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Tectona grandis (Teak Tree) Young Leaf Extract as a Histological Stain

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ABSTRACT

Stains are applied to impart contrast to the tissue and identify particular features of interest. However, the use of synthetic dyes as staining reagents has been associated with significant human health challenges and pollution of the ecosystem. These developments have necessitated a shift towards using natural dyes that are eco-friendlier and readily available. We investigated the staining reaction patterns of teak tree leaves (*Tectona grandis*) dye extracts and explored their suitability as a cytoplasmic stain in micromorphological assessments. Dye extracts were prepared using acetone, methanol, and ethanol as solvents from air-dried (under shade) teak tree young leaves. The dye extracts were applied as a counterstain and evaluated against eosin in formalin-fixed paraffin-embedded (FFPE) bovine tissue sections at varying concentrations and different staining times. Teak tree leaves (*Tectona grandis*) dye extracts produced relatively varying staining intensities of reddish-brown cytoplasmic coloration when used on bovine tissue at different concentrations and staining times comparable to eosin and with blue-purple hematoxylin nuclear stain. The present study showed that *Tectona grandis* leaf dye extracts provide an excellent cytoplasmic staining pattern and can be used as an alternative counterstain in routine H&E staining techniques.

1. INTRODUCTION

Staining of histological sections permits observation of characteristics otherwise not distinguishable.

Staining depends on the physicochemical structure of dyes, tissues, and the composition of cells [1-3]. Tissue components get stained by absorbing the appropriate wavelength of light and allowing the microscopic study of tissue morphology to diagnose abnormalities. There are two types of stains based on the production method, natural and synthetic [4]. Natural dyes are obtained from plants, animals, and minerals. Examples include hematoxylin, carmine, saffron, indigo, Berberine, Orcein, and Litmus [5]. Hematoxylin, a routine nuclear stain employed in histology, is obtained from the logwood called Hematoxylin campechianum [6]. Carmine, a dye from the insect Dactylopius Cacti, demonstrates nuclei, chromosomes, or mucins, depending on the formulations. Synthetic dyes, on the contrary, are produced by chemical Reactions and include Eosin Y, Basic fuchsin, Picric acid, Aniline blue, and Toluidine blue.

Natural dyes were the only ones available to scientists for histological staining until the first synthetic dye was discovered in 1856 [7]. However, rapid research in chemistry has led to synthetic dye alternatives pushing the natural dyes into oblivion. Despite their wide use, some synthetic dyes have been shown to cause hazards to human and animal health and have been withdrawn. Increased risks of occupational morbidity and mortality have been associated with the use of synthetic dyes globally [8]. The preparations of synthetic dyes are very hazardous and laborious. The hazardous effects of these reagents of synthetic dyes by frequent usage pose a health threat to laboratory professionals. Hence, replacing them with biofriendlier and less toxic natural dyes is necessary for sustainable development.

However, natural dyes are often times more expensive than synthetic dyes, in that regard most users of dyes prefer synthetic dyes to natural dyes not considering the hazardous nature of these synthetic dyes. Nonetheless, there is the belief that in the near future natural dyes will be an economically and environmentally viable option. Despite their frequent use, synthetic dyes are becoming increasingly expensive, and many small laboratories in developing countries cannot afford them.

Dyes produced from the root, bark, and fresh leaves of the Teak plant have been used for paper products, matting, and clothes producing yellow-brown or red-brown colors. Dyes from the teak tree leaves have been employed in the textile industry to print colorful woolen and cotton fabrics.

Teak leaves have been used to impart color to cotton and woolen fabrics in the textile industry. Also, recent studies have demonstrated that fungal structures such as mycelium stained reddish-brown with *T. grandis* leaves extract and stood out well against a suitable background stain [8].

However, it has not been shown that this extract can be used to stain animal tissues; therefore, this research sought to explore the staining properties of the teak tree's young leaves extracts in histopathological applications.

2. MATERIALS AND METHODS

2.1. Study Design

The research was an exploratory, experimental study conducted at the histopathology laboratory of the Ho Teaching Hospital in the Volta Region of Ghana. Ethical approval was sought from the University of Health and Allied Sciences, Research Ethics Committee (UHAS-REC A.10 [48] 20-21) and permission to use the bovine tissue from the Veterinary Services, Ho (NoMA/VR/VSD/VAL/84/1).

