Sumerianz Journal of Medical and Healthcare, 2019, Vol. 2, No. 7, pp. 80-88
ISSN(e): 2663-421X, ISSN(p): 2706-8404
Website: https://www.sumerianz.com

Original Article Open Access

Evaluation of Acute Oral Toxicity Induced By N-Hexane Extract of *Leptadenia Hastata* Leaves on the Histology of the Pancreas and Spleen in Albino Rats

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Abstract

Introduction: This study was undertaken to investigate the toxicological profile of the n-hexane extract of *Leptadenia hastata* after acute administration on the histology of the pancreas and spleen of albino rats. Methodology: A total of seventeen (17) albino rats were used for the study. For the acute toxicity study, Lorke's method was adopted. Twelve rats were grouped into two groups comprising of six rats each, which received a dosage of 10mg/kg, 100mg/kg and 1000mg/kg in the first phase. The second phase consisted of three groups of two rats each being administered a dosage of 1600mg/kg, 2900mg/kg and 5000mg/kg. Five rats were given 1000mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg of n-hexane extract of *Leptadenia hastata* to determine the effect of the extract on the pancreas and spleen at these concentrations. The rats were observed for general behavioral changes, adverse effects and mortality up to 7 days post-treatment. Body weight, food intake and water intake were monitored throughout the experimental period and the pancreas and spleen were removed and evaluated at the end of the experiment. Results: In the acute toxicity study, there was no any mortality or significant behavioural changes observed after administering the highest dose (5000mg/kg). Histological analysis showed normal histological appearance in the pancreatic and splenic tissue at a dosage below 2000mg/kg but tissue heamolysis at a concentration greater than 3000mg/kg. Conclusion: These results demonstrate that the extract may not have any single dose toxicity. The LD50 value is greater than 5000 mg/kg. However, at tissue level, there was observable heamolysis at a concentration above 3000mg/kg.

Keywords: *Leptadenia hastate*; Acute toxicity; Heamolysis; Pancreas; Spleen.

1. Introduction

Medicinal plants constitute an important part of the health care system for the prevention and treatment of various disorders, since the ancient civilization. Previous studies have shown that 30 % of the marketed drugs contain active principles that were isolated for the first time from plants [1]. Medicinal plants have served as sources of many active ingredients used in the manufacture of conventional drugs and are also taken in its natural form as medication. Medicinal plants are preferred by individuals that are unable to afford the conventional drugs manufactured or are unable to have to access these drugs. The knowledge of the medicinal properties of these plants are often passed down through generations and traditional stories and these plants are gathered up and ingested usually without a consideration for proper dosage. This could lead to more problems as these plants may possess active substances that could have adverse levels at tissue level. Acute toxicity can be defined as the toxic effects produced by single exposure of drugs by any route for a short period of time [2]. The main objective of acute toxicity studies is to identify a single dose (lethal dose) that would have a major adverse effect or life threatening toxicity, it also involves an estimation of the minimum dose which would cause lethality. Acute (single dose) toxicity study is often carried out on laboratory animals by administering a high dose (sufficient to produce death or morbidity) of the substance in question and/or based on previous report on its toxicity or toxicity of structurally related compounds [3, 4]. The result of the acute study provides a guideline for selecting doses for the sub-acute and chronic low dose study [5, 6].

Leptadenia hastata (Pers) Decne (Family-Asclepiadaceae), commonly known as *yadiya* in Hausa language is an edible non-domesticated vegetable collected in the wild throughout Africa. It is a voluble herb with creeping latex stems, glabescent leaves, glomerulus and racemus flowers as well as follicle fruits. Wild plants like *Leptadenia hastata* provide food security during seasonal changes and are used medicinally in many areas. The breeders

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commonly used the leaf and stems for their parasitic activity and against placental retention [7]. Ethno-botanical information obtained from traditional medical practitioners in northern Nigeria and during the course of this study revealed that it is used locally for the treatment of diabetes mellitus, and has been reported to have antimicrobial effect, hypo-glycaemic, hypo-lipidaemic, as has been reported by Aliero and Wara [8], Bello, *et al.* [7], Sanda, *et al.* [9] and Umaru, *et al.* [10]. It has also been considered beneficial against milk drying, cough, sexual impotency, trypanosomiasis, acute rhinopharygitis, hypertension and wound healing in humans [11]. The crushed leaves have been also used as dressing for fresh cuts, wounds and ulcers [12].

2. Methodology

2.1. Collection, Identification and Storage of Plant Material

Leptadenia hastata was collected from a garden in the University of Maiduguri, Borno State, authenticated by a plant taxonomist, from the Department of Biological Sciences, Faculty of Science, University of Maiduguri. The leaves were harvested, washed and shade-dried for a period of two weeks and then ground to powder using a mortar and pestle. The powder was sieved to obtain the fine powder, it was then labeled and stored for use.

2.2. Extraction of Plant Material

Maceration technique as described by Azwanida [13] was used for extraction in the current study. The leaf powder weighing 500g was dissolved in 3 liters of n-hexane in a 5 liter stoppered container. Maceration involved soaking the plant which is allowed to stand at room temperature for a period of 3 days at the minimum with periodic agitation. The process softened and broke the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture was filtered using Whatman's filter paper. The resulting n-hexane filtrate was concentrated to dryness in-vacuo using an evaporator and the resulting powder was kept in an air–tight container and refrigerated.

2.3. Preparation of Extract Stock Solution

Stock solution of 200mg/ml of the extract was prepared by dissolving 1g of the extract in 5ml of olive oil which served as the vehicle. From this stock solution, different concentrations of 10, 100 and 1000mg/ml were prepared and administered to the rats.

For the second phase of the acute toxicity study, hexane extract of the leaves of *Leptadenia hastata* (4.5g) was dissolved in 7.5ml of olive oil to produce a solution with a concentration of 600mg/ml. From this stock solution, different concentrations of 1600, 2900 and 5000mg/ml were prepared still using olive oil.

