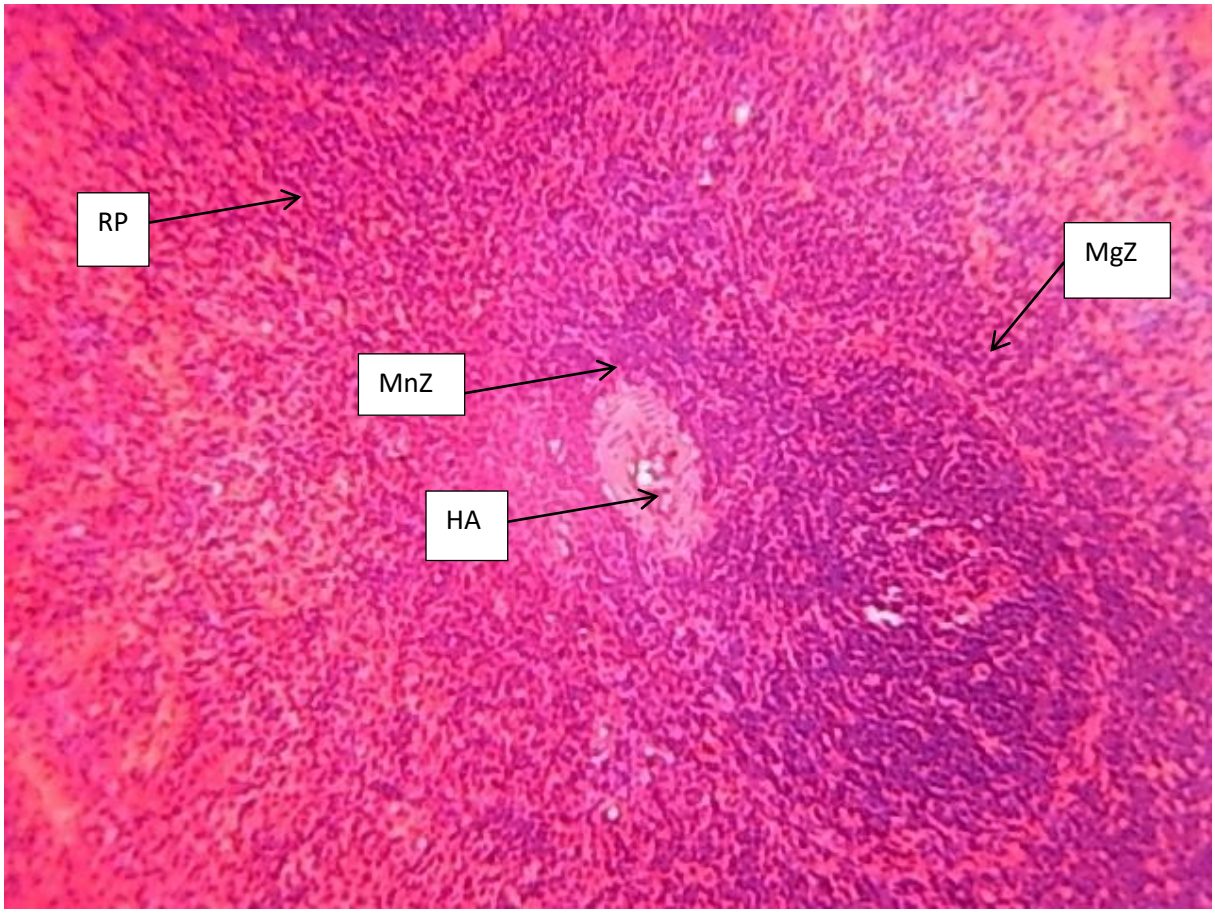

Figure 2 Micrograph (T/S) of spleen of rat administered with 2000mg/kg of PCM showing, hypertrophic arteriole with fibrosis and smooth muscle cell proliferation (HA), proliferation of cells in the mantle (MnZ), and marginal (MgZ) zones of white pulp and red pulp (RP) with disorganization of splenic compartments X100. H & E stains.