2.2. Collection of *Tectona grandis* Leaf

Fresh teak young leaves were obtained from the Titrinu teak plantation, located on the outskirt of Ho Municipality. The teak tree's young leaves are brown in color while the mature leaves are green (**Figure 1**).

The harvested leaves were rinsed thrice with tap water and twice with distilled water to remove impurities, including soil dust and avian fecal droplets that settled on the leaves. The prewashed teak leaves were shade dried in a dust-free environment for ten days.

2.3. Powderisation of *Tectona grandis* Leaf

The shade-dried leaves were finely ground into powder form, using a motor-operated domestic mixer

Figure 1. **Mature (blue arrow) and young (black arrow) leaves of the Teak plant (project site).**

grinder at 220V/2200 rpm, then stored in an amber-colored dry container for further usage.

2.4. Differential Extraction of *Tectona grandis* Leaf Dye

In an airtight brown bottle, three extracts were prepared using ethanol, methanol, and acetone at four varying concentrations (7.5g, 15g, 20g, and 25g in 100mls of the solvents) and stored for six days with intermittent shaking. Different extracts constituting twelve (12) dye solutions were obtained from the teak tree leaf extract and filtered (with Whatman No.1 filter paper) to eliminate remnants of plant material and debris. The pH for the various extracts was measured using the Digital pH meter (JENWAY 3510, Staffordshire, UK) and recorded. The various solutions were stored in brown bottles and kept away from direct sunlight until use.

2.5. Tissue Sampling

Fresh bovine of the West African short horn breed internal organs, including liver, spleen, and kidney obtained from the Ho Central Slaughterhouse, were used for the study. The organs after removal from the bovine were immediately placed in labeled 10% neutral buffered formalin-filled (in-house) containers and sent to the histopathology laboratory. The blood-stained formalin solutions on the organs were discarded and replaced with fresh 10% neutral buffered formalin (in-house) solutions. The organs were left in the formalin for 48 hours before processing.

2.6. Tissue Processing

The organs were sliced into 3mm thicknesses, and representative sections were placed into graphite pencil-labeled tissue cassettes denoted by the various experimental organs (liver, spleen, and kidney). The accessioned tissues were processed manually following standard tissue processing protocol. Briefly, the tissues were dehydrated by transferring into 70%, 80%, 90%, and three changes of absolute ethanol for 1hr:30 minutes each. The tissues were cleared in three changes of xylene for 1hr:30 minutes each. The tissues were further infiltrated in two changes of molten paraffin wax (58˚C) for 45 minutes each. After infiltration, the tissues were embedded on a semi-automated embedding station (KUNZ Instrument Giralt,

Sweden) with molten paraffin wax using embedding molds with appropriate orientation as stipulated by best practice [9]. After which they were placed quickly on ice to solidify (block). The tissue blocks were left at room temperature until ready for sectioning.

2.7. Tissue Sectioning

One hundred and fourteen (114) sections of 3μm thickness were cut from the various tissue blocks using the manual rotary microtome (ACCU-CUT SRM 200 CW, Sakura, Japan). Thirty-eight sections were cut from each of the three organs. Ribbons of sections were floated out on warm water in the thermostat-controlled water bath (JP SELECTA, Barcelona, Spain). The sections were picked onto clean and grease-free frosted-end glass slides, labeled as "liver", "spleen", and "kidney" for the organs. Sections were dried on a slide rack at room temperature for 30 minutes and transferred into the hot-air oven (GENLAB, Widnes, UK) for 30 minutes to fix the sections onto the slides. Tissue sections were grouped into four, with three groups containing 36 slides each and the fourth containing six slides. Unique IDs were generated according to the composite extracts, extract concentrations and duration of staining for each of the designated tissue sections. For example, "liver M25(15)" means that the liver tissue section is stained with methanolic extract at a concentration of 25g per 100 ml for 15 minutes. The fourth group, designated as controls, were also labeled according to the type of organ; for example, "liver CONT" represents the liver tissue section for control (**Supplementary 1**).

2.8. Staining

The sections (groups 1, 2, and 3) were stained manually with the dye extracts of different solvents and concentrations at different times (**Appendix 1**).

The sections (group 4) were stained manually with H&E as indicated in the standard protocol (**Appendix 2**). Both the Harris hematoxylin and the eosin used were prepared in-house following the standard protocol [10].