2.4. Acute Oral Toxicity Study

An acute toxicity study was performed using modified Lorke's method according to the Organization of Economic Co-operation and Development (OECD) guide for testing of chemicals Organization for Economic Co-operation and Development (OECD) [14], and Salawu, *et al.* [15]. The route of administration was oro-gastric. In the first phase, six rats of either sex were divided into three groups (I - III) containing two rats each. The first, second and third groups received 10 mg/kg, 100 mg/kg and 1000 mg/kg of *Leptadenia hastata* extract respectively. These animals were monitored for 7 days to observe any mortality. In the second phase, six rats were used and divided into three groups of two rats each. Each of the group received different doses of the extract which were 1600 mg/kg, 2900 mg/kg and 5000 mg/kg. These rats were also monitored for 7 days [15, 16]. In addition, 5 animals were administered a dosage of the extract at concentrations of 1000mg/kg, 2000mg/kg, 3000mg/kg, 4,000mg/kg and 5000mg/kg to determine the effect of the extract at microscopic level on several tissues. At the end of 7 days, these animals were sacrificed and tissue samples from the spleen and pancreas were carefully removed and histologically prepared and stained with Heamatoxylin and Eosin to observe histopathological changes at these concentrations. This method adopted was as described by Porwal, *et al.* [17].

3. Results

3.1. Acute Oral Toxicity of Leptadenia Hastata Extract

In the first phase, no mortality was reorded in all the treated rats (Table 1a). After administration, animals did not display any significant changes in behavior, skin effects, respiration, impairment in food and water consumption nor postural abnormalities. No lacrimation, salivation nor diarrhea was also detected in the animals tested.

No mortality was also recorded in the second phase of the study after administering the highest dose of the extract. The animals in this phase also did not display significant changes in mucous membrane nor behavioral patterns. There was also no salivation, lacrimation, tremors, diarrhea nor lethargy observed in any of the animals in the groups. No deaths were also recorded in the rats (Table 1b).

The hexane extract of *Leptadenia hastata* was found to be safe as the highest dose did not kill any of the experimental animals. Hence, the LD_{50} value was estimated to be greater than 5000 mg/kg.

Table-1. Outcome of first phase (a) and second phase (b) acute oral toxicity study

(a)

Dose (mg/kg)	Number of Rats Used	Mortality (%)
10	2	0 (0.0)
100	2	0 (0.0)
1000	2	0 (0.0)

(b)

Dose (mg/kg)	Number of Rats Used	Mortality (%)
1600	2	0 (0.0)
2900	2	0 (0.0)
5000	2	0 (0.0)

3.2. Histological Observations in the Pancreas

The animals that received 1000mg/kg and 2000mg/kg revealed a normal histology with the endocrine pancreas - Islets of Langerhans (black arrow) and exocrine pancreas (pancreatic acinar cells) as shown by a white arrow (Figure 1A and B)

The animals that received 5000mg/kg of extract had hemorrhaging in the pancreatic parenchyma represented by black arrow head (figure 1E). The islet cells of Langerhans are observed as a cluster of cells which are pale staining in the micrographs and represented by black arrows. The pancreatic acinar cells are represented by white arrows in animals that ingested 3000mg/kg and 4000mg/kg of the extract (figure 1C and D).

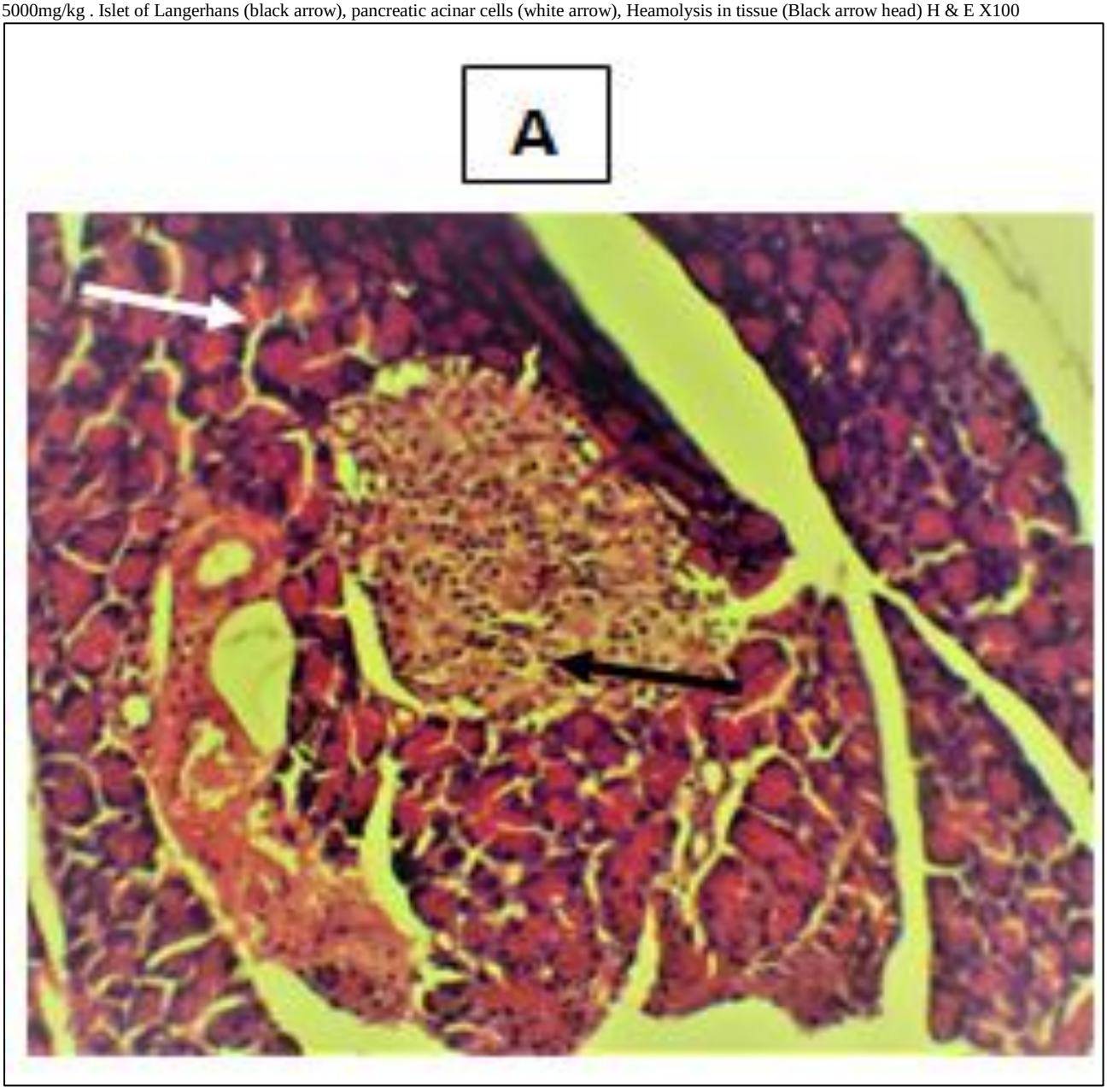
3.3. Histological Observations in the Spleen