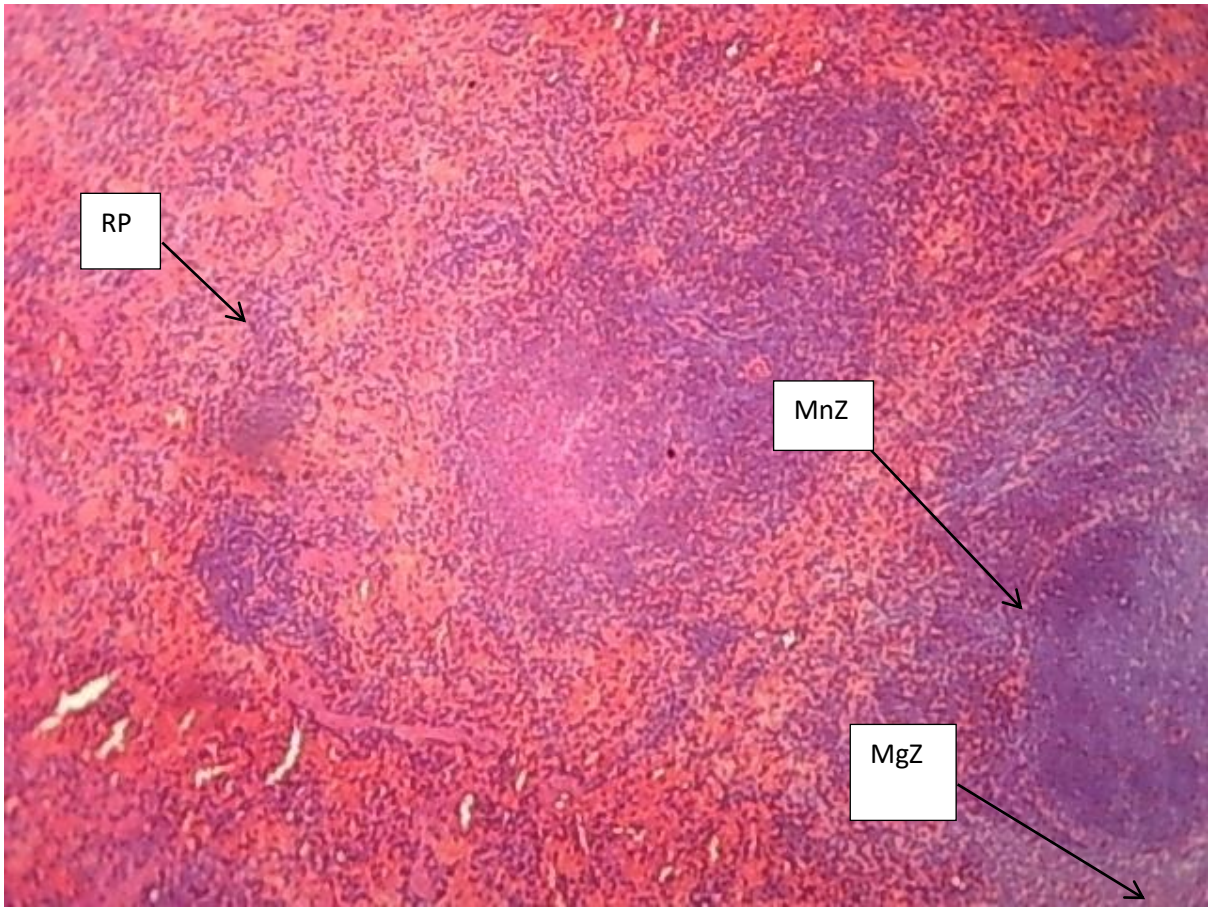

Figure 3 Micrograph (T/S) of spleen of rat administered with 40mg/kg of *Nauclea latifolia* extract for 7 days, and 2000mg/kg of PCM on the 8th day showing, reduction of cells in the mantle (MnZ) and, marginal zones (MgZ) of white pulp and, red pulp (RP) with improvement in white pulp architecture X100. H & E stains

