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HORMONAL AND HISTOMORPHOLOGIC ADVERSE EFFECTS OF CRUDE EXTRACT OF *ABELMOSCHUS ESCULENTUS* LEAF ON NON- GYNAECOLOGICAL ORGANS OF VIRGIN AND MULTIPAROUS WISTAR RATS

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ABSTRACT

Background: *Abelmoschus esculentus*, okra leaf, is an important, highly nutritious plant, especially its leaf and pods. Traditional beliefs about the fertility-enhancing property of this plant prompted an investigation into the impact of its leaf crude extract on serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, and non-gynecological histomorphology in virgin and multiparous female albino rats. **Methods:** Twenty adult female albino rats were equally divided into four (4) groups of virgin control, VC, virgin extract, VE, multiparous control, MC, and multiparous extract, ME. VC and MC were controls, while VE and ME were treated with 100mg/kg of the aqueous crude extract, twice daily by oral gavage for 15days. After the end of the extract administration, all female rats were bled and their serum analyzed for LH and FSH. Two (2) male rats were introduced into each cage for mating. After mating, all pregnant female rats were allowed to deliver their young ones within a gestation period of about 21-25days. The individual animal's litter number was counted. Thereafter, the female rats were sacrificed using chloroform anesthesia, and vital organs like liver, heart, kidney, and lungs were harvested and processed histologically to observe for any histomorphological change. **Results:** Hormonal assay showed that LH (Mean ± S.E.M VE- 1.35±0.11, ME-1.40±0.21) and FSH (Mean ± S.E.M VE- 1.27±0.13, ME-1.31±0.12) levels in the serum were higher in VE and ME with significant difference ($P<0.05$). Histomorphologic sections of the liver, kidney, heart, spleen, and lungs showed same intact histological structures as those of the controls. **Conclusion:** In conclusion, crude leaf extract of *Abelmoschus esculentus* increases litter size with corresponding effect on LH and FSH, and therefore said to have a fertility-boosting effect on the female wistar rats.

KEYWORDS: Histomorphology, wistar rats, luteinizing, multiparous, virgin.

INTRODUCTION

Having twins is one of the many delightful surprises and marvels that can make pregnancy truly special. Despite significant medical advancements, twins continue to captivate and intrigue families, and twins themselves, surrounding their arrival with an air of wonder.[6]

For centuries, women have sought ways to influence their fertility, with varying degrees of acceptance and support from society. Many herbal remedies have been traditionally used for reproductive health, including contraception, though their efficacy and safety can vary.[5] As concerns grow about industrial chemicals in plastics and resins, more women are exploring natural fertility boosters, such as herbs and plants. This underscores the importance of further research into medicinal plants, both for their potential benefits and to inform evidence-based use.[14]

In Oyo state, south-western Nigeria, there is a town called Igbo Ora, associated with high twin births.[13] Research suggests Igbo-ora might have one of the highest twinning rates globally. Some research explores potential links between diet and twinning rates, but none conclusively prove a direct connection.[6] The high twinning rate in this town remains somewhat mysterious.

It is not uncommon to encounter multiple sets of twins in the town, making it a unique aspect of the community.[10] The locals attribute the high twinning rate to their traditional diet of lifestyle including the consumption of specific foods like okra leaves known as ewe ilasa.[7]

Okra (*Abelmoschus esculentus*), is indeed a versatile plant with various uses, and its origins are debated among scholars. It is widely cultivated and consumed globally, particularly in typical and subtropical regions.[12]

MATERIALS AND METHODS

Fresh *Abelmoschus esculentus* leaves (1500g) were collected from a local farm in Igbo –Ora, Oyo state, Nigeria. The plant material was identified and authenticated at Forestry Research Institute (FRIN), Ibadan. The leaves were then dried under shade, and ground into fine powder. Standard methods were employed to prepare crude aqueous extract.

Phytochemical constituents of the leaves were determined according to the methods described by Kokate (2001) and Harbone (1998). The phytochemicals tested were alkaloids, flavonoids, steroids, anthraquinones, tannins and glycosides.

Female Wistar albino rats (weighing 100-150g) were used for the experiment. The animals were kept and maintained under standard laboratory conditions of temperature (25 ± 2^0C), a 12hour light, 12hour dark cycle, and were fed with standard commercial diet, and allowed to drink clean water ad libitum. The Wistar albino rats were allowed to acclimatize for two weeks before the commencement of the studies.

Animals were divided into 4 groups, and each group comprised 5rats. The grouping details are as follows:
Group 1 is made up of virgin rats, with 100mg/kg weight of the extract given orally, twice daily. This group is denoted VE.
Group 2 comprises virgin rats, with no extract given. This serves as control, and denoted VC.
Group3 includes multiparous rats, with 100mg/kg weight of the extract given twice daily. This is denoted ME.
Group4 comprises multiparous rats, with no extract given. This is another control, and denoted MC.

The animals were weighed daily to observe for weight gain or loss, which is a pointer for their well being throughout the period of the experiment. Groups VE and ME which were the treatment groups comprising the virgin and multiparous rats respectively were treated with 100mg/kg body weight of the aqueous crude extract, twice daily by oral gavage for 15days. At the end of the extract administration, animals were bled via the retro-orbital sinus of the medial canthus and their serum analyzed for luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Two (2) male rats were introduced into each cage for mating. After mating, all pregnant female rats were allowed to deliver their young ones within a gestation period of about 21-25days. The individual rat's litter number was counted. Thereafter, the female rats were sacrificed using chloroform anesthesia, and vital organs like liver, heart, kidney, uterus and lungs were harvested and processed histologically to observe for any histomorphological change.

The organs harvested were promptly fixed in 10% neutral-buffered formol saline for at least, 48hours, and then processed using automatic tissue processor. Sections obtained at the end of the processing were stained using Haematoxylin and Eosin staining technique.

RESULTS

The influence of the crude extract of *Abelmoschus esculentus* on the uterus of virgin and multiparous albino rats was assessed over a period of 15days, and stopped when they were assumed to be pregnant. Weight and size of liver, heart and kidney of test group did not show marked differences compared to controls. Within the treatment group, there was also a non significant ($P>0.05$) difference, and all the animals maintained a healthy appearance throughout the period of investigation.

The histology of the liver, heart and kidney of all the rats involved (test and control groups) was evaluated after staining with haematoxylin and eosin staining technique. In all, there is no difference within the animals of all the groups. So, in all the organs observed, they all show normal structural components. See photomicrographs.

Litter Size

Table 1: Virgin Albino Rats.

VC1	5	VE1	10
VC2	4	VE2	6
VC3	6	VE3	8
VC4	6	VE4	8
VC5	4	VE5	10

VC- Virgin Control, VE- Virgin Extract

Table 2: Multiparous Albino Rats.

MC1	6	ME1	8
MC2	8	ME2	8
MC3	6	ME3	8
MC4	6	ME4	10
MC5	8	ME5	8

MC-Multiparous Control, ME-Multiparous Extract

Table 3: Mean of litter size.

Group	VC	MC	VE	ME
Mean ± S.E.M	5.0 ± 0.09	6.8 ± 0.11	8.4 ± 0.07	8.4 ± 0.09

Results are presented as Mean ± S.E.M. P < 0.05 is regarded significant.

Table 4: Hormonal Evaluation.

	VC	MC	VE	ME
Luteinizing Hormone (LH)	1.31 ± 0.08	1.33 ± 0.11	1.35 ± 0.11	1.40 ± 0.21
Follicle Stimulating Hormone (FSH)	1.24 ± 0.18	1.29 ± 0.13	1.27 ± 0.13	1.31 ± 0.12

Results are presented as Mean ± S.E.M. P < 0.05 is regarded significant.

The result of female hormone profile in the control and Abelmoschus esculentus extract administered animals is reported. As can be seen on the table, treatment with A. esculentus extract caused a significant increase in the serum levels of the follicle stimulating hormone (FSH) and luteinizing hormone (LH).