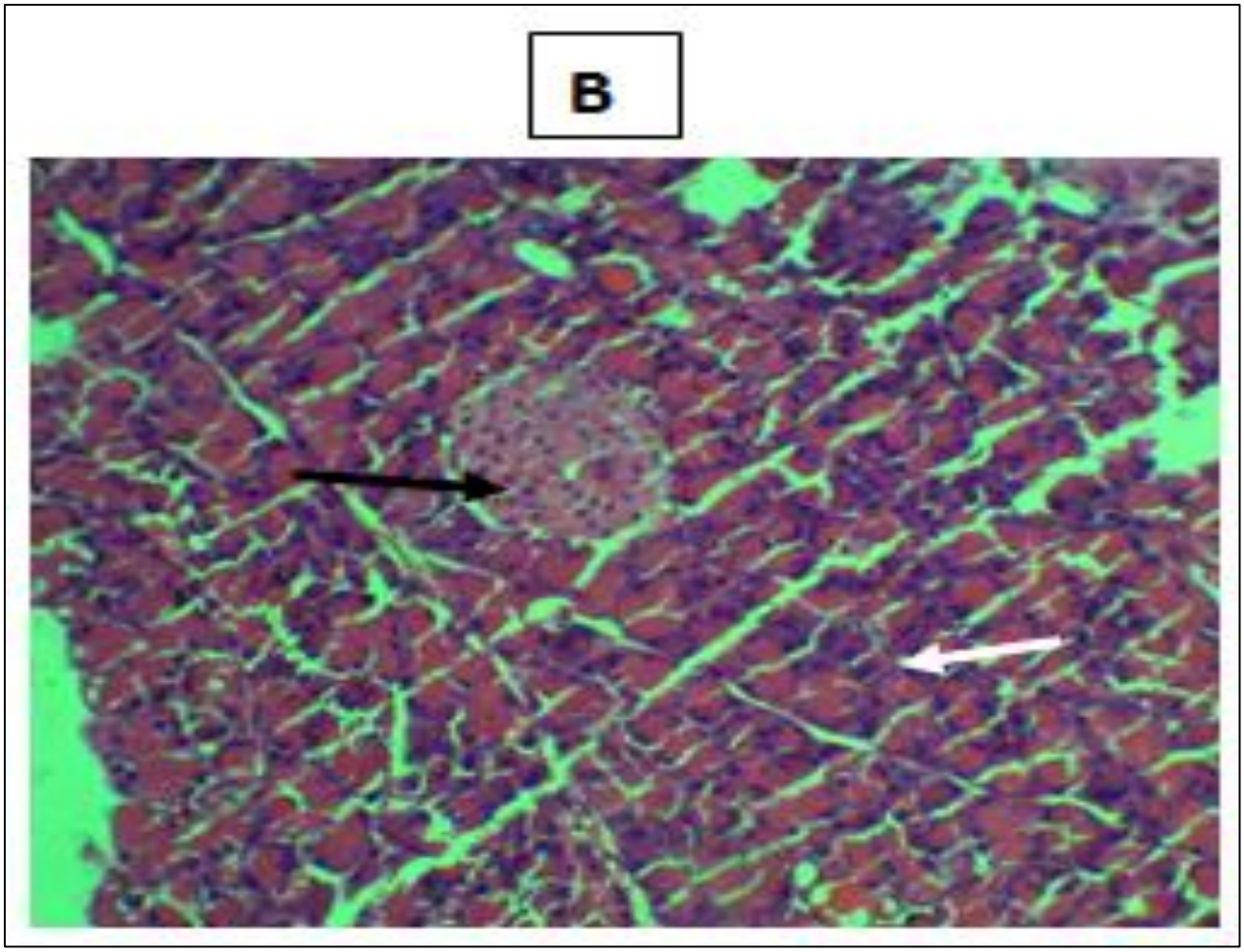
The animals that received 1000mg/kg and 2000mg/kg revealed a normal histology with the white pulp (white arrow) differentiated from the red pulp (black arrow) (Figure 2 A and B).

The animals that received 3000mg/kg revealed a disorganization in the architecture of the splenic tissue with the white pulp (white arrow) being invaded by blood cells (Figure 2C)

The animals that were administered 4000mg/kg and 5000mg/kg of extract had hemorrhaging in the splenic parenchyma. This is represented by blue arrow head (Figure 2D and 2E).

Figure-1. Micrographs of pancreas of rats exposed to the acute toxicity study. A- 1000mg/kg, B- 2000mg/kg, C- 3000mg/kg, D- 4000mg/kg, E- 5000mg/kg . Islet of Langerhans (black arrow), pancreatic acinar cells (white arrow), Heamolysis in tissue (Black arrow head) H & E X100