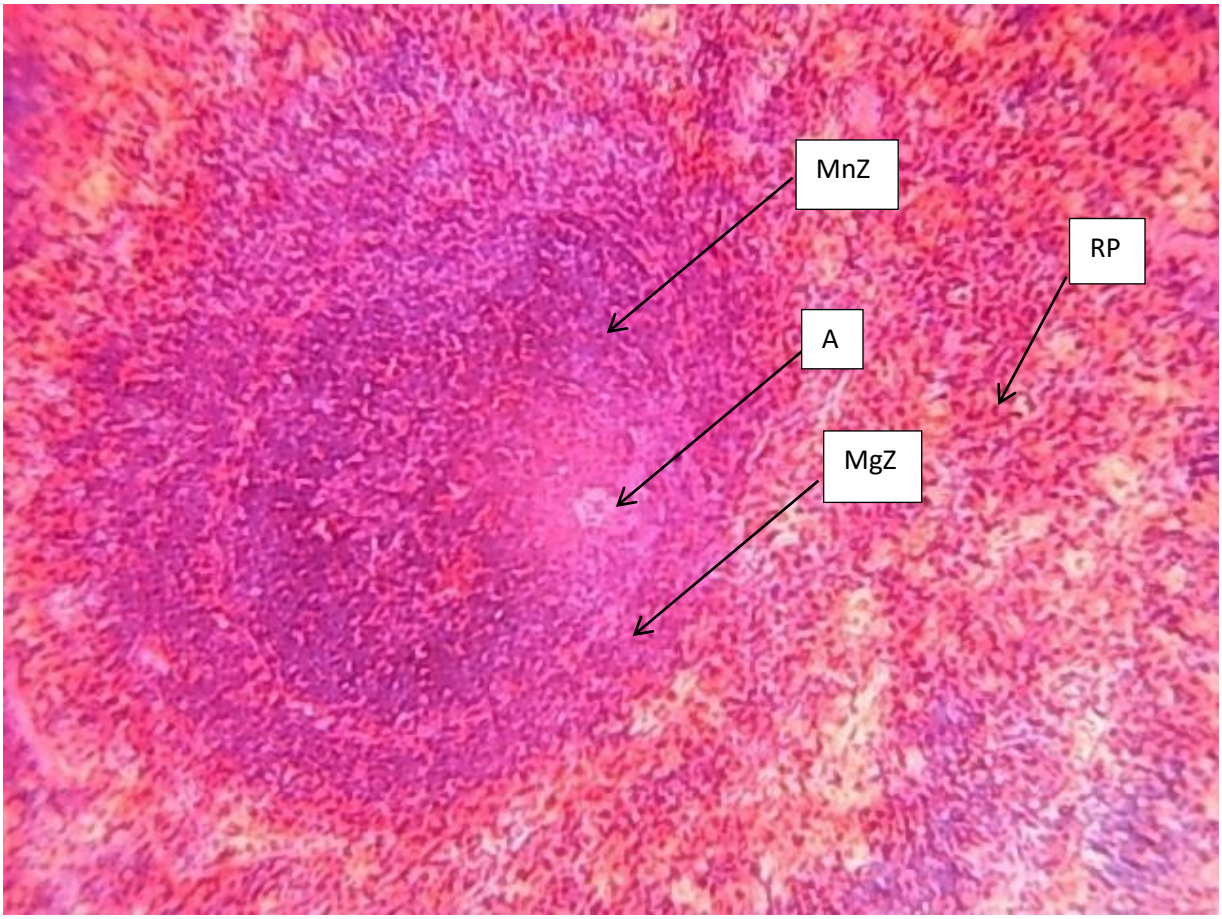

Figure 4 Micrograph (T/S) of spleen of rat administered with 80mg/kg of *Nauclea latifolia* extract for 7 days, and 2000mg/kg of PCM on the 8th day showing, reduced smooth muscle hypertrophy in the arteriole (A), reduction of cells in the mantle zone (MnZ), marginal zone (MgZ) of white pulp and red pulp (RP) with improvement in the splenic organization X100. H & E stains.