The qualitative analysis of the phytochemical constituents of the plant leaves showed the presence of alkaloids, flavonoids, steroids, and glycosides. However anthraquinones, saponins and tannins were not detected in this study. It also showed high concentrations of flavonoids and steroids. Glycosides were present in moderate amounts while alkaloids and triterpenoids were detected in trace amounts. Each of these phytochemicals is known for various protective and therapeutic effects.[3] For instance, flavonoids are known to possess antibacterial, anti-inflammatory, anti-allergic, antiviral and anti neoplastic activity. They have antioxidation effects in animals. Stereoidal compounds are of importance due to their relationship with some compounds such as sex hormones.[17] Steroids, Glycosides, and alkaloids have been reported to exert inhibiting activity against most bacteria.[2]

PHOTOMICROGRAPHS

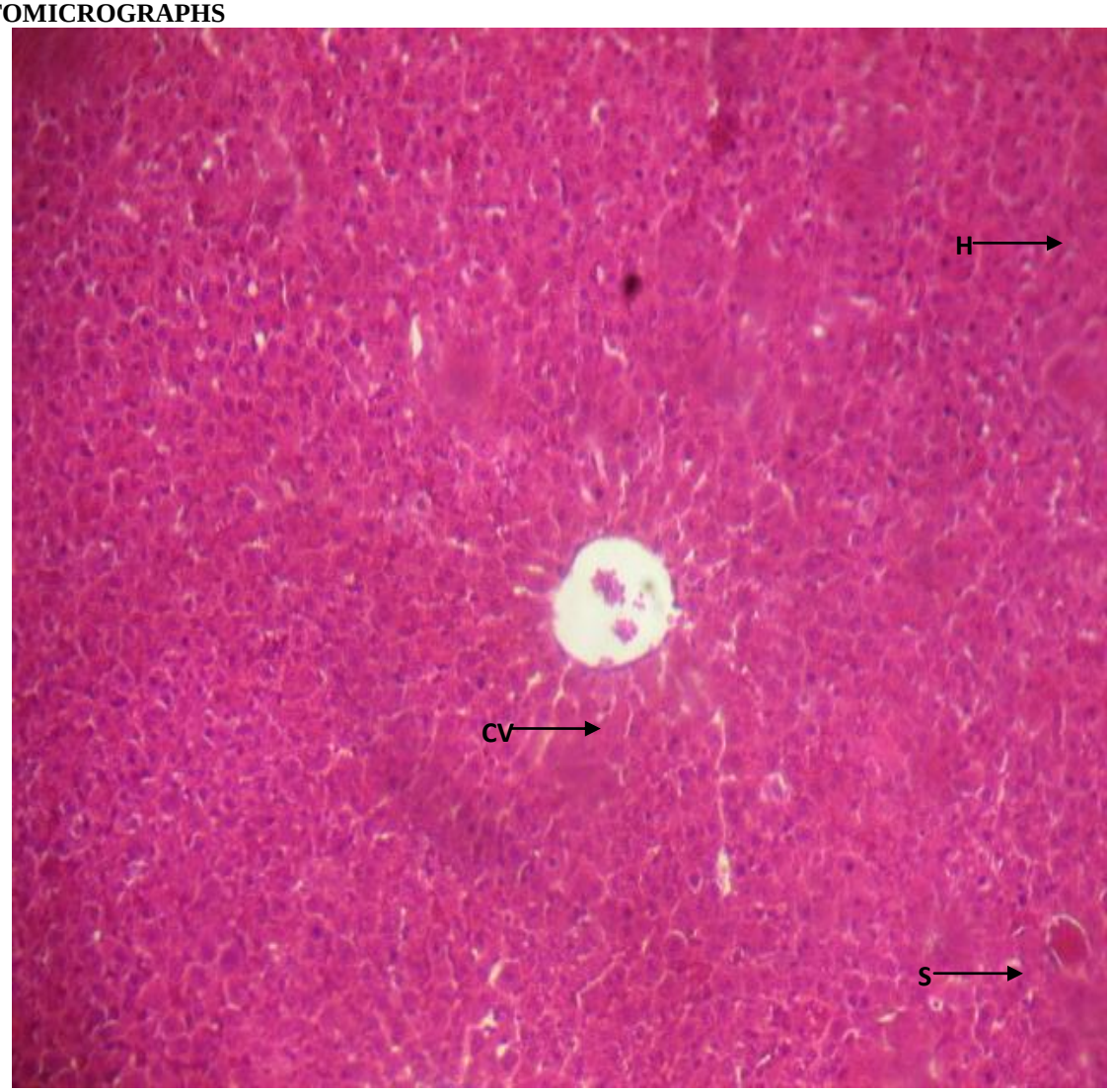

Figure 1: Photomicrograph of VC liver, H&E stain. X400.

CV-Central vein. S-Sinusoid. H-Hepatocyte

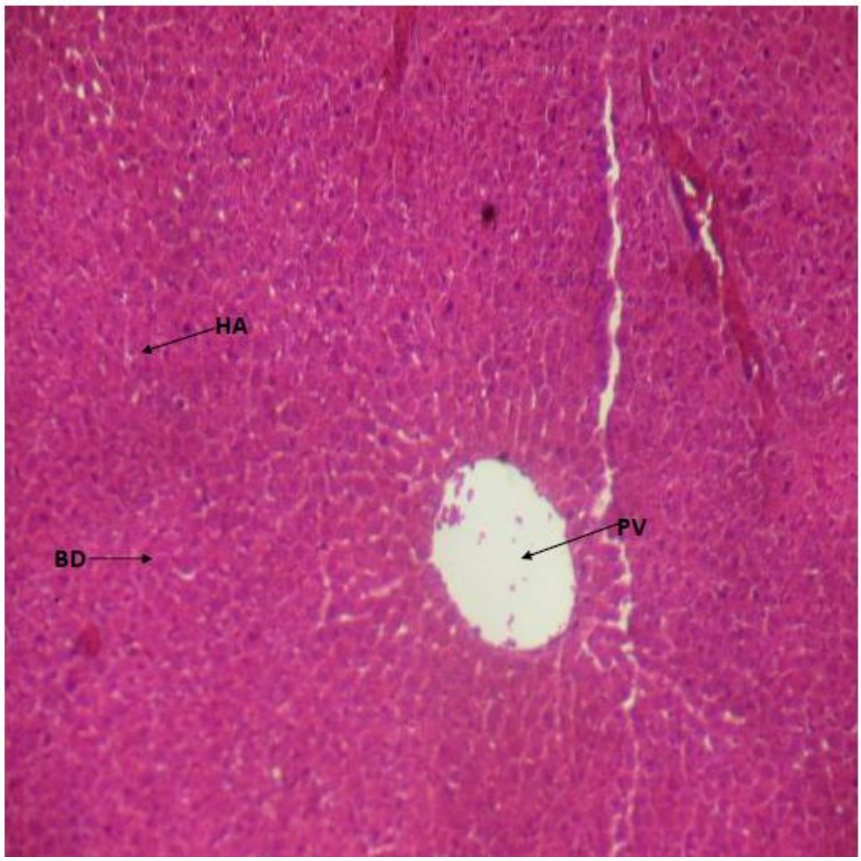

Figure 2: Photomicrograph of VE liver, H&E stain. X400.
HA- Branch of hepatic artery. BD- Biliary duct. PV-Branch of portal vein