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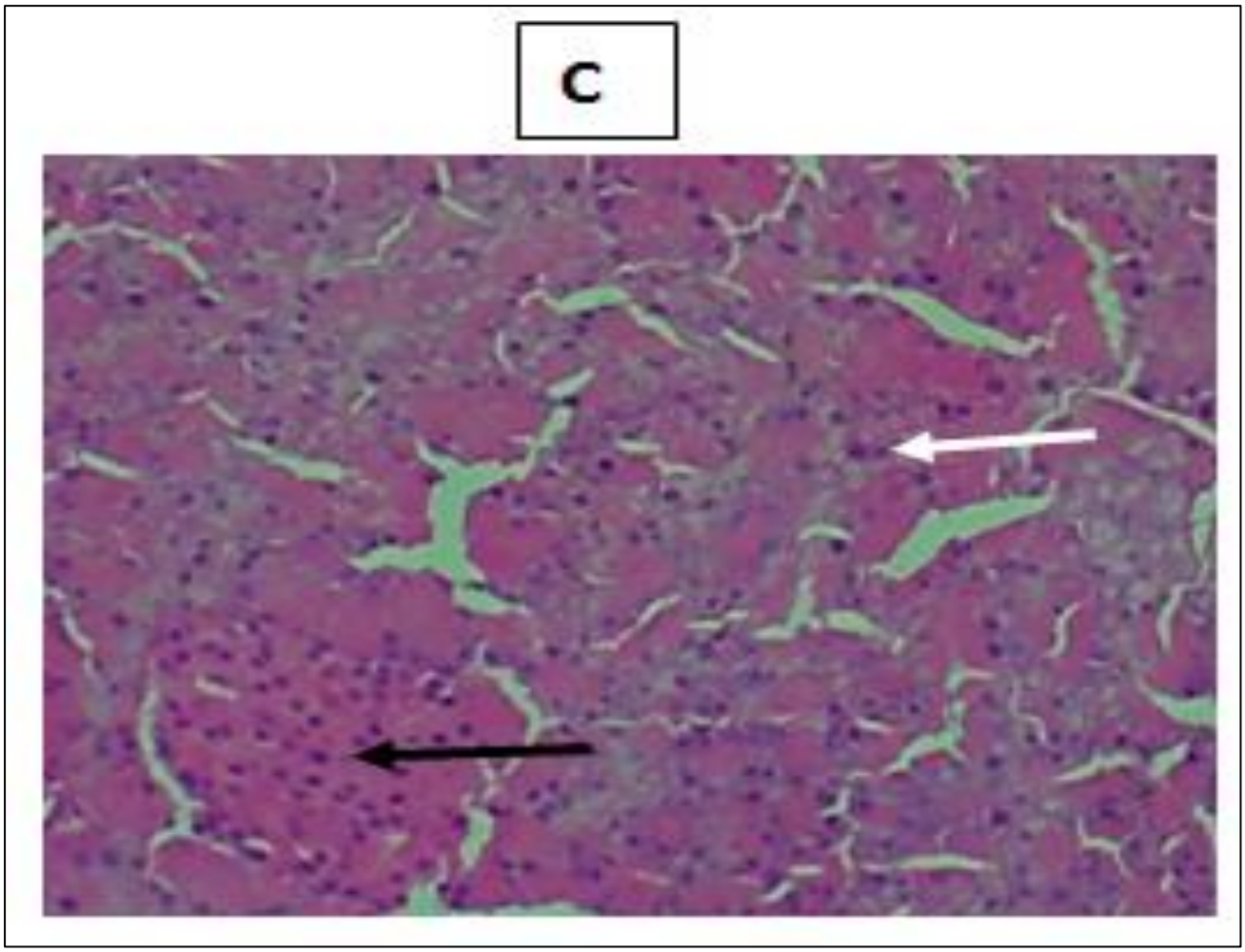
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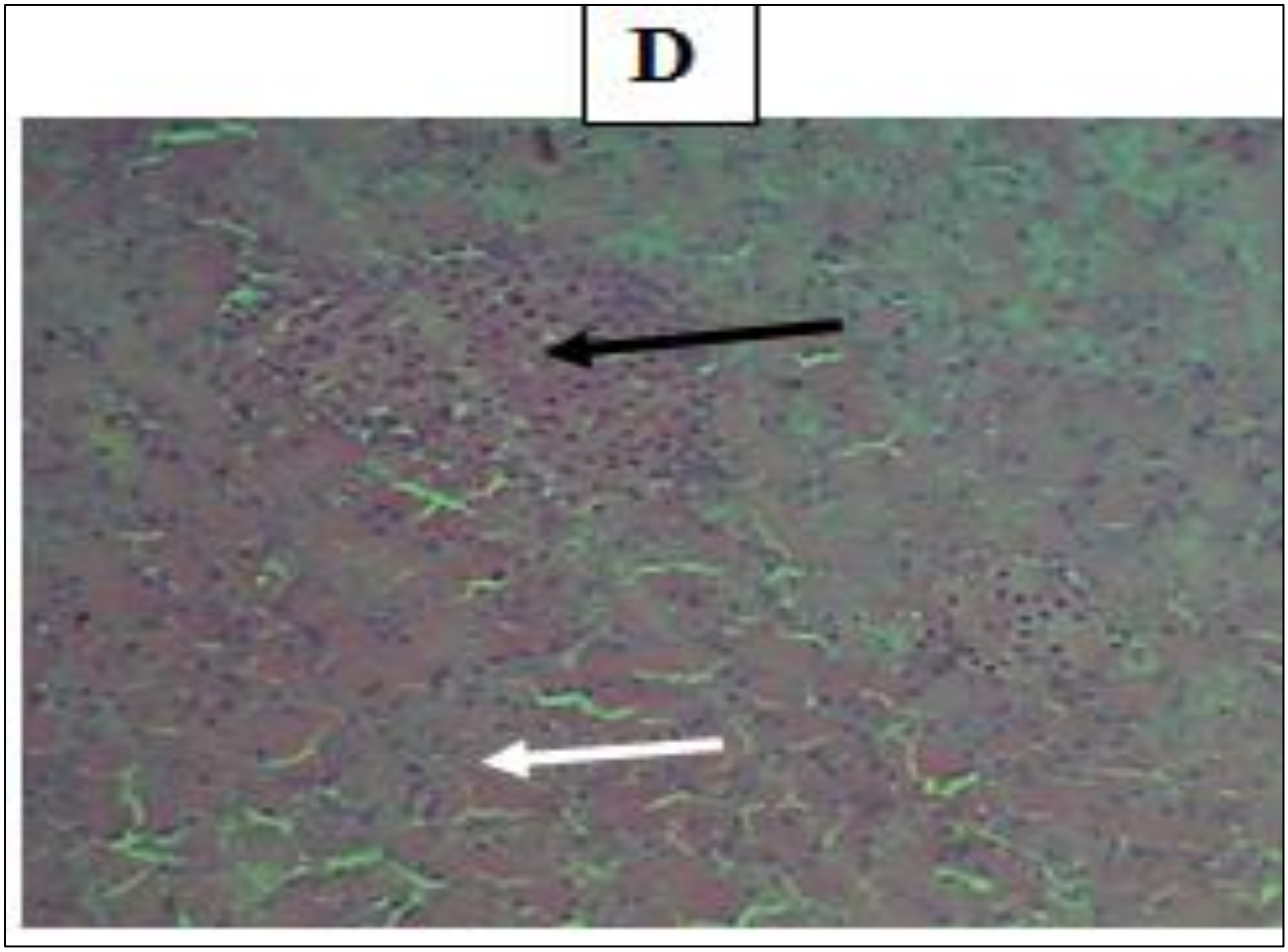
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