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Extract of Ginger (*Zingiber Officinale*) on the Histology of the Spleen Using Adult Male Rats

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Abstract
Usage of botanical medicine is ancient and plant chemicals are still the backbone of our pharmacopoeia because more than 50% of drugs used in Western pharmacopoeia are isolated from herbs or derived from modification of chemicals first found in plants. *Zingiber officinale* is one of the most widely used herbs and food flavouring agent and commonly known as ginger. This study was done to evaluate the possible histological effect(s) of ethanolic extract of *Zingiber offinale* on the liver of adult male albino rats. Twenty five (25) adult wistar rats weighing between 125-200g were divided into five groups of five (5) rats each. Group C, D and E served as experimental groups while group A and B served as the control groups. Group C was administered a dose of 100mg/kg of the extract, group D was given oral dose of 250mg/kg of the extract while group E was given oral dose of 500mg/kg of *Zingiber officinale* extract. Administration of extract lasted for fourteen days at the end of which the animals were sacrificed using chloroform-inhalation method. The spleen was harvested as tissue samples from sacrificed animals for pathological examination using routine histological procedure and stained with the haematoxylin and eosin stains. Histological examinations of spleen showed that after treatment with low and medium doses (100, 250mg/kg) the ginger extract produced little damaging effects on the spleen histology, indicating moderate lymphoid hyperplasia in the splenic cells. But at higher dosage (500mg/kg), reactive lymphoid hyperplasia in the splenic cells was extensive. Therefore, *Zingerber officinale* should be used with caution because it may have harmful effects on the spleen cells at high doses.
Keywords: Ginger, Histology, Ethanolic extract, Spleen, Wistar rat

INTRODUCTION

Plants are the basic source of knowledge of modern medicine. The burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care (Ahmed M. and Hussain, 2013). Several plants of diverse origins have been exploited by trial and error over many generations for therapeutic purposes. In Africa and in most of the developing countries, plants' properties are empirically appreciated.

Ginger is the underground rhizome of the ginger plant with a firm striated texture. The flesh of the ginger rhizome can be yellow, white or red color, depending upon the variety. It is cover with a brownish skin that may either be thick or thin, depending upon whether the plant was harvested when it was mature or young. It lends its name *Zingiber officinale* to its genius and family (*zingibera ceae*) (Akoachere, 2002), other notable members of this plants family are turmeric, cardamom and galangal.

Phytochemical studies have shown that the unique culinary and medicinal properties of ginger are due to the presence of phytochemicals like zingerone, shogaols , gingerols, pardols, β-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, β-elemene, zingiberol, linalool, α-zingiberene, β-sesquiphellandrene, β-bisabolene, zingiberenol and α-farmesene (Baliga *et al.,* 2011; Haniadka *et al.,* 2013). Scientific studies carried out in accordance to the principles of modern system of medicine have convincingly shown that ginger possesses numerous health benefits like antimicrobial, antiviral, gastroprotective, antidiabetic, anti-hypertensive, cardioprotective, anticancer, chemopreventive and immunomodulatory effects (Baliga *et al.,* 2011; Ali *et al.,* 2008).

The spleen is an organ found in virtually all vertebrates. Similar in structure to a large lymph node, it acts primarily as a blood filter. It is possible to remove the spleen without jeopardizing life. The spleen plays important roles in regard to red blood cells (also referred to as erythrocytes) and the immune system.[3] It removes old red blood cells and holds a reserve of blood, which can be valuable in case of hemorrhagic shock, and also recycles iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is removed in the liver. (Mebius and Kraal 2005)

The spleen synthesizes antibodies in its white pulp and removes antibody-coated bacteria and antibody-coated blood cells by way of blood and lymph node circulation. A study published in 2009 using mice found that the spleen contains, in its reserve, half of the body's monocytes within the red pulp. (Swirski et al., 2009) These monocytes, upon moving to injured tissue (such as the heart), turn into dendritic cellsand macrophages while

promoting tissue healing. (Swirski et al., 2009; Jia, T and Pamer, EG, 2009). The spleen is a center of activity of the mononuclear phagocyte system and can be considered analogous to a large lymph node, as its absence causes a predisposition to certain infections. (Brender et al., 2005)

In humans, the spleen is brownish in color and is located in the left upper quadrant of the abdomen. (Mebius and Kraal2005; Loscalzo et at., 2008)

The spleen, in healthy adult humans, is approximately 7 centimetres (2.8 in) to 14 centimetres (5.5 in) in length. It usually weighs between 150 grams (5.3 oz) and 200 grams (7.1 oz). (Spielmann et al., 2005) An easy way to remember the anatomy of the spleen is the 1×3×5×7×9×11 rule. The spleen is 1" by 3" by 5", weighs approximately 7 oz, and lies between the 9th and 11th ribs on the left hand side.