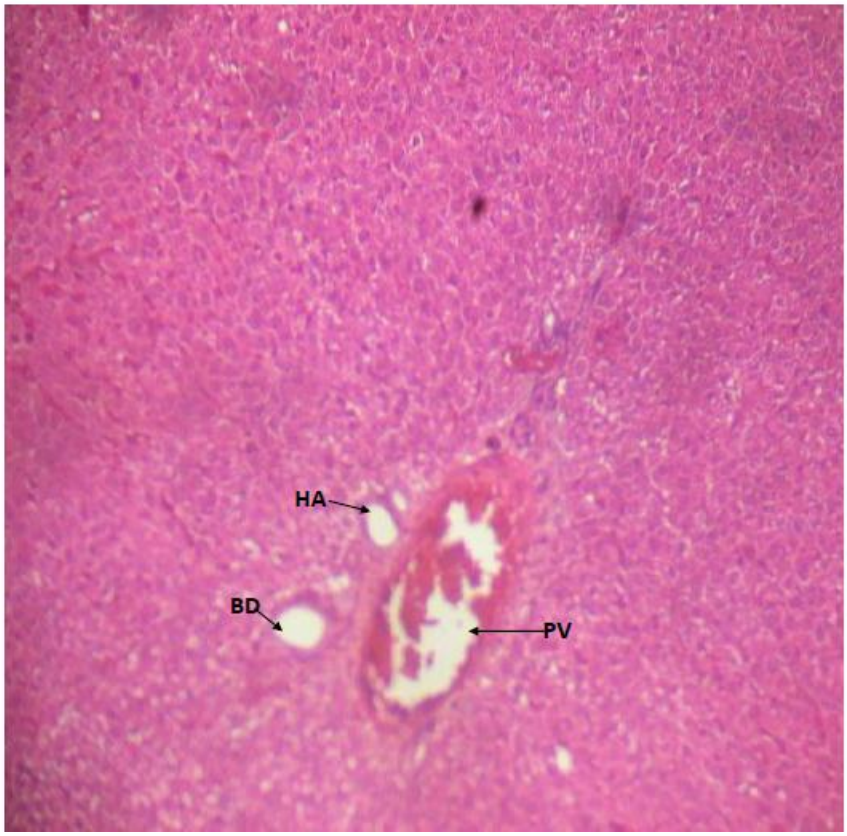

Figure 3: Photomicrograph of ME liver, H&E stain. X400.
HA- Branch of hepatic artery. BD- Biliary duct. PV-Branch of portal vein

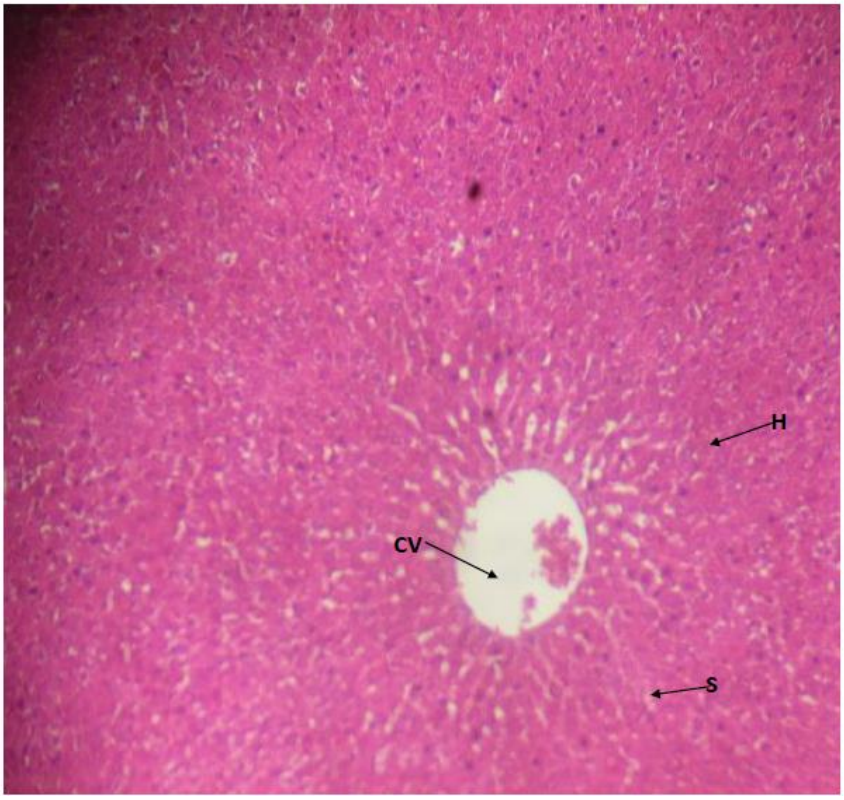

Figure 4: Photomicrograph of MC liver, H&E stain. X400.
CV-Central vein. S-Sinusoid. H-Hepatocytes

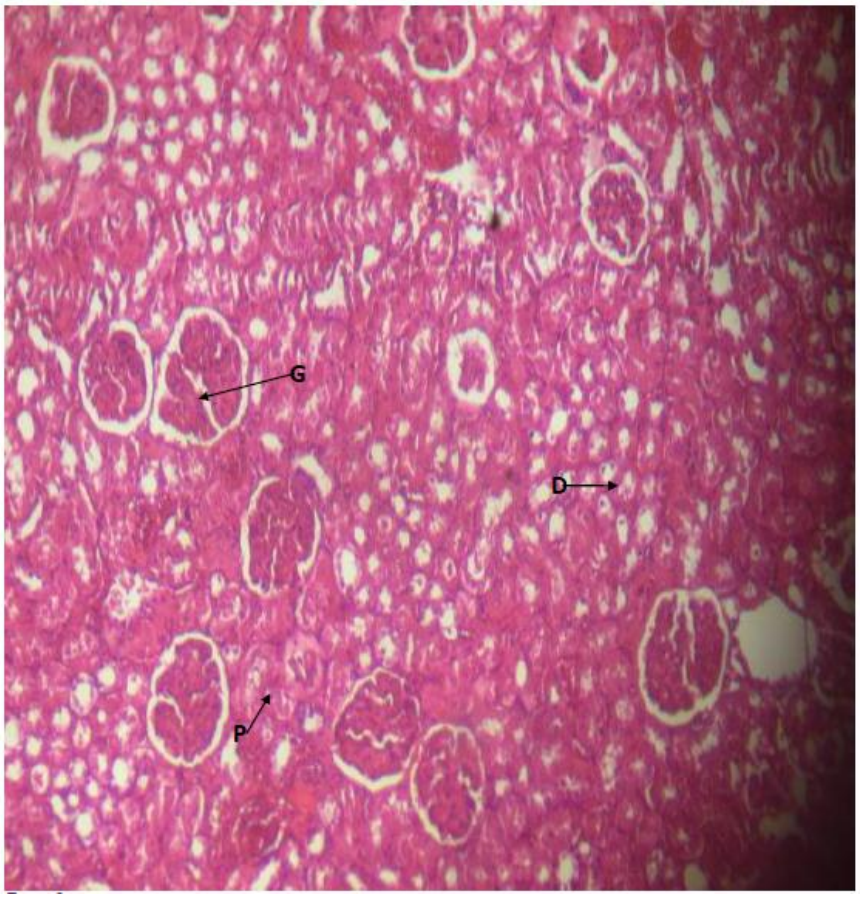

Figure 5: Photomicrograph of MC kidney. H&E stain. X400.
G-Glomerulus. P-Proximal convoluted tube, D-Distal convoluted tube