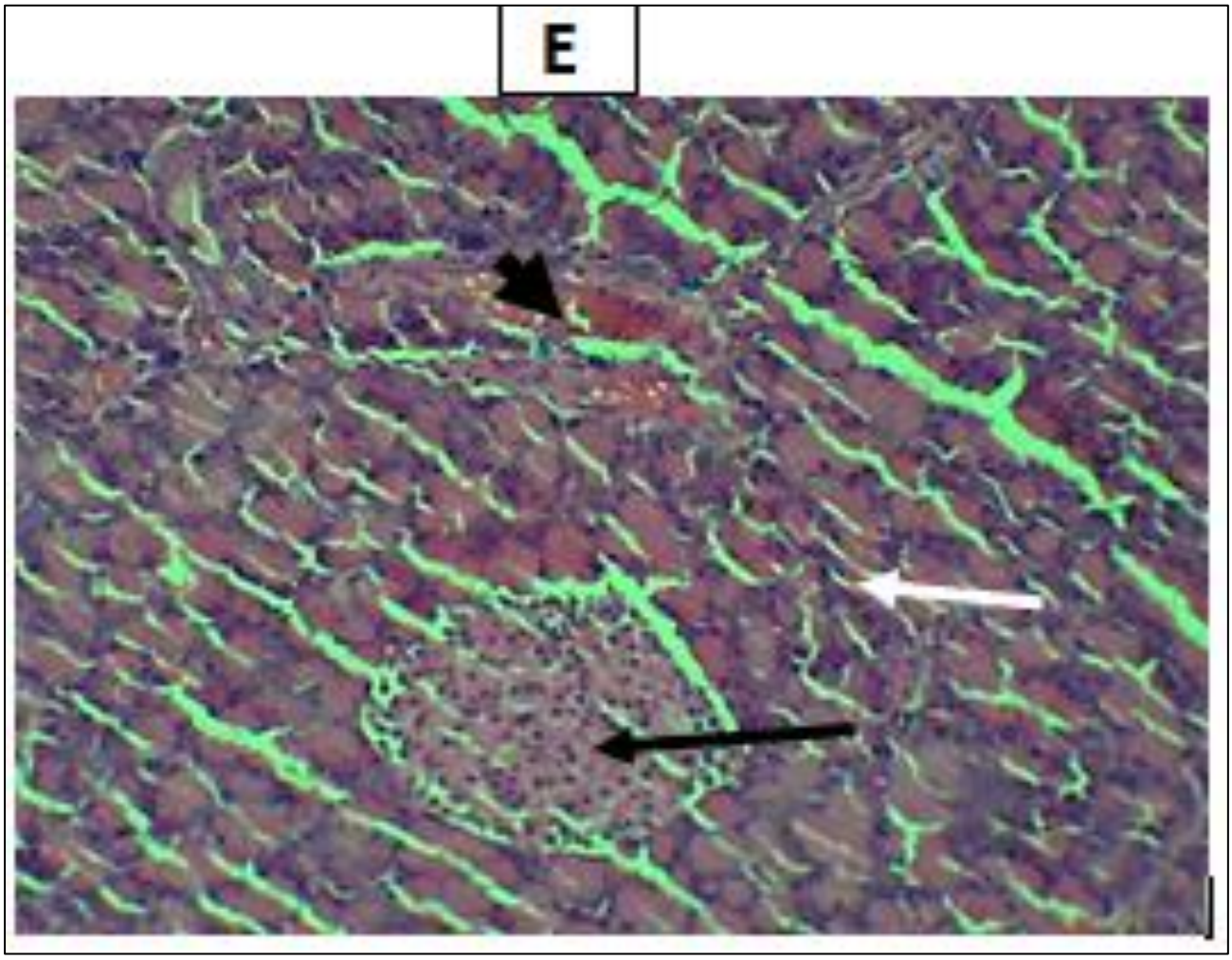
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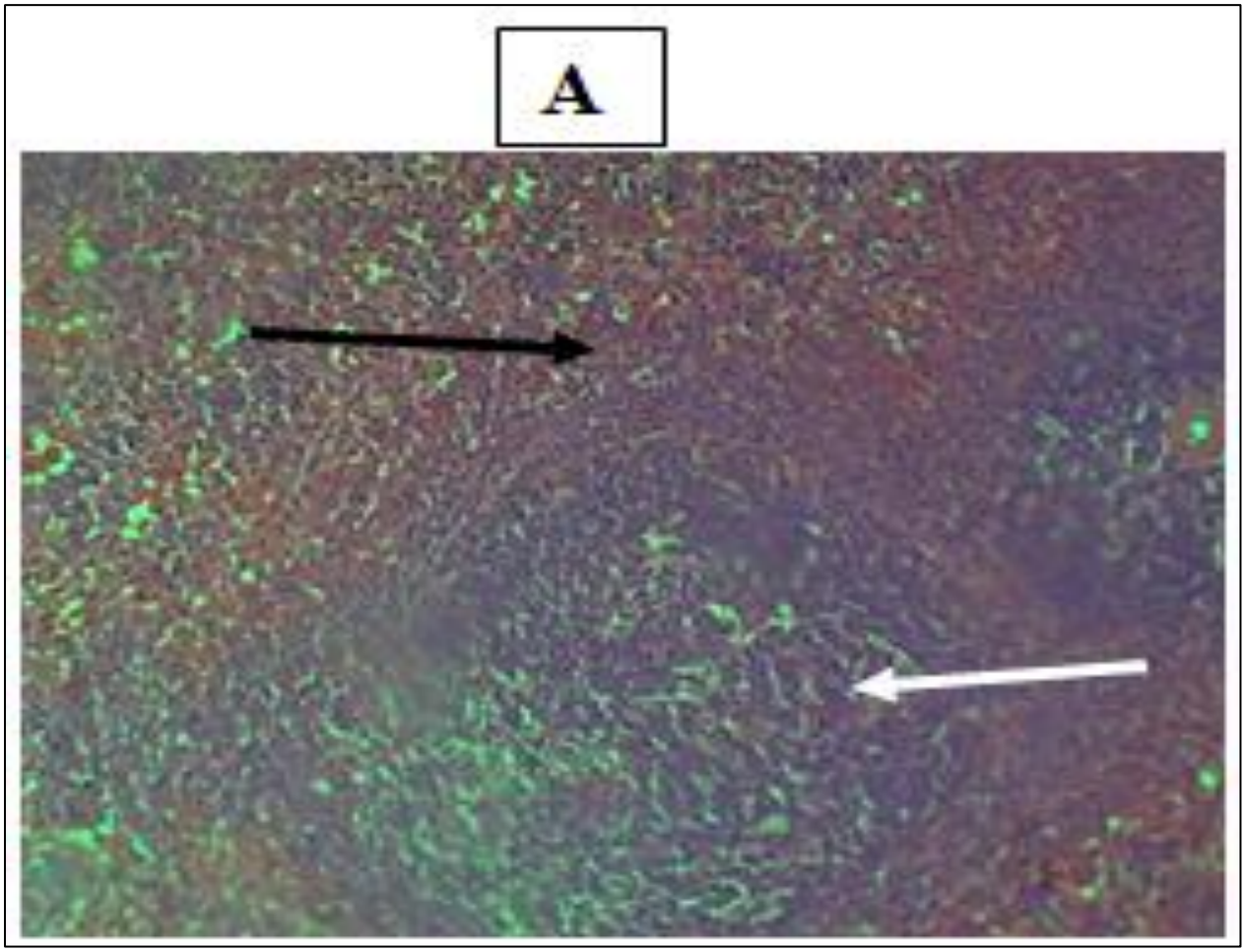
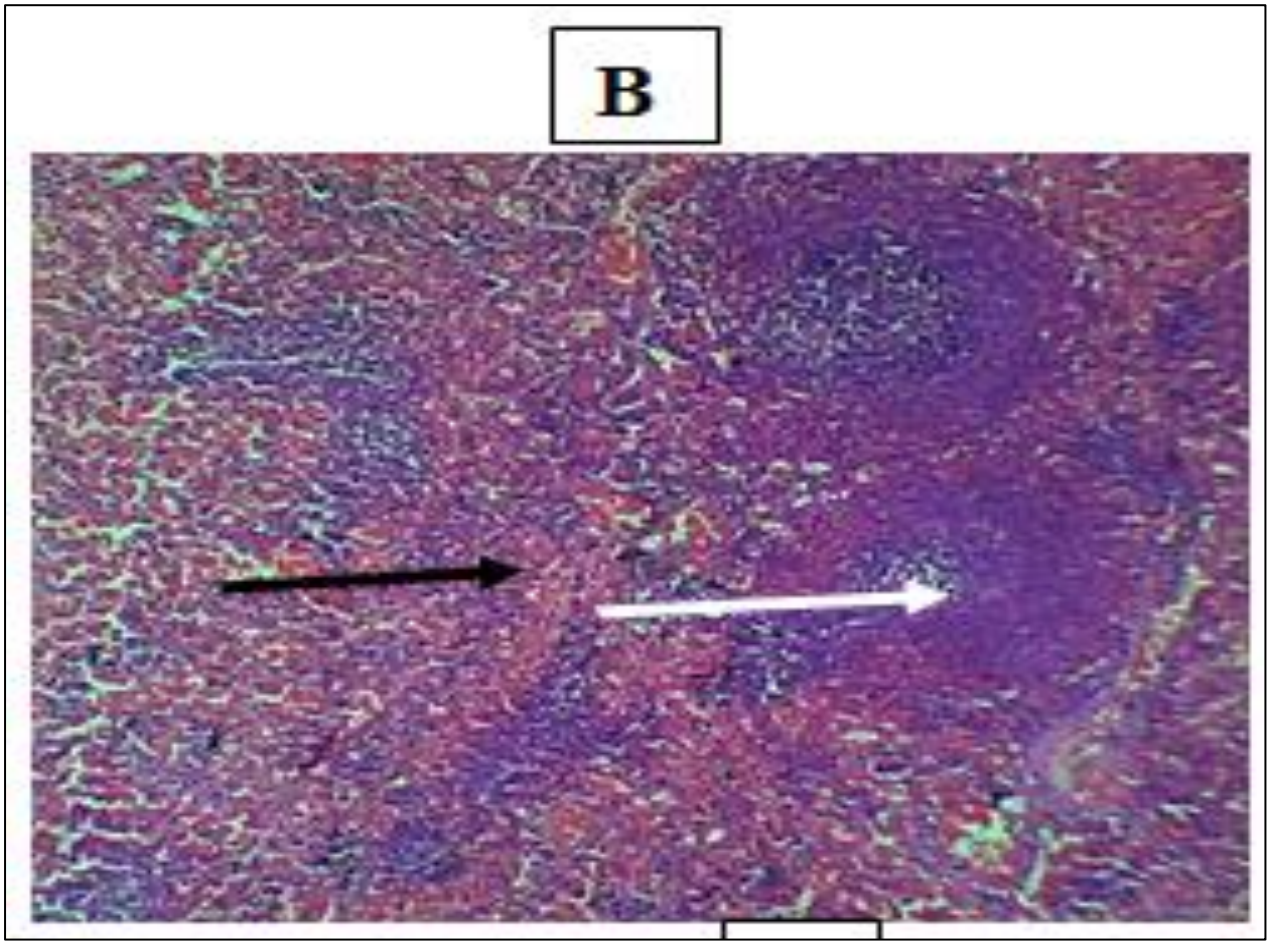
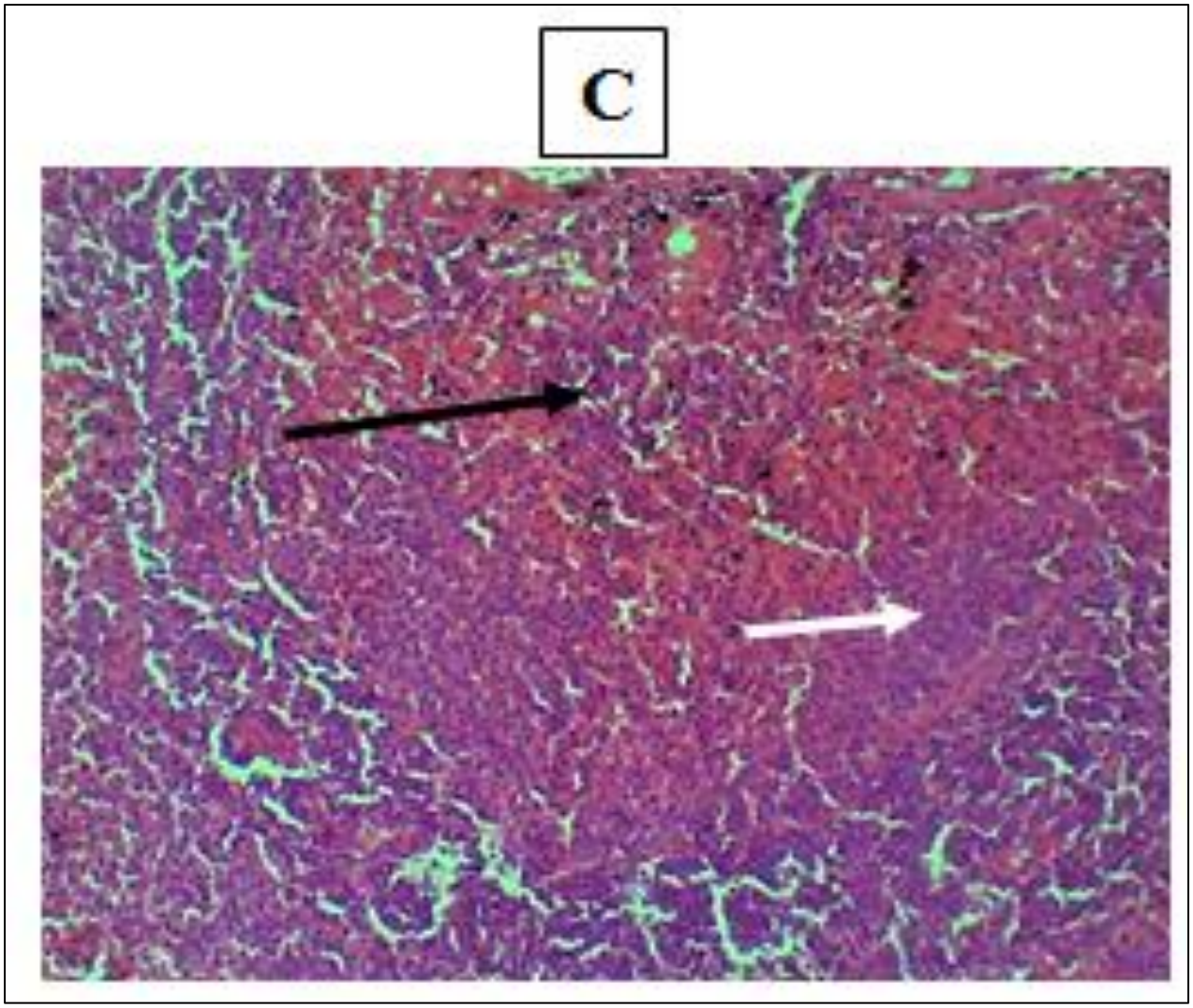
Figure-2. Micrographs of spleen of rats that were exposed to the acute toxicity study. A- 1000mg/kg, B- 2000mg/kg, C- 3000mg/kg, D- 4000mg/kg, E- 5000mg/kg . White pulp (white arrow), Red pulp (Black arrow), Heamolysis in tissue (Blue arrow) H & E X100