Functions of the spleen include the following

1. Production of opsonins, properdin, and tuftsin.
2. Creation of red blood cells. While the bone marrow is the primary site of hematopoiesis in the adult, the spleen has important hematopoietic functions up until the fifth month of gestation. After birth, erythropoietic functions cease, except in some hematologic disorders. As a major lymphoid organ and a central player in the reticuloendothelial system, the spleen retains the ability to produce lymphocytes and, as such, remains a hematopoietic organ.
3. Storage of red blood cells, lymphocytes and other formed elements. In horses, roughly 30% of the red blood cells are stored there. The red blood cells can be released when needed. (Carey, 2006) In humans, up to a cup (236.5 ml) of red blood cells can be held in the spleen and released in cases of hypovolemia. It can store platelets in case of an emergency and also clears old platelets from the circulation. Up to a quarter of lymphocytes can be stored in the spleen at any one time.

MATERIALS AND METHOD

Preparation of ethanolic extract of *zingiber officinale* plant

Fresh ginger (*zingiber officinale* roscoe) rhizome was purchased from the local market at Yala Local Government, Cross River State, Nigeria. The roots were identified and authenticated by the botanist in the botany department, University of Calabar, Calabar.

2.5kg of fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried for two weeks and crushed into powdered form using an electric blender

2000g (2kg) of this powdered ginger was macerated completely in 5000ml of 99.9% ethanol and shaken vigorously. It was allowed to stand for 48 hours at room temperature and was stirred at intervals.

After 48 hours, the dissolved ginger in ethanol was filtered using at first a material with small pores after which it was filtered again using No1 whatmann paper (filter paper) and funnel. The filtrate was collected in a tray and was air dried for 5 days. This was to ensure the complete evaporation of the ethanol used.

The ginger paste obtained was collected from the tray with the aid of a spatula into a container and was measured using an electric weighing balance. 50g of ginger paste was extracted and was then dissolved in 100ml of extra virgin olive oil (which served as the vehicle). This extract was kept in a dry place at room temperature.

Breeding/grouping of animals

Twenty-five adult albino male wistar rats weighing between 90g to 130g were purchased from the department of pharmacology animal farm, university of Calabar, Calabar. These animals were housed in well ventilated animal cages and were kept in the animal house of the department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar. The animal house was properly fitted with bright light and environmental temperature always kept at a range of 28 to 32 degrees Celsius. The house was constantly kept clean and disinfected.

The animals were fed with growers mesh obtained from vital feed located in Calabar and Distilled water daily with the aid of water bottles and were allowed to acclimatize for a period of 14 days.

After the fourteenth day of acclimatization, the rats weighed between 125-200g. They were then randomly selected into five groups with each group containing five rats in well labelled cages.

Plant extract administration

The animals were divided into five groups with five rats each.

GROUP A: was the normal control group. The animals were administered distilled water and growers mesh (normal laboratory diet) only.

GROUP B: was the vehicle control group (virgin olive oil control group). They were administered 2ml of virgin olive oil.

GROUP C: was the low dose group. They were administered 100mg/kg body weight of the ethanolic extract of Zingiber officinale.

GROUP D: was the medium dose group. The animals were administered 250mg/kg body weight of the ethanolic extract of Zingiber officinale.

GROUP E: was the high dose group. The animals were administered 500mg/kg body weight of the ethanolic extract of Zingiber officinale.

Each animal in the experimental groups was administered the plant extract based on its body weight and administration was done using the oral route throughout the period of the experiment (which lasted for 14 days) after which the animals were sacrificed, liver harvested and processed for histological observation.

RESULT

The following results were obtained from the histological process using Haematoxylin and Eosin staining method.

CONTROL (GROUP A): This group received no extract of *zingiber officinale* but was given feed and distilled water. Section shows a preserved cytoarchitecture of the spleen consisting of white pulp (WP) and red pulp (RP). The white pulp has a centrally located blood vessel known as the central arteriole surrounded by aggregates of lymphocytes. Red pulps consist of sinusoidal spaces and mild population of lymphocytes with red blood cells Figure 1.