2.9. Microscopy and Scoring of Staining Intensity

The staining reaction of each stain category on the tissue morphology was examined and micrographs were taken using a Da Vinci Medical Microscope; Phase contrast and Darfield microscope (LEICA DM750, LEICA ICC50 W). Nuclear materials within the tissues were stained blue to purple with hematoxylin. The cytoplasm and its inclusions were stained reddish-brown by the extracts or pinkish to red by the eosin.

The intensities of the staining reactions of both hematoxylin/extracts and control were expressed using a scoring system based on the staining intensity. The intensity was considered excellent (+4) when all the following components were observed under the light microscope: cell nucleus, cytoplasm, cell membrane, and extracellular matrix. If three of these components were observed, the intensity was expressed as very good (+3). The intensity observed in two components was expressed as good (+2), and the intensity for one component as poor (+1). If no component was observed, it was expressed as no staining (0) (**Figure S1**).

3. RESULTS

3.1. Extracts Preparation

Three extracts of the dye were prepared: ethanol, methanol, and acetone extracts using different concentrations of the *Tectona grandis* powder (7.5 g, 15 g, 20 g, 25 g) per 100 mls. The pH of the extracts varied per the type of extract with a mean of 6.67 for methanol extract, 6.45 for ethanol extract, and 6.32 for acetone extract (**Appendix 3**). The extracts were used to stain histologically processed sections from the kidney, liver, and spleen at different times (5 minutes, 10 minutes, and 15 minutes) (**Figure 2** and **Supplementary Figures S2-S37**).

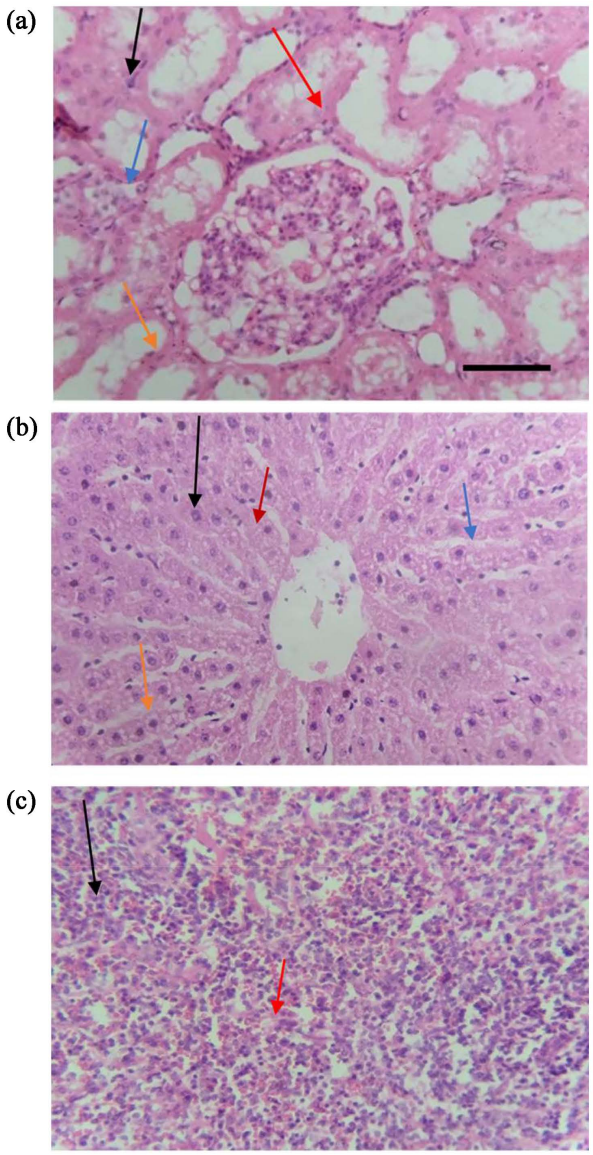

Figure 2. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections scored excellent staining (4+) stained with H&E at a Magnification of 400×.

3.2. Staining Intensity

Hematoxylin and eosin stains were used as the control. Hematoxylin stained the nuclei purple (black arrow) and eosin-stained cytoplasm pink (red arrow). The cell membrane is also stained pink (orange arrow) and the extracellular matrix-stained pink (blue arrow) (Figure 3).