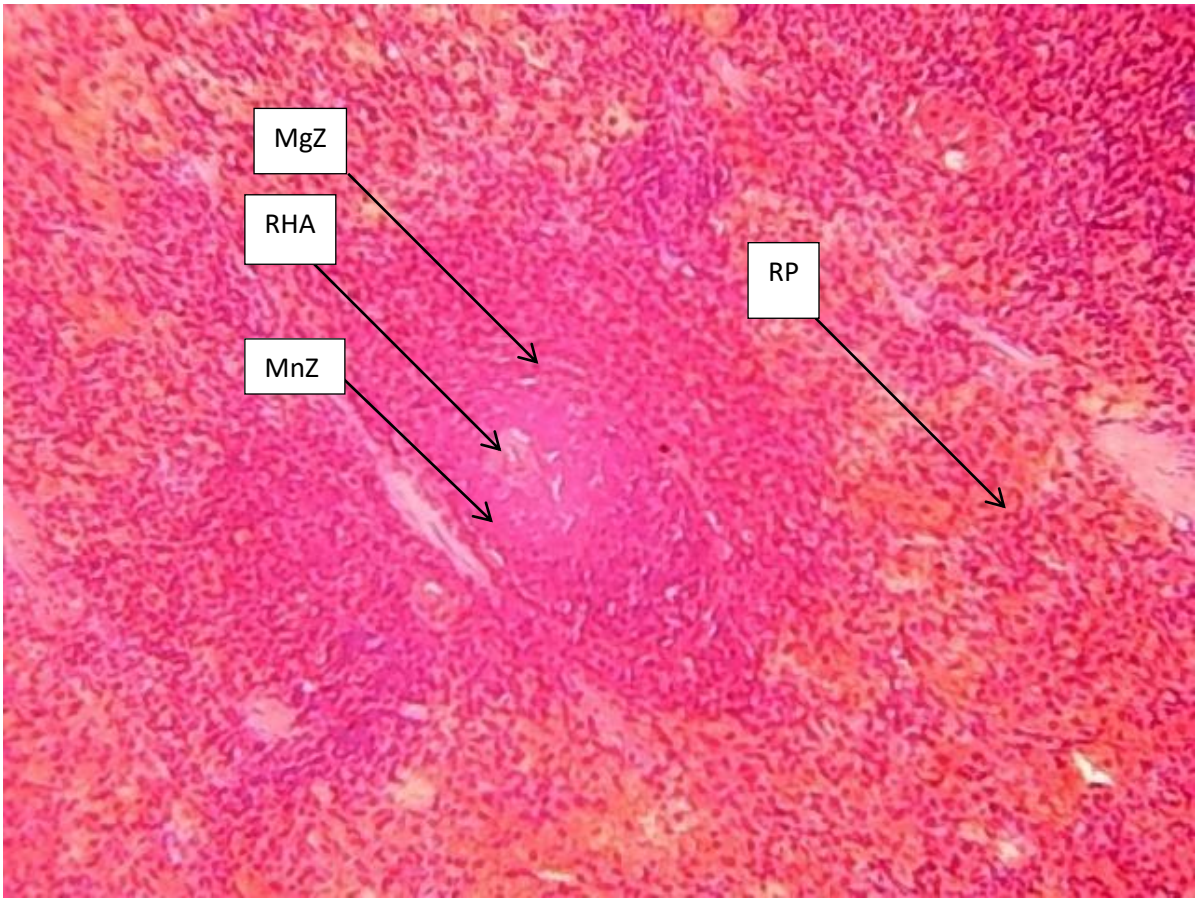

Figure 5 Micrograph (T/S) of spleen of rat administered with 120mg/kg of *Nauclea latifolia* extract for 7 days, and 2000mg/kg of PCM on the 8th day showing, reduced smooth muscle hypertrophy in the arteriole (RHA), reduction of cells in the mantle (MnZ) and marginal zones (MgZ) of white pulp and that of the red pulp (RP) X100. H & E stains