OLIVE CONTROL (GROUP B): This group received feed and distilled water as well as 2ml of the vehicle (olive oil) daily. Section of the spleen shows prominent splenic pulp consisting of the white pulp (WP) and the red pulp (RP). The white pulp consists of aggregates of lymphocytes with eccentrically placed blood vessel (BV). The red pulp consist of sinusoid spaces (SS), lymphocytes (LYM) and red blood cells. The white pulps are with a darker staining mantle zone and a lighter staining germinal zone Figure 2.

MEDIUM DOSE (GROUP D): The group received 250mg/kg body weight of the extract for a period of 14 days. Section of the spleen shows prominent lymphoid follicles with centrally to eccentrically located blood vessels. The follicles (white pulp) consist of aggregates of lymphocytes (LYM). The red pulps (RP) are prominent and shows a normal configuration. Congested dilated blood vessels (BV) are present. Finding shows a reactive lymphoid hyperplasia Figure 4.

HIGH DOSE (GROUP E): The high dose group received 500mg/kg body weight of the extract for a period of 14 days. Section shows prominent enlarged white pulp (WP) consisting of proliferating lymphocytes (LYM). The outer mantle zone is darker than the inner germinal centre. Cellular disintegration and loss of nuclei material are observed, thus the lymphocytes and the white pulp are decolourized. There is constriction of the central arteriole. There is detachment of white pulps from spleen parenchyma. Loss of cells in red pulp (RP) evidenced by dilated sinusoid and nuclei pyknosis. Findings are suggestive of reactive lymphoid hyperplasia. Figure 5

DISCUSSION

Zingiber officinale commonly known as 'ginger' has its origin traced to Asia. It has a lot of medicinal uses as far as herbal medicine is concerned. It has been proven to have anticovulsant, antidiuretic, anti-inflammatory, diuretic, antifungal, antihypertensive, antispasmodic, antitumor, and anti-cancer. It has other medicinal values which are too

Ginger is widely used in different parts of the world as a spice for cooking different kinds of food and also in the baking industries as flavour and spice for making biscuits, bread and cakes. It can be eaten raw and as food additive. Certain people also used zingiber officinale in ginger ale, ginger breads, ginger snaps, ginger cake and ginger biscuits (Hashimoto *et al* 2002). Ginger contains volatile oils (~1% to 3%) and non-volatile pungent components oleoresin Zick *et al.,* (2008). A variety of active components were identified in the oleoresin of ginger including gingerols and shogaols. Gingerols are a series of homologues with varied un branched alkyl chain length, whereas shogaols are a series of homologues derived from gingerols with dehydration at the C-5 and C-4 during long-term storage or thermal processing. Other active compounds from the oleoresin portion of ginger were also reported, such as 6-paradol; 6- and 10-dehydrogingerdione; 6-and 10-gingerdione; 4, 6, 8, and 10-gingerdiol; 6- methylgingerdiol; zingerone; 6-hydroxyshogaol; 6-, 8-, and 10-dehydroshogaol; and diarylheptanoids Sang *et al.,* (2009). Among these compounds, gingerols and shogaols are the major constituents of oleoresin, while the other compounds are present in a limited amount, accounting for 1-10% of the overall amount of gingerols and shogaols Sang *et al*., (2009). Gingerols (especially 6-gingerol) are the major components in the fresh ginger rhizome. The amount of shogaols is increased in the dried ginger, as evidenced by the reduction of the ratio of 6-gingerol to 6-shogaol from 10:1 in fresh ginger to 1:1 in dried ginger Wu *et al*., (2010). Since ginger extracts contain various components, it would be important to identify which compounds are responsible for their pharmacological effects. It was demonstrated that 6-, 8-, and 10-gingerols and 6-shogaol showed efficacy in anti-inflammatory, antibacterial, antipyretic, antilipidemic, antitumorigenic, and antiangiogenic effects Park *et al.*, (2009). In addition, 6- gingerol was shown to inhibit leukotriene A4 hydrolase (LTA4H) and suppress anchorage-independent cancer cell growth in colorectal cancer cells (HCT116 and HT29) with IC5o's of 50 and 35 uM, respectively Jeong *et al*., (2009). Sang *et al*., (2009) demonstrated that 6-, 8-, and 10- shogaols exhibited much higher antiproliferative potency than 6-, 8-, and 10-gingerols against human lung cancer cells (H-1299) with IC5o's of 8μM for 6-shogaol and 150 μM for 6-gingerol. In addition, 10-

gingerol was the most potent among the gingerol Sang *et al*., (2009) Furthermore, Dugasani *et al*., (2010) found that 6-shogaol showed the most potent efficacy of antioxidative activity with an IC50 of about 8μM, while 6, 8, and 10-gingerols had IC5o's of 28, 20, and 12μM, respectively.