3.3. Hematoxylin and Dye Extract

Hematoxylin stained the nuclei blue-black (black arrow) and dye extract-stained cytoplasm golden-brown (red arrow). The cell membrane was also stained golden-brown (orange arrow) and the extracellular matrix stained faintly brown (blue arrow) (Figure 3) depending on the density of the components. The hematoxylin's color and intensity remained the same for all sections whilst the cytoplasm-stained shades of golden-brown with varied intensity with the extracts (Figure 3 and **Supplementary** Figures S2-S37).

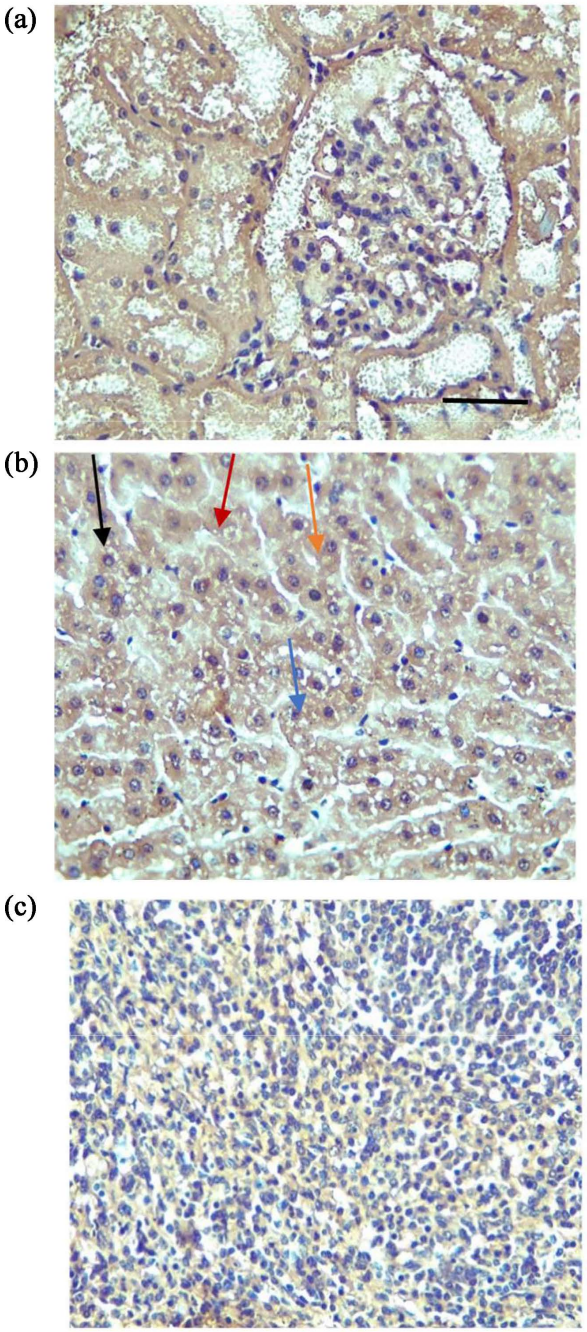

Figure 3.Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 25 g/100mls for 15 minutes (magnification 400×).

4. DISCUSSION

Tissue morphological appraisal is essential for clinicopathological correlation in diagnosing and managing diseases. The assessment of histological architecture to elucidate cellular details is achieved by using suitable dyes. Over the years, the use of synthetic dyes has gained traction, especially in the textile industry, and a significant application in laboratory medicine. Although synthetic dyes are easy to produce commercially, their usage poses a threat to human and aquatic lives due to heavy pollutants from their by-products released into the ecosystem [11]. Natural dyes, on the contrary, are eco-friendly and biode-

gradable with rich biodiversity and availability since most of their sources are consumed as medicinal products or present as constituents of the food chain [12]. However, their use as tissue colorants in laboratory medicine is yet to be explored.

The present study explored dye extract from *Tectona grandis* leaf as a potential cytoplasmic stain for histological studies. Dye extracts from acetone, ethanol and methanol prepared at different concentrations were used as counterstain with hematoxylin nuclear stain on liver, kidney, and spleen tissue sections. The various dye extracts obtained were acidic (pH of 6.28 to 6.72). The acidity of the extracts varied among the different solvent solutions used. Acetone extracts were more acidic, followed by the ethanol extracts, while the methanol extract was increasingly towards the neutral pH. Within the same solvent extract, it was noted that the pH decreased with an increase in the concentration. Thus, the highest concentration (25g) in all the solvents had the lowest pH in their respective solvents (**Appendix 3**). This observation is indicative that the solvent and concentration of the extract influenced the acidity of the dye, making it preferably a cytoplasmic stain.