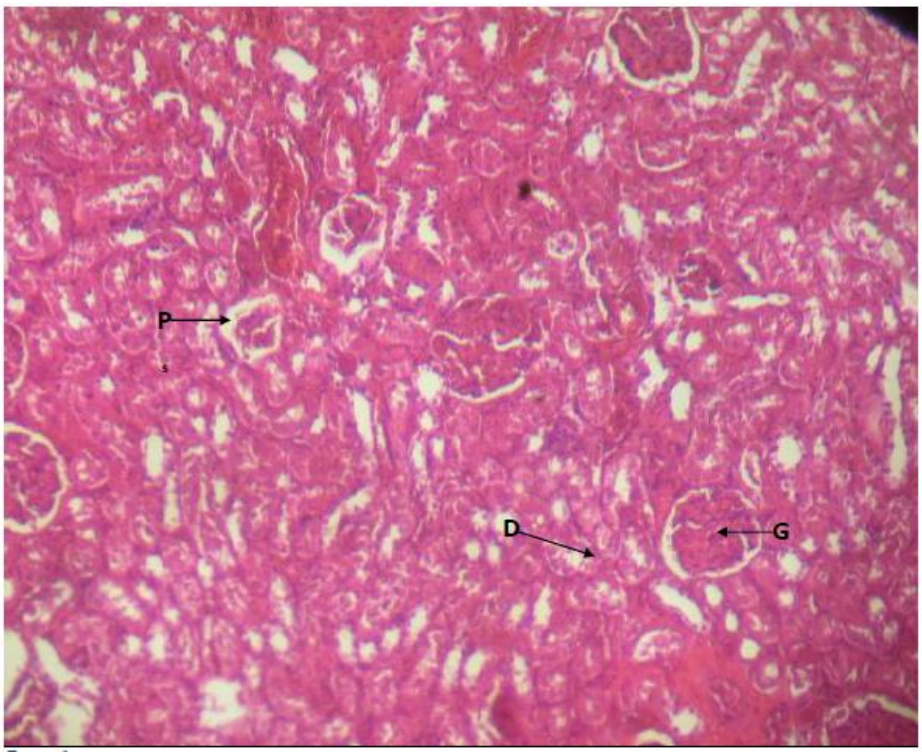

Figure 6: Photomicrograph of ME kidney. H&E stain. X400.
G-Glomerulus. P-Proximal convoluted tube. D-Distal convoluted tube

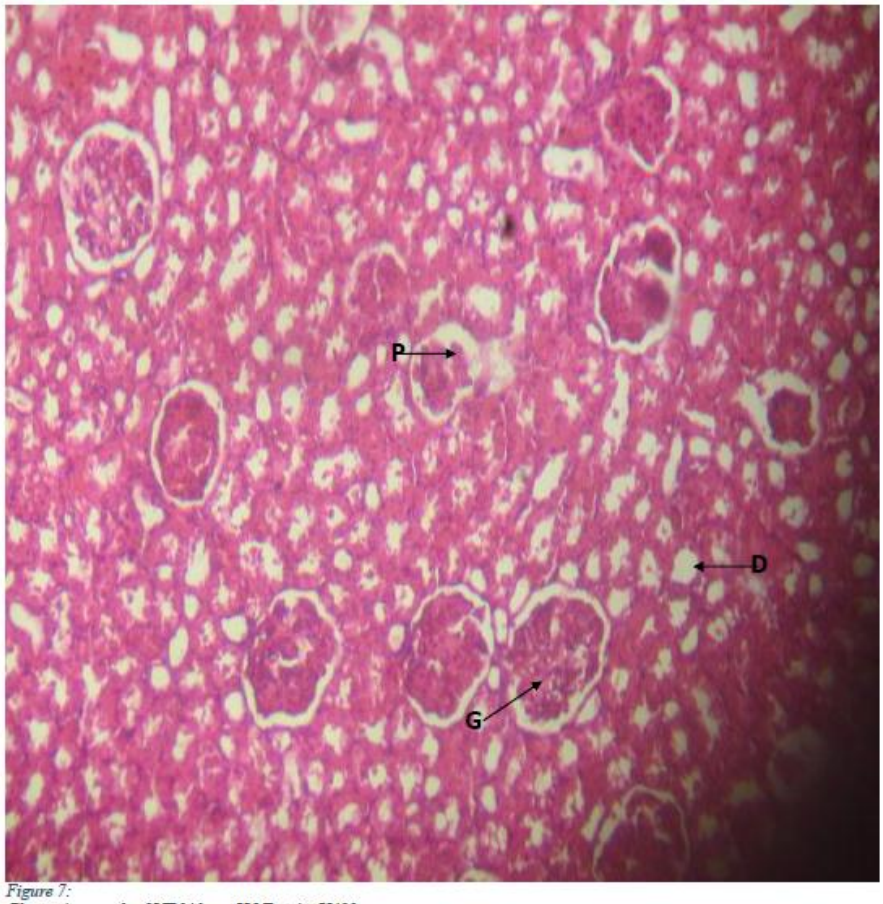

Figure 7:
Photomicrograph of VE kidney. H&E stain. X400.
G-Glomerulus, P-Proximal convoluted tube, D-Distal convoluted tube

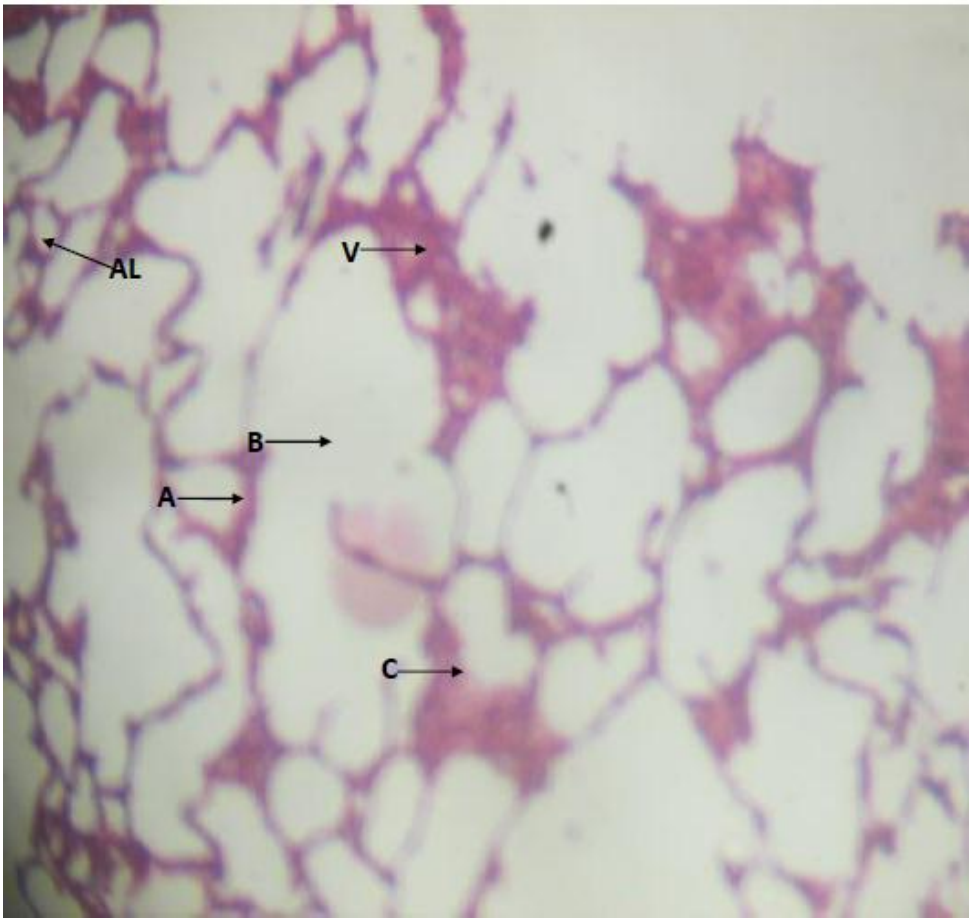

Figure 7: Photomicrograph of VC lungs. H&E stain. X400.
B-Bronchiole. AL- Alveolus. C-Cartilage. A-Artery. V-Vein.