From the above result, it can be observed that at high dosage, ginger extract can be deleterious to the spleen as observed in the alteration of the cytoarchitecture of the splenic histology. The observed effect is largely due to the presence of 6-8-10 gingerols and 6-10-shogaols which are the affective components responsible for its pharmacology effect. Park *et al* (2007). However, this result is not in line with documented effects on ginger in the immune system which has been found to help balance the immune system and restore it to proper functioning, thus enhancing the protective function of the immune system. Ahui *et al* (2008) documented that ginger had been found to suppress the Th2-mediated immune response thereby reducing the effect of asthma. Also in a study by Nirmala *et al* (2010) ginger was shown to protect against the harmful effects of xenobiotics in rats by helping the body climiate xenobiotics identified as cancer-causing agents.

CONCLUSION

The observed effect from the result, is dose dependent as greater effects were seen in animals that were administered high doses. This effect may be due to the highlighted phytochemicals contained in the plant.

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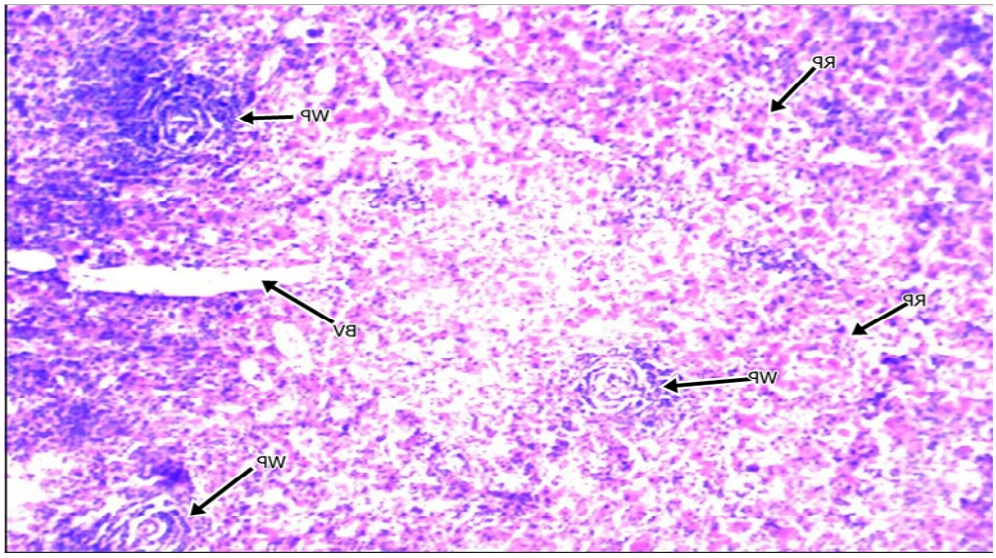

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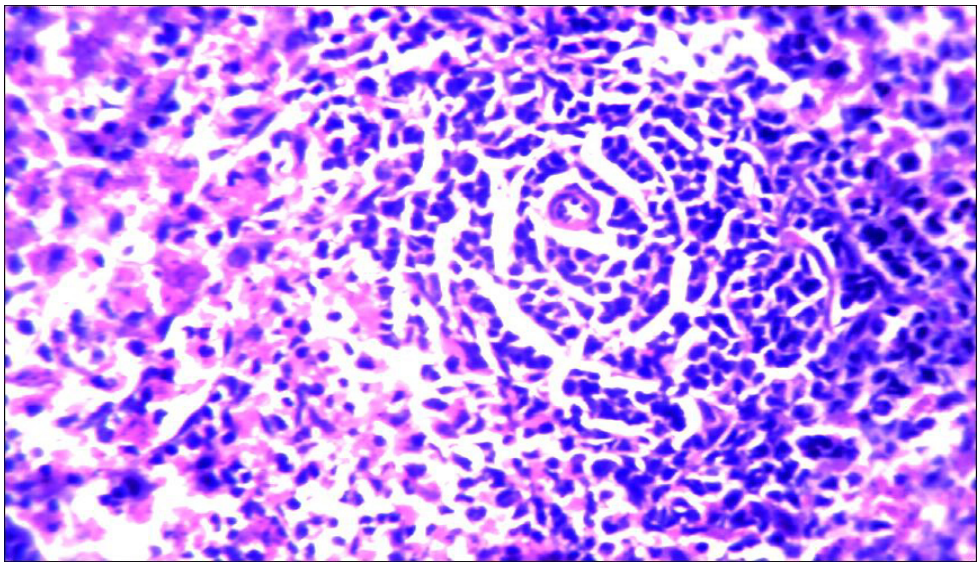

Figure 1: Control group: section shows a preserved cytoarchitecture of the spleen consisting of white pulp (WP) and red pulp (RP). The white pulp has a centrally located blood vessel known as the central arteriole surrounded

by aggregates of lymphocytes. Red pulps consist of sinusoidal spaces and mild population of lymphocytes with red blood cells.