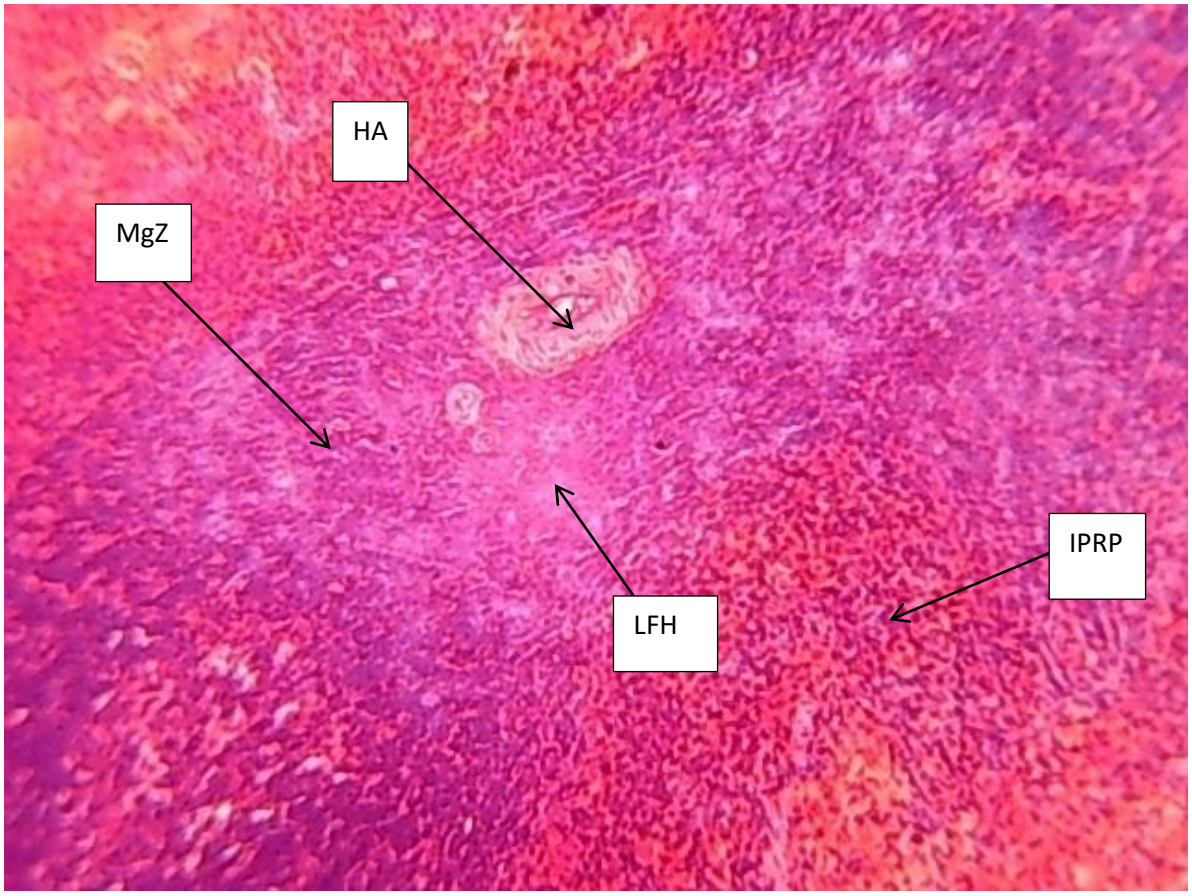

Figure 6 Micrograph (T/S) of spleen of rat administered with 100mg/kg of Silymarin for 7 days and 2000mg/kg of PCM on the 8th day showing, hypertrophic arteriole (HA), lymphoid follicle hyperplasia (LFH) in the white pulp and infiltration of plasma cells in the red pulp (IPRP) X100. H & E stains.