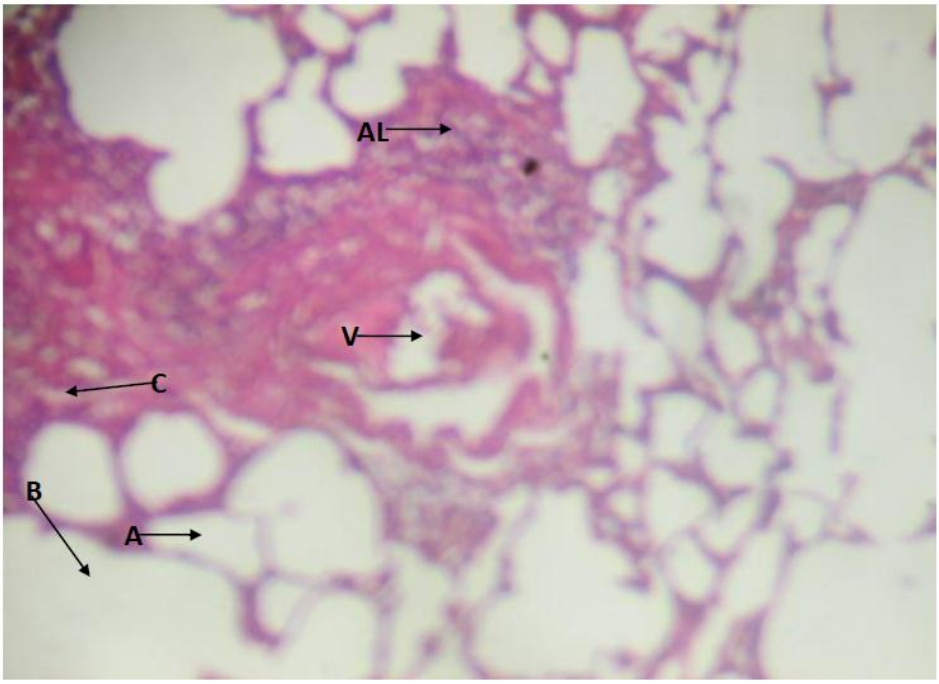

Figure 8: Photomicrograph of MC lungs. H&E stain. X400.
B-Bronchiole. AL- Alveolus. C-Cartilage. A-Artery. V-Vein.

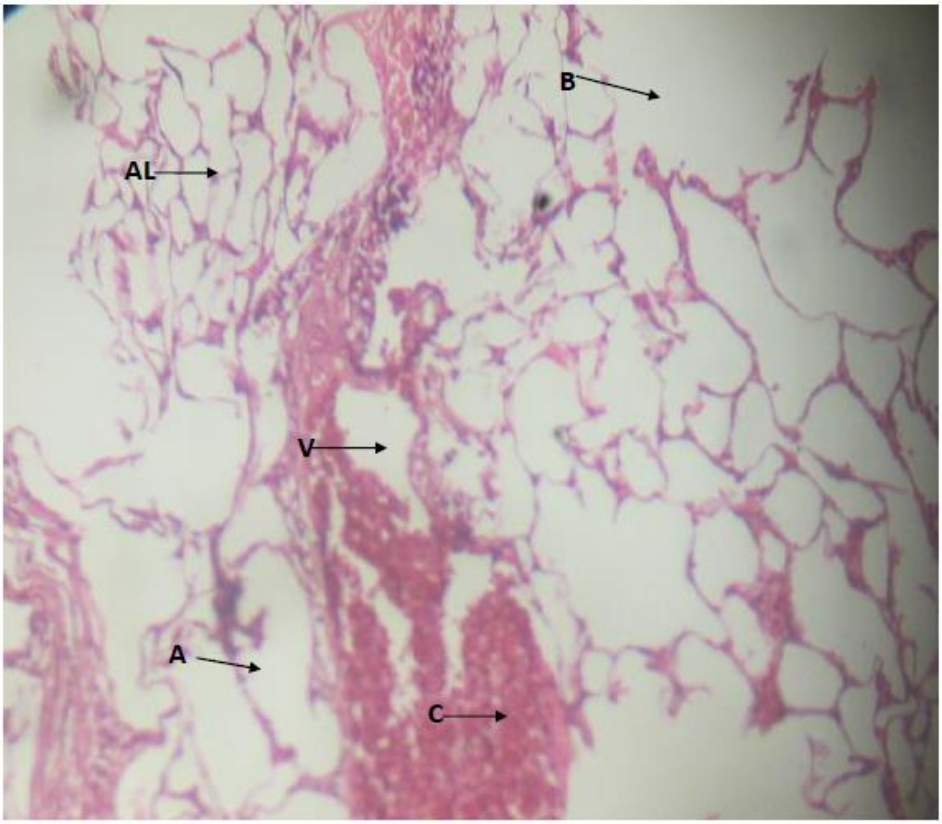

Figure 9: Photomicrograph of MC lungs. H&E stain. X400.
B-Bronchiole. AL- Alveolus. C-Cartilage. A-Artery. V-Vein.

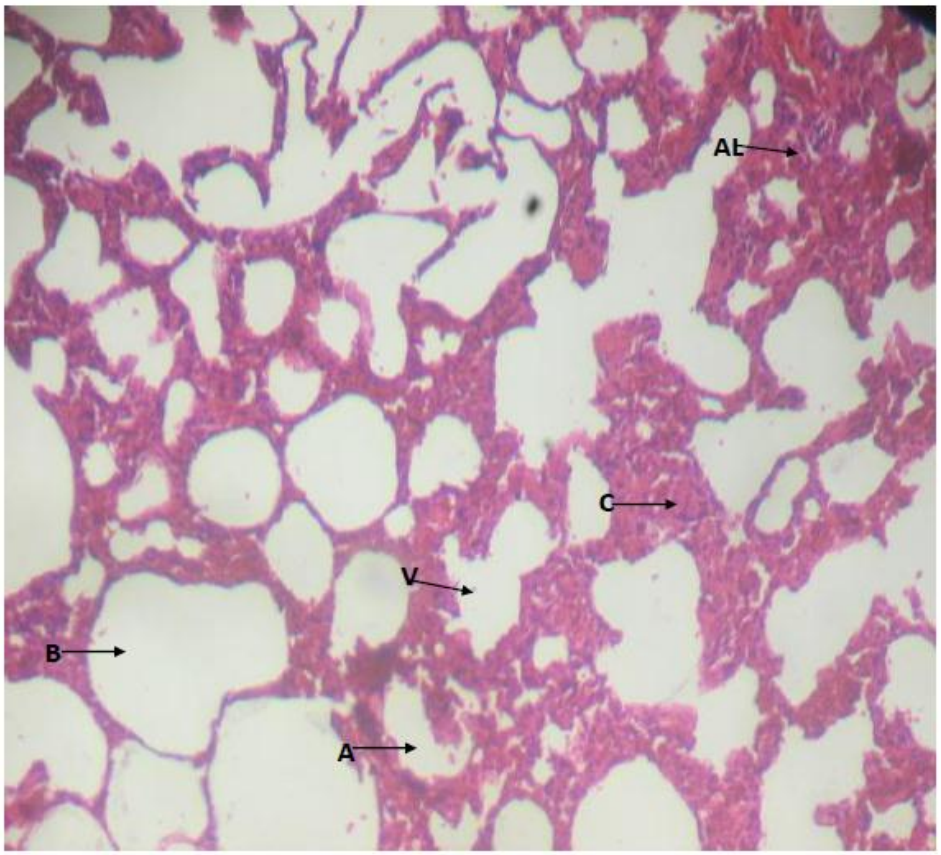

Figure 12: Photomicrograph of VE lungs. H&E stain. X400.
B-Bronchiole. AL- Alveolus. C-Cartilage. A-Artery. V-Vein.