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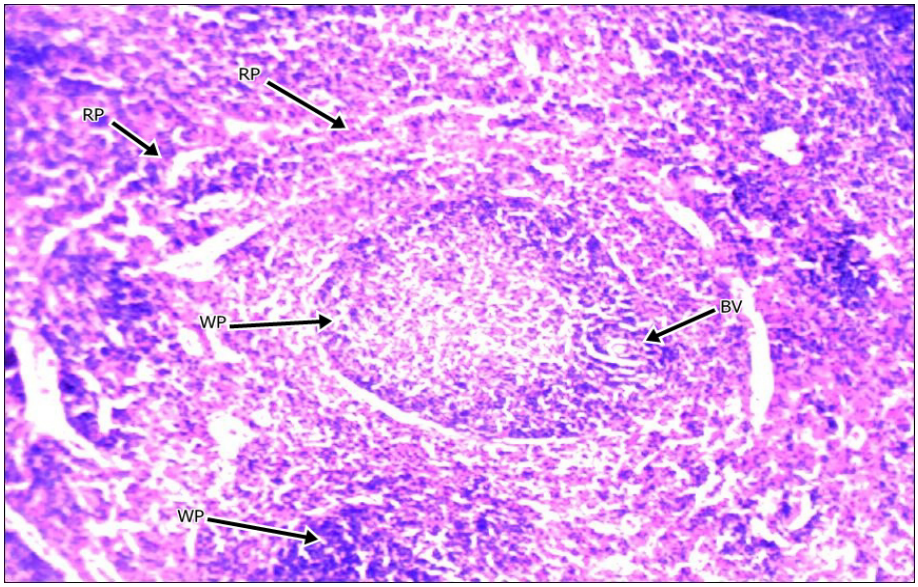

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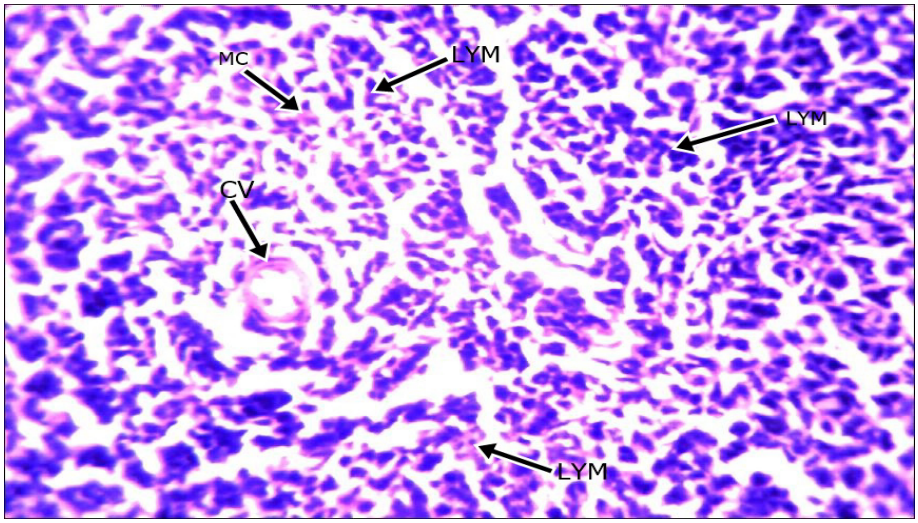

Figure 2: Olive control group: Section of the spleen shows prominent splenic pulp consisting of the white pulp (WP) and the red pulp (RP). The white pulp consists of aggregates of lymphocytes with eccentrically placed blood vessel (BV). The red pulp consist of sinusoid spaces (SS), lymphocytes (LYM) and red blood cells. The white pulps are with a darker staining mantle zone and a lighter staining germinal zone.