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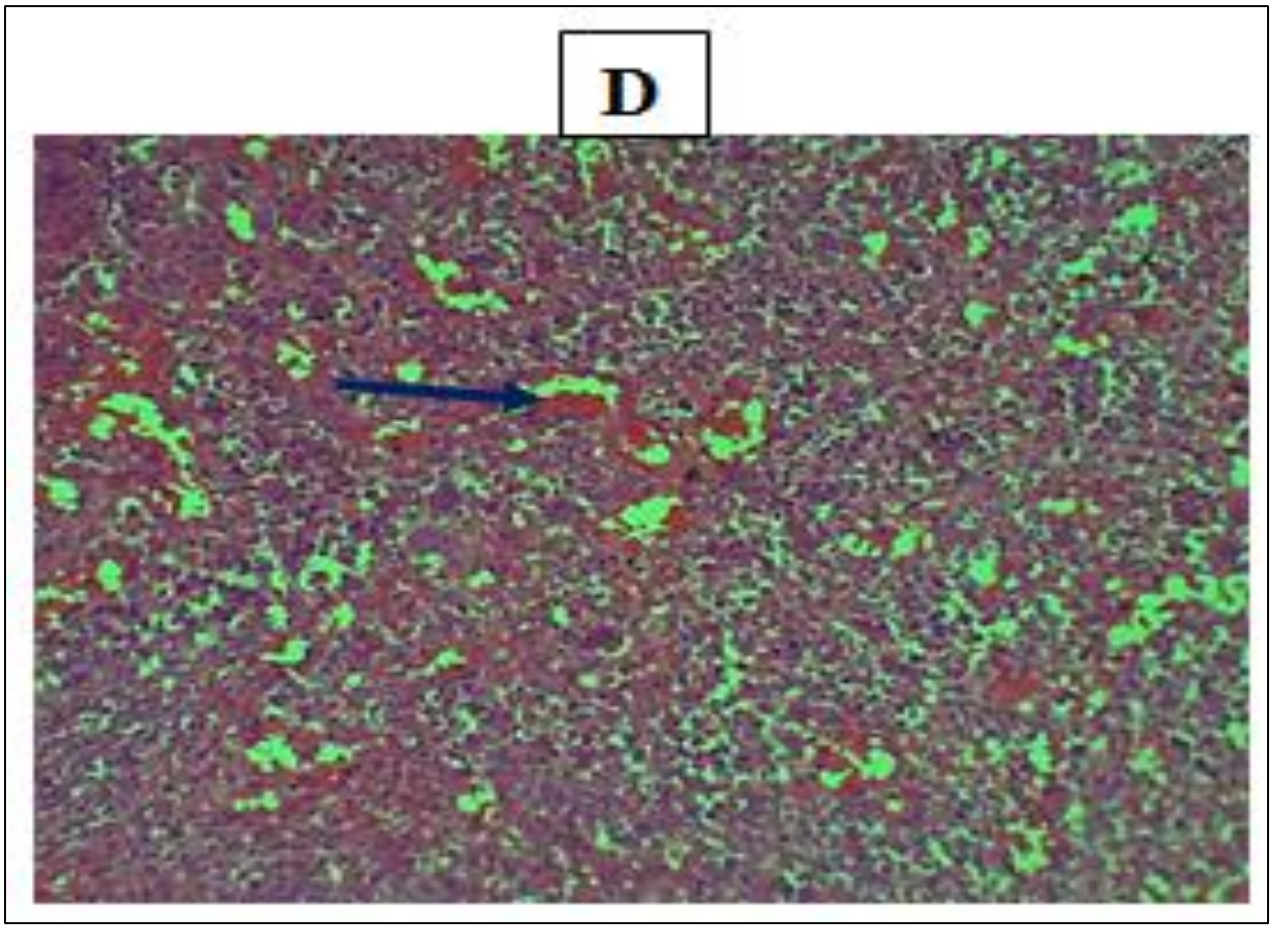
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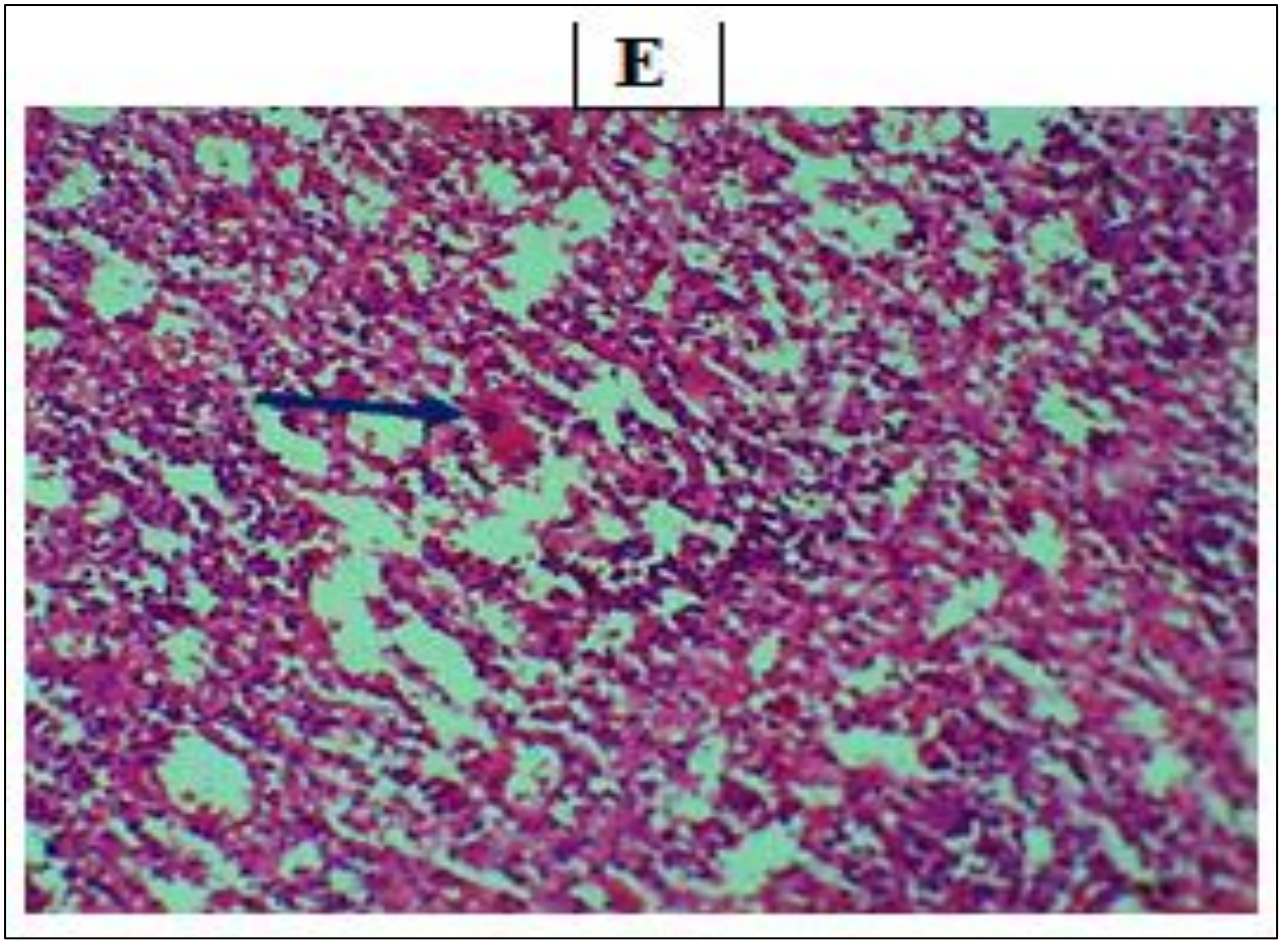