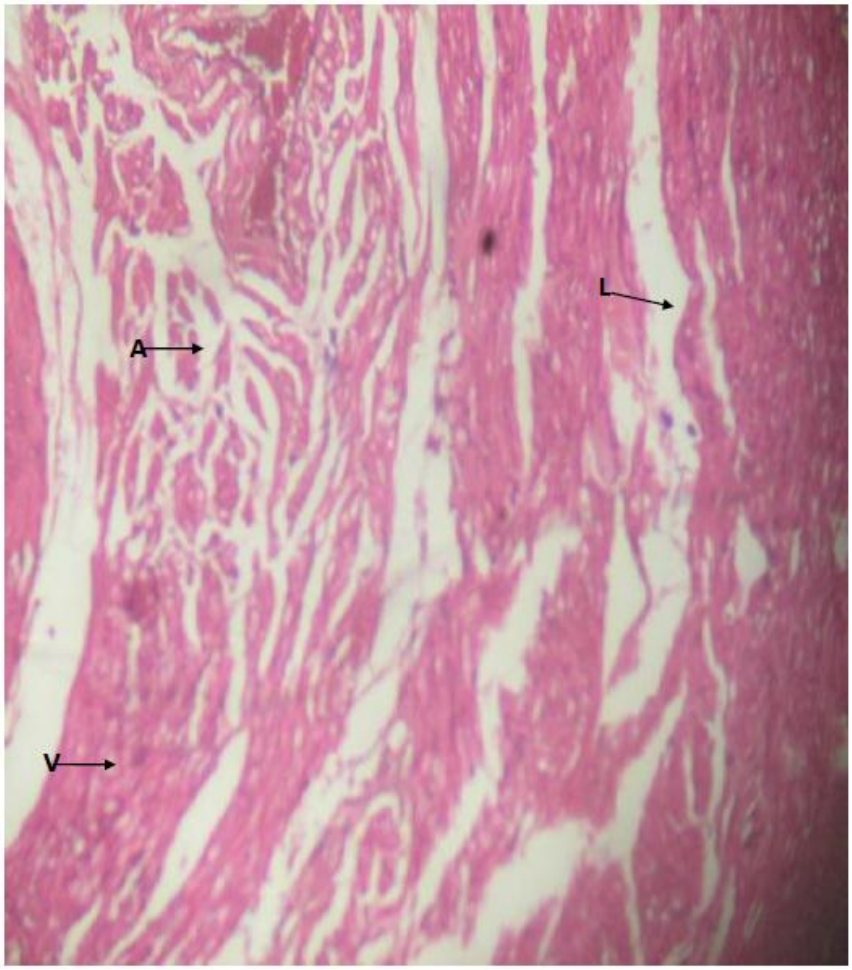

Figure 13: Photomicrograph of VE heart. H&E stain. X400.
L-Lymphatic vessel. A-Arteriole. V-Venule

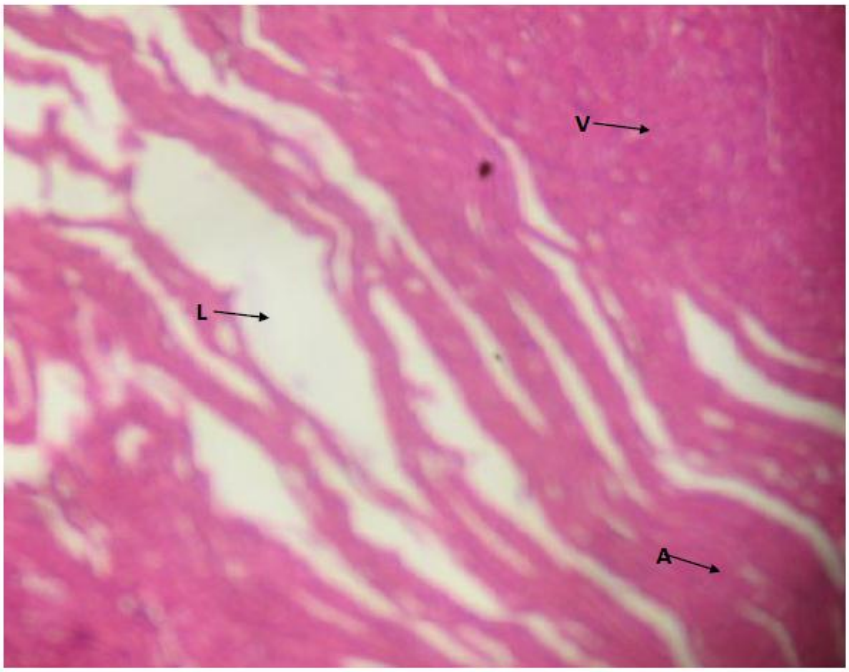

Figure 14: Photomicrograph of VE heart. H&E stain. X400.
L-Lymphatic vessel. A-Arteriole. V-Venule

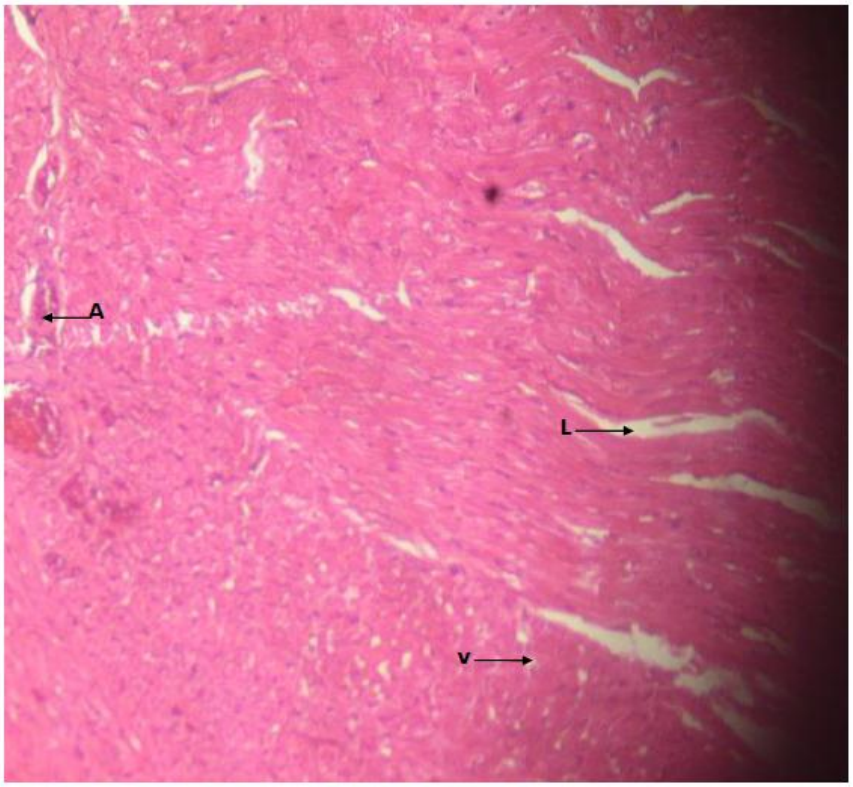

Figure 15: Photomicrograph of VE heart. H&E stain. X400.
L-Lymphatic vessel. A-Arteriole. V-Venule