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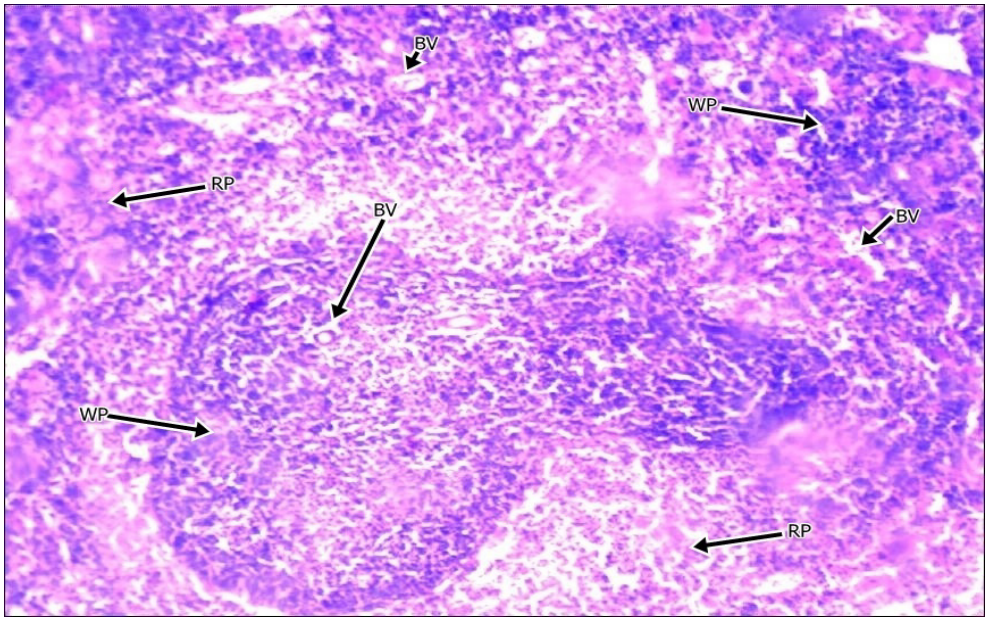

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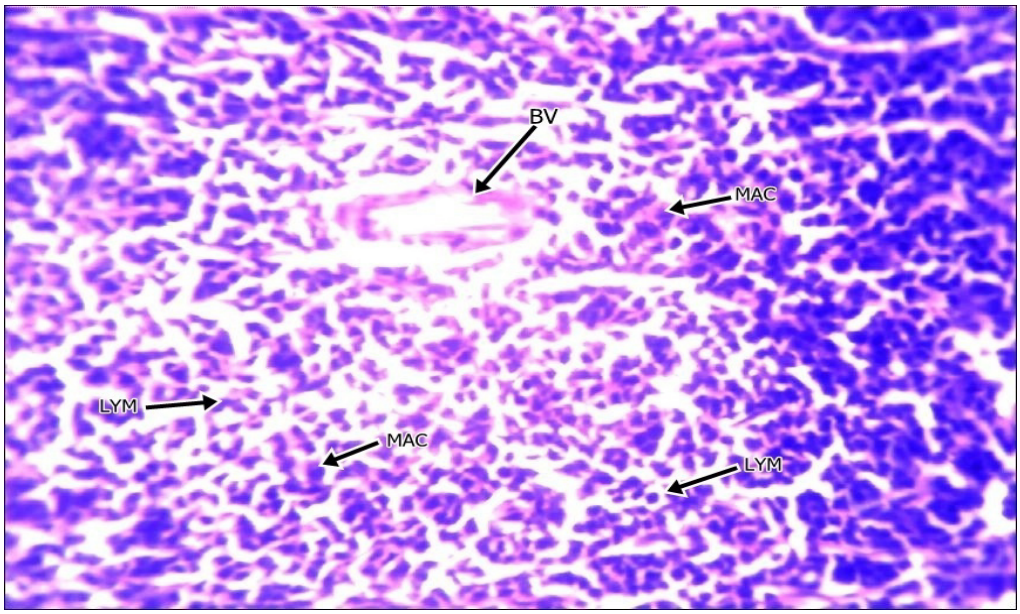

Figure 3: Low dose: Section of the spleen shows enlarged lymphoid follicles in the white pulp (WP) with reactive germinal centres containing lymphocytes (LYM) and tangible body macrophages (MAC) surrounded by a darker mantle zone. White pulp contains a central blood vessel (BV). The red pulp (RP) contains sinusoid spaces (SS) and moderate population of lymphocytes and red blood cells. Finding shows a reactive lymphoid hyperplasia.