The overall staining reaction of the hematoxylin and various dye extracts in the bovine organs showed tissue contrast with distinct blue to purple coloration of the nuclei and a bright staining pattern of the cytoplasm. This phenomenon is critical to identifying pathological derangement at the cellular level. The cytoplasm showed different shades of reddish-brown, which could probably be due to the acidophilic nature of the cytoplasmic components. Additionally, varying cytoplasmic colorations may be attributed to the differences in the pH of the extracts. This suggests that different extracts and their concentrations determine the intensity of the stains.

The methanol extracts exhibited the best staining reaction among the lot, showing clear reddish-brown coloration of the cytoplasm, with distinct morphological architecture (**Supplementary Figure S16**). Conversely, ethanol extracts showed a bright reddish-brown coloration of the cytoplasm with well-preserved tissue morphology (**Supplementary Figure S28**). This observation further supports the fact that ethanol is a cytological fixative with increased preservation of cytoplasmic inclusions. In most sections, the acetone extracts showed a brick-red coloration of the cytoplasm (**Supplementary Figure S7**). However, it caused some tissue section degeneration, which amounts to technical error rather than pathological derangement interfering with the tissue morphology (**Figure 3**). The changes in the morphology of the tissue cells may be attributed to the relative acid strength of the extracts.

The cytoplasmic staining showed varied intensity according to the concentrations of the extracts and the staining time. Increased concentration of the extracts and prolonged staining time resulted in increased cytoplasmic staining intensity comparable to the nuclear staining intensity, with resultant tissue contrast and distinct morphological architecture (**Supplementary Figure S37**).

The outcome of this study is promising. Further studies will afford the need to elucidate the staining reactions of the extract in pathological tissues and reinforce the validity of its wider application, especially in resource-limited settings, Hematoxylin stained the nuclei blue-black (black arrow) and dye extract-stained cytoplasm golden-brown (red arrow).

5. CONCLUSION

In conclusion, the extracts demonstrated the various components of the tissues that are used for the morphological diagnosis of tissues. Also, methanol extracts exhibited good staining intensity compared to the other extracts though all the extracts produced good staining quality and the staining reactions were influenced by the concentration of the extracts. Furthermore, *Tectona grandis* young leaf extract has excellent prospects as a natural dye that could substitute for eosin in standard histological staining techniques.

ACKNOWLEDGEMENTS

We are grateful to Ho Teaching Hospital's management, the Laboratory Department's staff members, and the technologists at the School of Allied Health Sciences Laboratory Unit, the University of Health and

Allied Sciences.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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APPENDICES

Appendix 1: Staining Protocol for Teak Extracts

ACTIVITY	REAGENT	DURATION
Deparaffinization and rehydration	Xylene I	10 minutes
	Xylene II	10 minutes
	100% Ethanol	5 minutes
	90% Alcohol	5 minutes
	Water wash	5 minutes
Nuclear staining	Harri's hematoxylin	20 minutes
	Water wash	2 minutes
Differentiation	1% acid alcohol	3 dips
	Water wash	5 minutes
Bluing	Scott's tap water	10 minutes
	Water wash	5 minutes
Cytoplasmic staining	Teak extracts (group 1)	15 minutes
	Teak extracts (group 2)	10 minutes
	Teak extracts (group 3)	5 minutes
	Water wash	1 minutes
Dehydration	Room temperature	30 minutes
Clearing	Xylene I	5 minutes
	Xylene II	5 minutes
Mounting	DPX mountant	

Appendix 2: Staining Protocol for H&E

ACTIVITY	REAGENT	DURATION
Deparaffinizationand rehydration	Xylene I	10 minutes
	Xylene II	10 minutes
	100% Alcohol	5 minutes
	90% Alcohol	5 minutes
	Water wash	5 minutes
Nuclear staining	Harris hematoxylin	20 minutes

Continued

	Water wash	2 minutes
Differentiation	1% acid alcohol	3 dips
	Water wash	5 minutes
Bluing	Scott's tap water	15 minutes
	Water wash	3 minutes
Cytoplasmic staining	1% Eosin	20 seconds
Dehydration	90% Alcohol	30 seconds
	70% Alcohol	30 seconds
Clearing	Xylene I	5 minutes
	Xylene II	5 minutes
Mounting	DPX mountant	