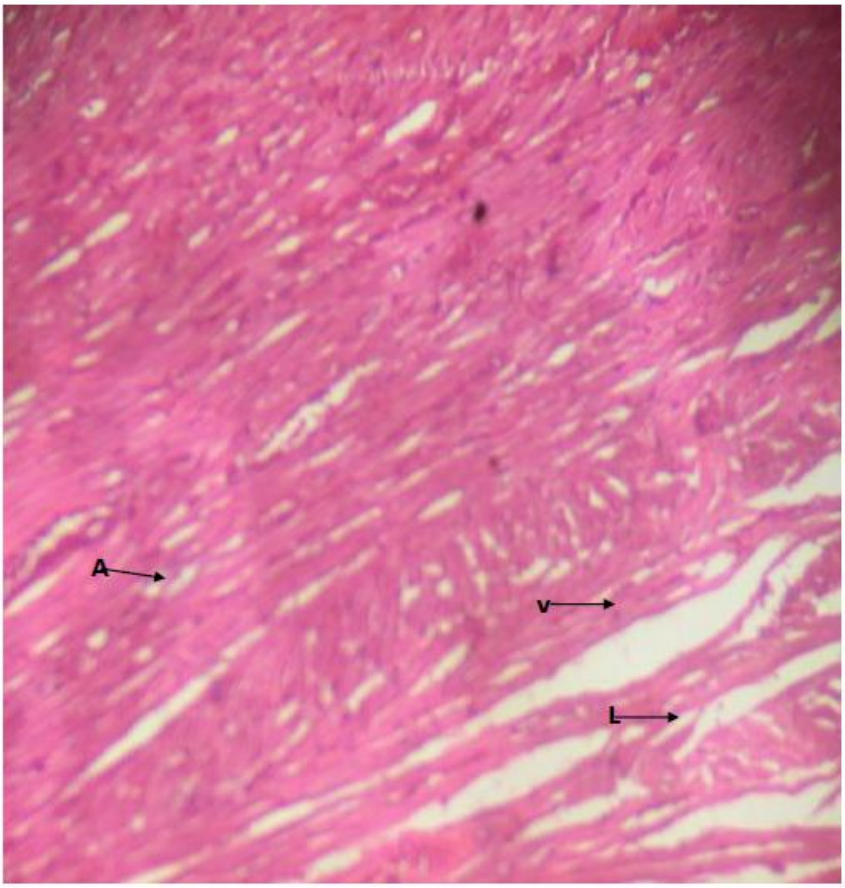

Figure 16: Photomicrograph of VE heart. H&E stain. X400.
L-Lymphatic vessel. A-Arteriole. V-Venule

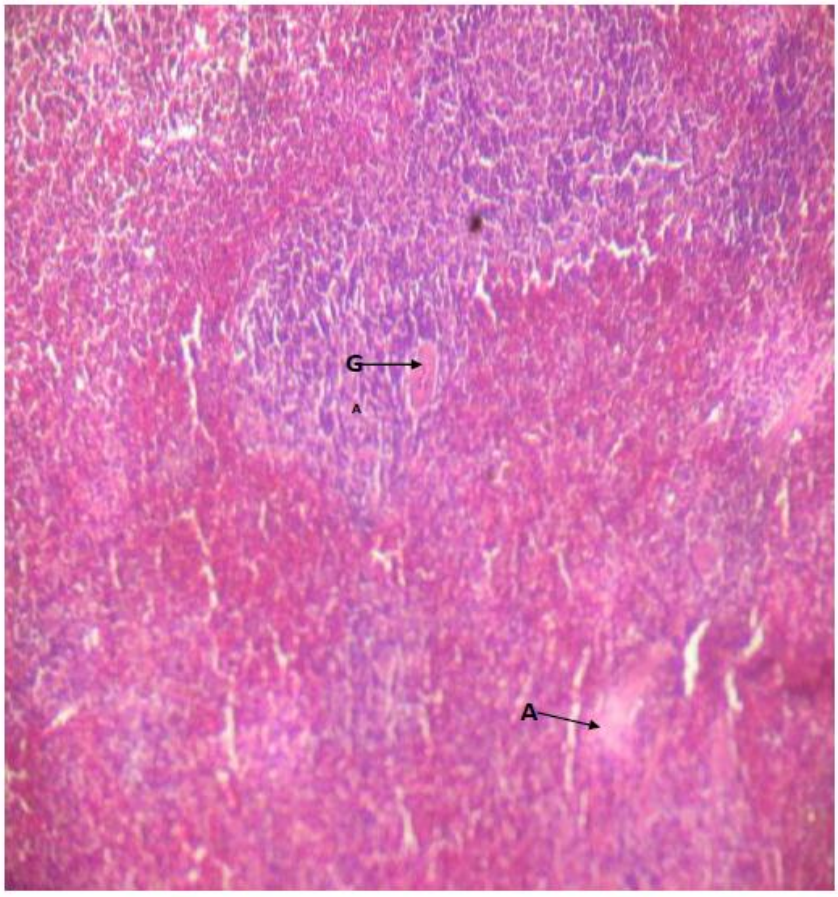

Figure 17: Photomicrograph of VE spleen. H&E stain. X400.
A-Central artery. G-Germinative center

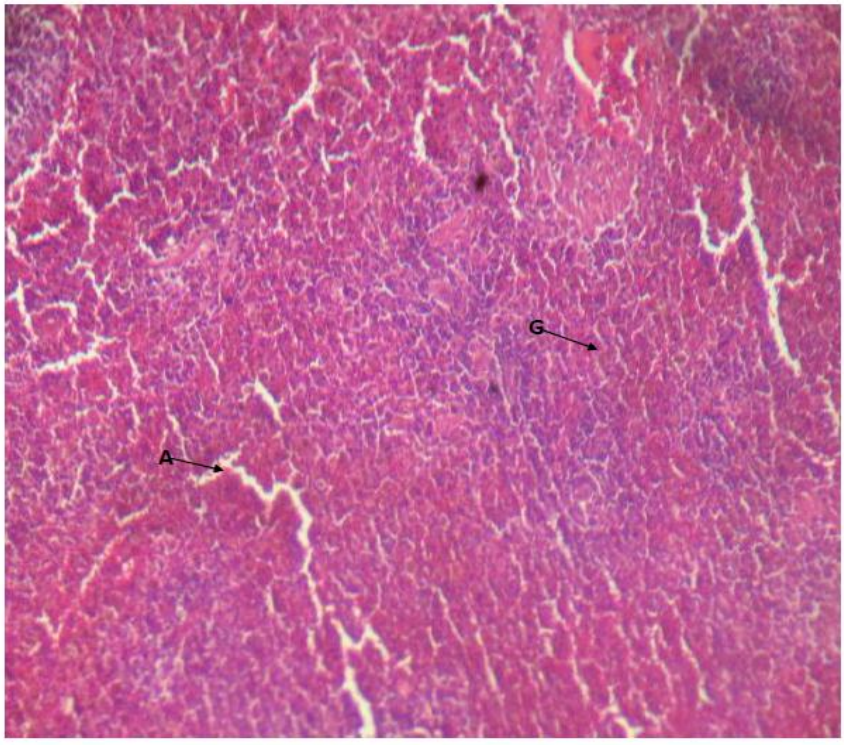

Figure 18: Photomicrograph of VE spleen. H&E stain. X400.
A-Central artery. G-Germinative center

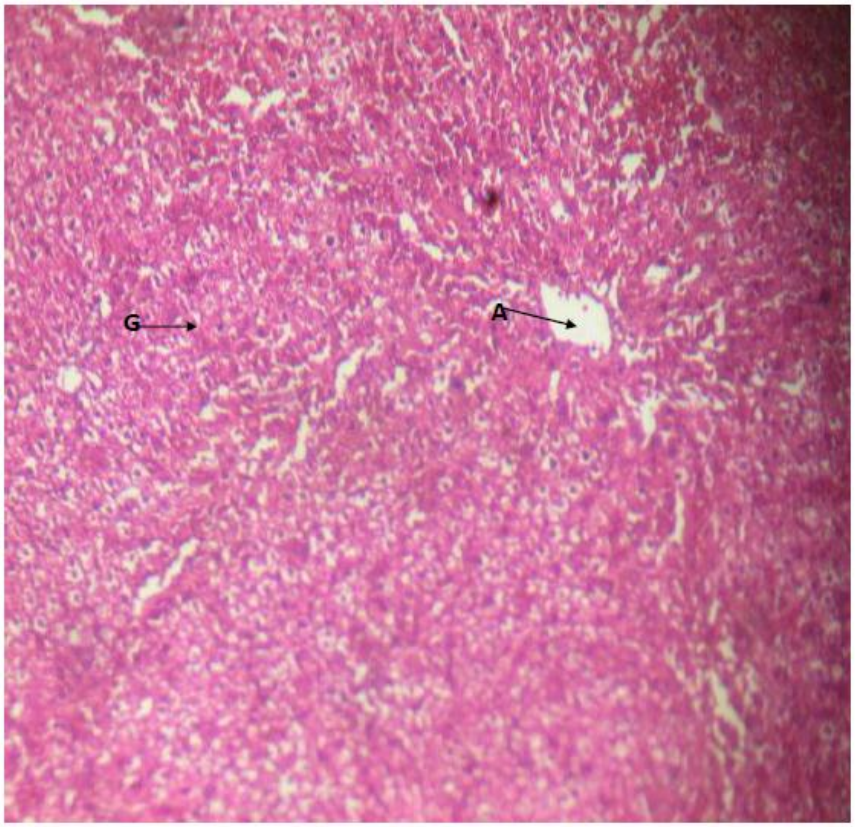

Figure 19: Photomicrograph of VE spleen. H&E stain. X400.
A-Central artery. G-Germinative center