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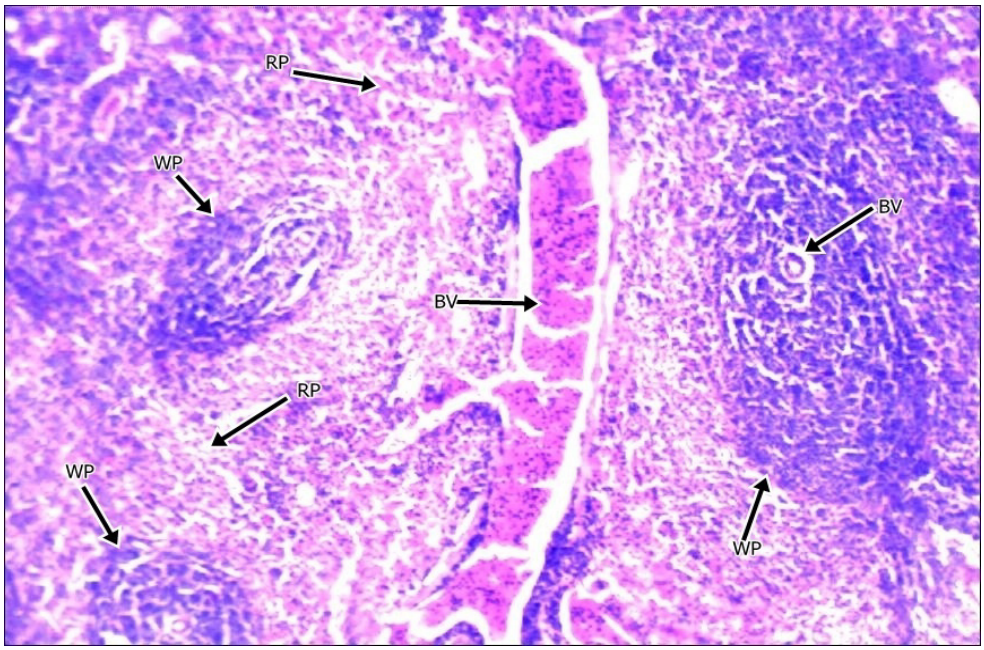

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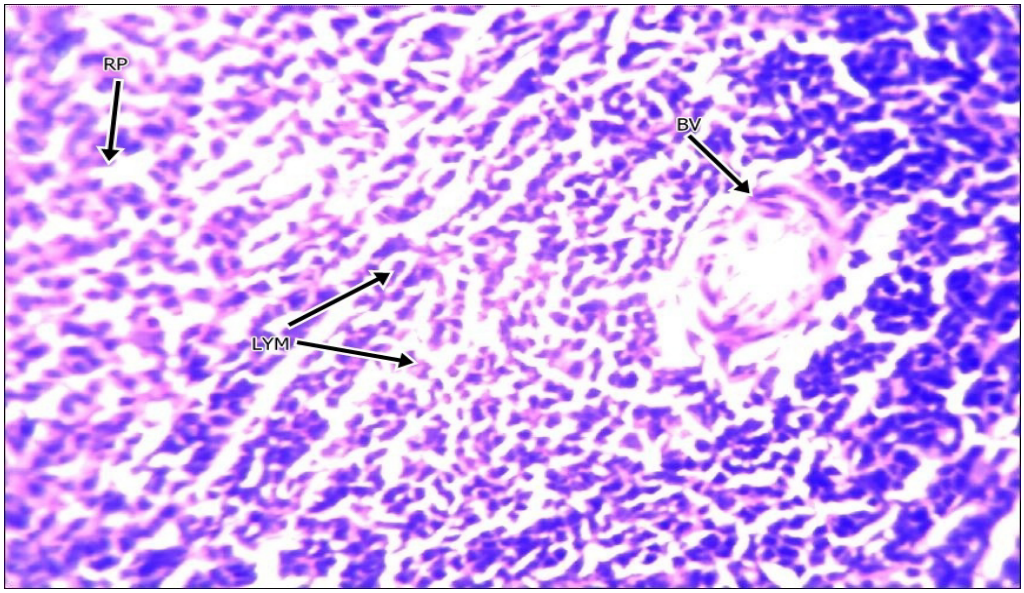

Figure 4: Medium dose Section of the spleen shows prominent lymphoid follicles with centrally to eccentrically located blood vessels. The follicles (white pulp) consist of aggregates of lymphocytes (LYM). The red pulps (RP) are prominent and shows a normal configuration. Congested dilated blood vessels (BV) are present. Finding shows a reactive lymphoid hyperplasia.