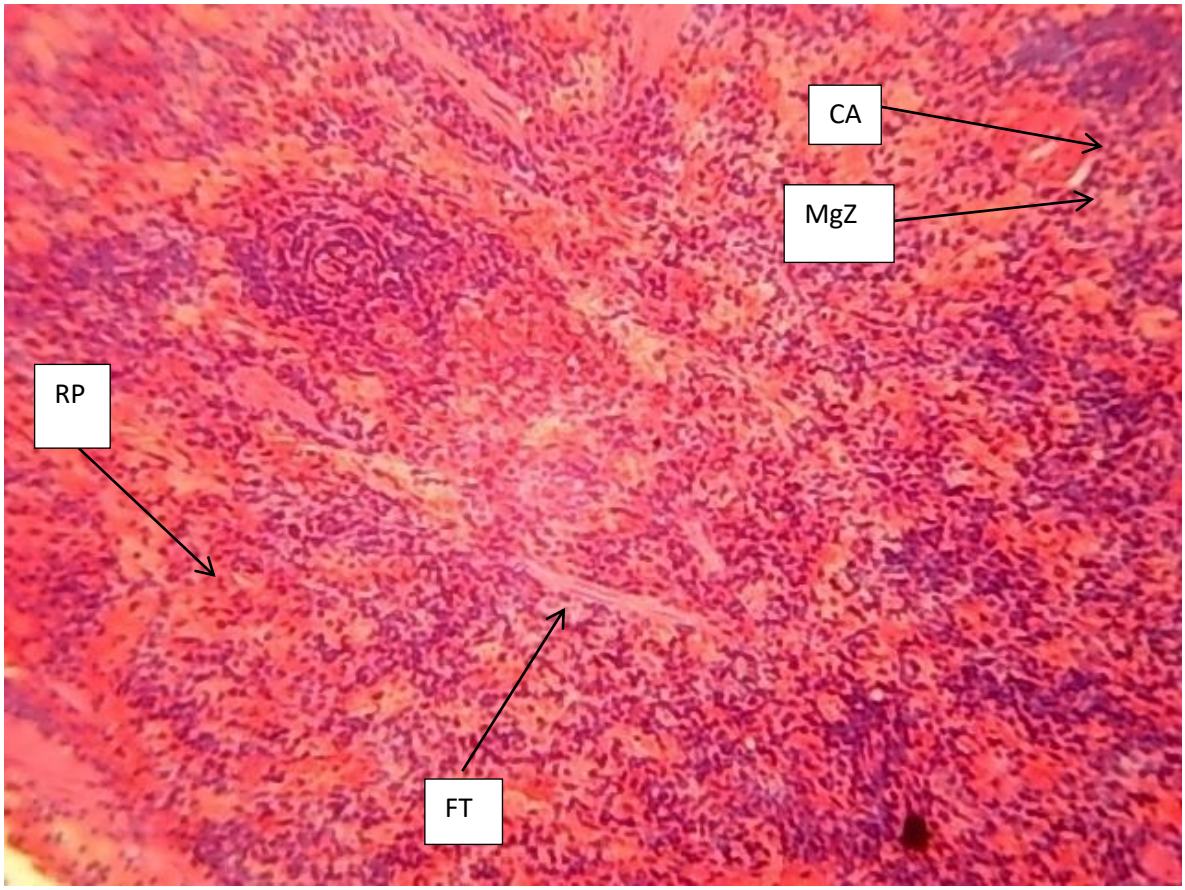

Figure 7 Micrograph (T/S) of spleen of rat administered with 80mg/kg of *Nauclea latifolia* for 7 days, 100mg/kg of Silymarin for 7 days, and 2000mg/kg of PCM on the 8th day showing, congested arteriole (CA), complete depletion of cells and disorganization of compartment in the marginal zones (MgZ) of white pulp, an increase fibrous activity (FT) in the red pulp (RP) X100. H & E stains.