4. Discussion

For many centuries, the use of herbal medicines and their preparations as medication has continued through generations and have been available to many individuals. These medicinal plants have been considered to be safe and effective due to their negligible side effects [17]. This assumption that these substances have minimal adverse effect may have influenced the indiscriminate use of these formulations to a large extent amongst the rural populace. These formulations are usually administered over a long period of time and most often without proper dosage monitoring by the experts and lack of awareness of the toxic effects that might result from such prolonged usage [18]. Therefore, scientific knowledge towards oral toxicity is much needed, which will not only help identify doses that could be used subsequently, but also to reveal the possible histopathological effects on tisues when ingested at high concentrations.Acute toxicity studies in animals are considered necessary for any pharmaceuticals intended for human use. The results of the study suggested that *Leptadenia hastata* is relatively non-toxic when administered orally as indicated with LD_{50} greater than 5000mg/kg. This is in agreement with studies carried out by Maina, *et al.* [19], who recorded no mortalities after a dosage of 5000mg/kg of *Leptadenia hastata* was administered. In the present study, however, haemolysis in the hepatic, pancreatic, renal and splenic parenchyma indicated that the extract may be harmful at concentrations above 3000mg/kg at tissue level. The result of these findings corresponds to the study carried by Maurice, *et al.* [20] who suggested that though safe, but at a higher dosage, *Leptadenia hastata* may be poisonous or lethal to rats at a concentration greater than 2,320mg/kg [20].

5. Conclusions

The result of these findings corresponds to the study carried by Attah, *et al.* [21], Maurice, *et al.* [20] who determined that the LD50 of *Leptadenia hastata* was found to be as high as 2320 mg/kg body weight. The outcome of his experiment and the present study suggested that though safe, but at a higher dosage, *Leptadenia hastata* may be poisonous or lethal to rats.

5.1. Conflict of Interests

The authors declare no conflict of interests.

5.2. Financial Contributions

The study was privately funded and received no contribution from any institution or governmental organization.

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