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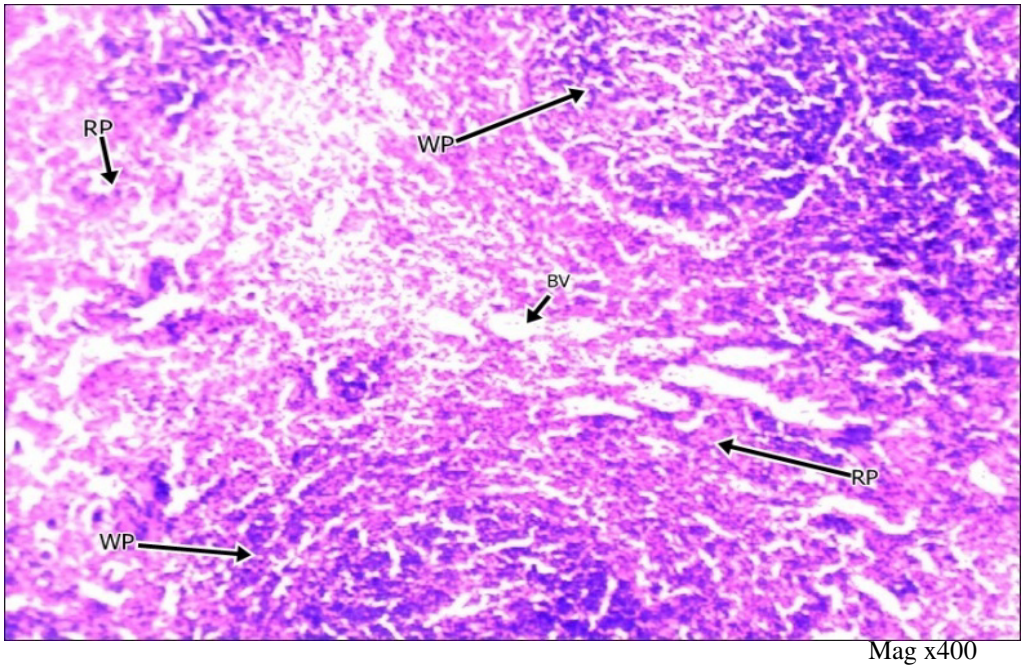

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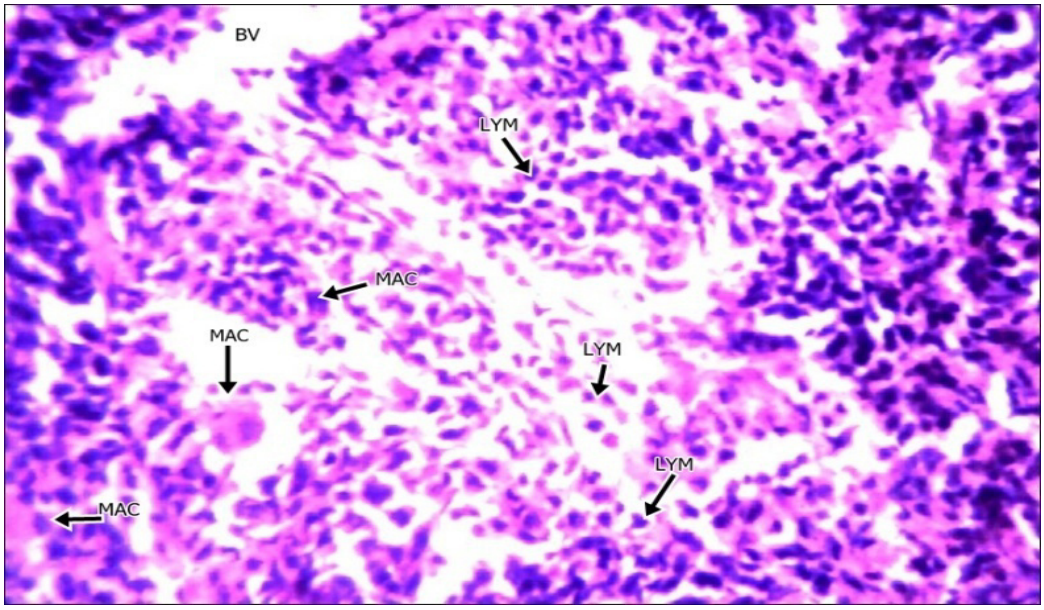

Figure 5: Section shows prominent enlarged white pulp (WP) consisting of proliferating lymphocytes (LYM). The outer mantle zone is darker than the inner germinal centre. Cellular disintegration and loss of nuclei material are observed, thus the lymphocytes and the white pulp are decolourized. There is constriction of the central arteriole. There is detachment of white pulps from spleen parenchyma. Loss of cells in red pulp (RP) evidenced by a dilated sinusoid and nuclei pyknosis. Finding is suggestive of reactive lymphoid hyperplasia

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