4. DISCUSSION

The spleen from the control group administered 10ml/kg normal saline had normal histological presentation showing the central arteriole, mantle zone, white and red pulps. However, the spleen of rats administered 2000mg/kg of paracetamol showed increase and proliferation in the concentration of lymphoid cells in the mantle and marginal zones, hypertrophic arteriole with fibrosis and infiltration of plasma cells. This is because lymphoid cells have been mobilized into the body tissues in the presence of the toxicant, paracetamol, which have been implicated in various organ disorders such as the kidney, etc (Hook, 1980). Similarly, it has been reported that paracetamol can adversely have unintended impacts on the immune system. Sections of animals that received 40mg/kg of extract plus 2000mg/kg of paracetamol on the 8th day, showed a reduction of lymphocytes in the marginal zone as well as improvement of the white pulp ultrastructure.

Similarly, sections of animals that were given 80mg/kg of the extract plus 2000mg/kg of paracetamol also had reduced concentration of lymphoid cells seen in both the mantle and marginal zones of the spleen, reduced smooth muscle hypertrophy in the arteriole and improvement in the splenic architecture. This indicated that the extract has some protective effect against paracetamol toxicity. *Nauclea latifolia* has been reported to exert both positive and negative impacts on specific organs in the body. It has been found to protect the prefrontal cortex from a toxicant such as valproic acid (Lucky and Yibala, 2019). Moreover, sections of animals that received 120mg/kg of extract for 7 days and 2000mg/kg of paracetamol only on the 8th day showed a reduction of cells in the mantle zone of the white pulp, as well as red blood cells in the marginal zone of the red pulp and reduced hypertrophy in the arteriole.

This result indicated time and dose-dependent action of the plant extract following paracetamol toxicity. Sections of the spleen that received 80mg/kg of the extract and, 100mg/kg of silymarin and 2000mg/kg of PCM on the 8th day, showed lymphoid follicle hyperplasia in the white pulp, smooth muscle hypertrophy in the arteriole, and infiltration of plasma cells. This is because, silymarin was unable to protect the spleen from the adverse impact of the toxicant, and is believed to counteract the protective activity of the plant extract on the spleen. Silymarin is an antioxidant specific to treating various forms of hepatic issues.

Environmental toxicants such as drugs can alter the immune response and enhance immune-mediated diseases, some of which are allergies and fibromyalgia. Similarly, the pro-inflammatory immune response initiated by exposure to some toxicants can bring about immune dysfunction, which is a common factor in many autoimmune diseases. Our study also showed that, the extract in the presence of silymarin could not protect completely the pressure exerted by paracetamol. This may be due to the counter-therapeutic effect caused by the interaction between the extract and silymarin.

5. CONCLUSION

The results obtained from this study indicated that *Nauclea latifolia* root extract contains substances that can protect the spleen from the effect of the toxicant (paracetamol). Similarly, the spleen was unprotected in the presence of silymarin, which is a known and specific hepatoprotective substance.

Authors' Contribution

All authors were involved from the beginning of the work till the completion. They were all involved in the experimental set up and design, interpretation of results, writing and editing of the manuscript.

Informed consent

Not applicable.

Ethical approval

All the rats were handled according to American Physiological Society standard guidelines for the care and use of laboratory animals. The Animal ethical guidelines are followed in the study for experimentation. The ethical guidelines for plants & plant materials are followed in the study.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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