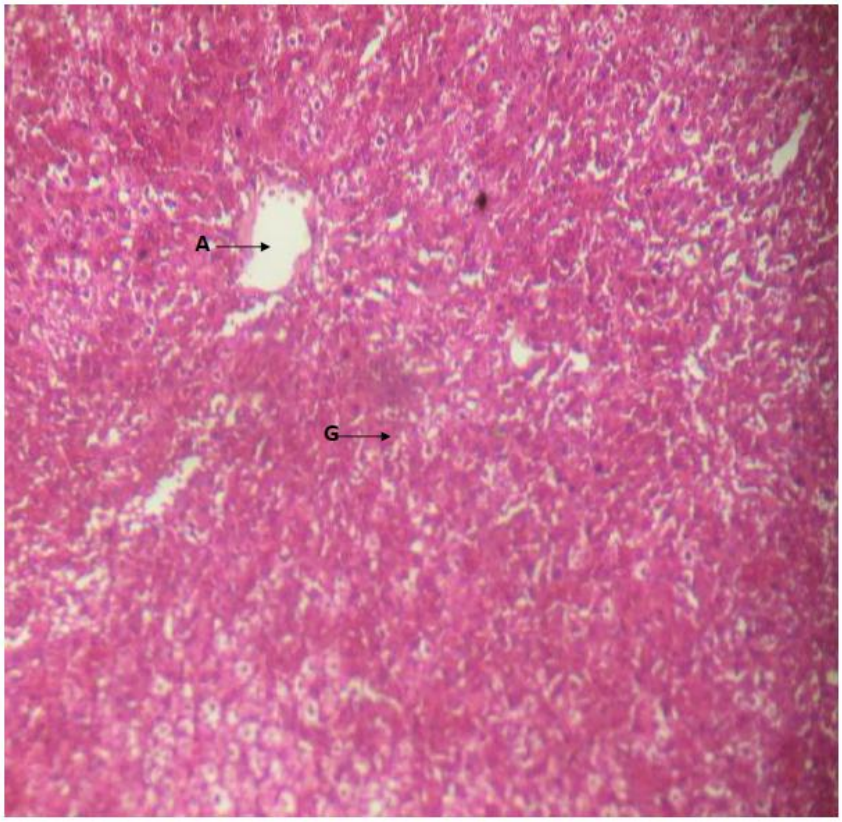

Figure 20: Photomicrograph of VE spleen. H&E stain. X400.
A-Central artery. G-Germinative center.

DISCUSSION

Multiple births are much more common today than they were in the past. According to the US Department of Health and Human Services, the twin birth rate has increased by over 75% since 1980, and triplet, quadruplet, and high-order multiple births have increased at an even higher rate. This increase can be attributed to two major factors; increased use of fertility treatments and delayed childbearing, as twin births are more common among women over 35 years old.[1]

As it can be seen from the tables 1 and 2 above, the litter size of test animals (VE and ME) is quite significantly different in number ($P<0.05$), compared to those of control groups (VC and MC). This may have been due to the effect of the extract as it is believed to contain gonadotropins which may greatly influence stimulation of the rats' ovary, causing release of more than usual number of eggs. As for multiparous control, MC, the history shows that their previous average litter size is 6, which is still repeated here, compared to those of test, ME, which is 8-10. This is in line with ASRM report that older women are more likely than younger women to get pregnant with multiples, especially with fertility treatment. Also, the frequency of twins increases with maternal age and number of pregnancies. Women between 35 to 40 years of age with 4 or more children are 3 times more likely to have twins than a woman under 20 without children.[11]

Comparing VC and VE, it can be seen that the litter size for VC is 4-6, while those with extract, VE, is 6-10, which statistically, is a significant difference, and this can also be traced to effect of the crude extract given.

Assay of the serum levels of gonadotropins (LH and FSH) revealed higher levels in the two treatment groups, which could facilitate the release of more than usual no of eggs during ovulation. Gonadotropins (or glycoprotein hormones) are protein hormones secreted by gonadotrope cells of the anterior pituitary of vertebrates.[13] This is a family of proteins, which include the mammalian hormones follicle-stimulating hormone (FSH), luteinizing hormone (LH), placental chorionic gonadotropins hCG and eCG and chorionic gonadotropin (CG), as well as at least two forms of fish gonadotropins. These hormones are central to the complex endocrine system that regulates normal growth, sexual development, and reproductive function. The hormones LH and FSH are secreted by the anterior pituitary gland, while hCG and eCG are secreted by the placenta. The two principal gonadotropins in vertebrates are luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Gonadotropin receptors are embedded in the surface of the target cell membranes and coupled to the G-protein system. Signals triggered by binding to the receptor are relayed within the cells by the cyclic AMP second messenger system.[15]

Gonadotropins are released under the control of gonadotropin-releasing hormone (GnRH) from the arcuate nucleus and preoptic area of the hypothalamus. The gonads — testes and ovaries — are the primary target organs for LH and FSH. The gonadotropins affect multiple cell types and elicit multiple responses from the target organs. As a simplified generalization, LH stimulates the Leydig cells of the testes and the theca cells of the ovaries to produce testosterone (and indirectly estradiol), whereas FSH stimulates the spermatogenic tissue of the testes and the granulosa cells of ovarian follicles.[7] Gonadotropin directly stimulates the ovaries so that they can form eggs, and because of the direct stimulation, the risk of giving birth to twins or more babies is increased. To be more specific, 10 to 40 percent of the women who use gonadotropins get multiple pregnancies.[9]

As fertility issues have recently become increasingly common, couples confronted with them have resorted to various solutions. When natural methods do not work, women opt for fertility drugs or ART (Assisted Reproduction Technology) in order to increase their chances of becoming mothers. It is true that women who use this kind of treatment are more likely to get pregnant, but at the same time they might encounter multiple births. Nowadays, due to advanced fertility treatments, including fertility drugs, one in 32 births is multiple.[4]

Generally, these specific drugs are designed to improve the ovulation process, but there are variations regarding the effects depending on the nature and purpose of the drug.

All in all, in order to induce multiple births, women simply have to take fertility treatment, as it will cause the ovaries to release more eggs, which automatically means more chances to bring twins or more babies to the world.[10] It is then strongly believed that the crude extract of *Abelmoschus esculentus* behaved just like these injected fertility booster.

CONCLUSION

In conclusion, crude extract of *Abelmoschus esculentus* increases serum LH and FSH levels, thereby facilitating super ovulation which causes release of more than usual eggs in the ovaries, giving rise to multiple births. Also, due to its non-alteration of histomorphological structures, the leaf is considered to be safe for consumption. Hence, the leaf may be explored as a fertility booster to aid multiple births, therefore its consumption by the people of Igbo-Ora to aid multiple births is justified.

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