Appendix 3: PH Values of the Dye Extract at Different Mass Concentrations

Weight of extracts used	7.5g	15g	20g	25g
pH readings of extracts				
Methanol dye extracts	6.72	6.68	6.66	6.6
Ethanol dye extracts	6.52	6.45	6.42	6.41
Acetone dye extracts	6.36	6.33	6.3	6.28

Supplementary Material

Supplementary 1: Tissue Accession and Sectioning

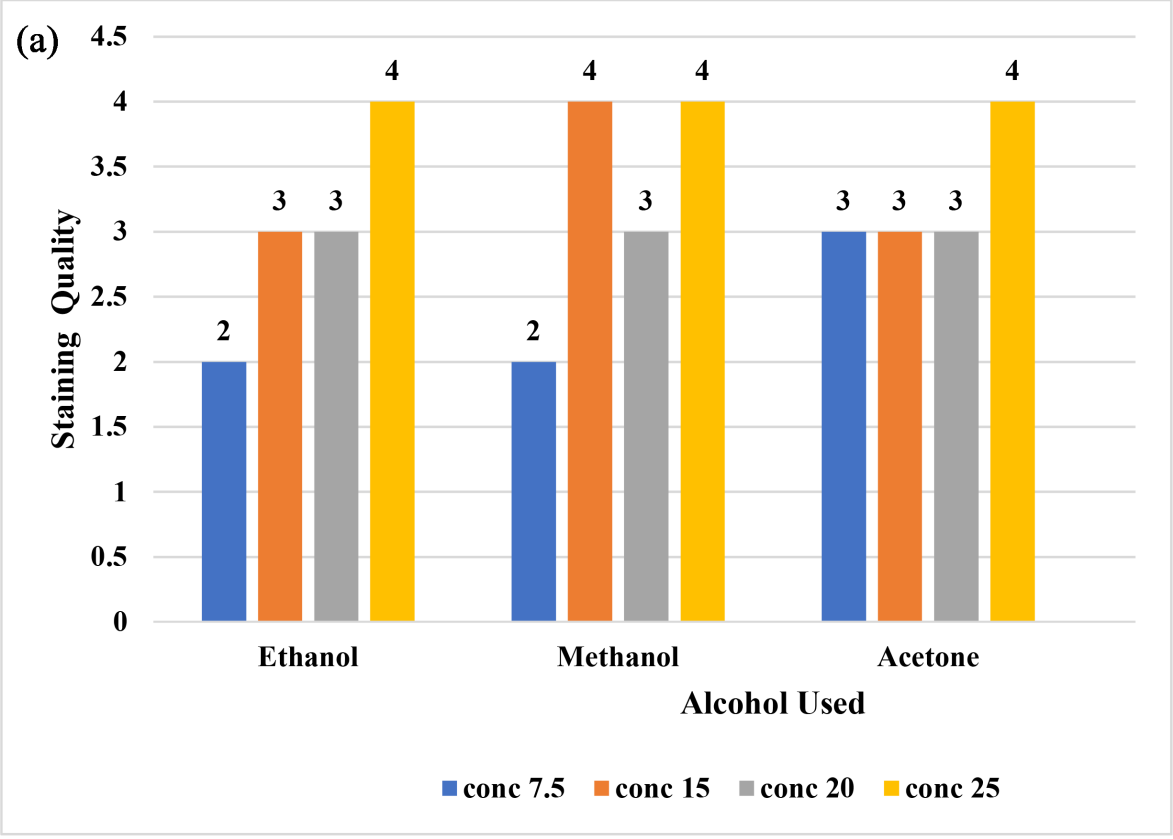
GROUP 1	GROUP 2	GROUP 3	GROUP 4
Kidney E25 (15)	Kidney E25 (10)	Kidney E25 (5)	Kidney control
Kidney E20 (15)	Kidney E20 (10)	Kidney E20 (5)	Kidney control
Kidney E15 (15)	Kidney E15 (10)	Kidney E15 (5)	
Kidney E7.5 (15)	Kidney E7.5 (10)	Kidney E7.5 (5)	
Kidney M25 (15)	Kidney M25 (10)	Kidney M25 (5)	
Kidney M20 (15)	Kidney M20 (10)	Kidney M20 (5)	
Kidney M15 (15)	Kidney M15 (10)	Kidney M15 (5)	
Kidney M7.5 (15)	Kidney M7.5 (10)	Kidney M7.5 (5)	
Kidney A25 (15)	Kidney A25 (10)	Kidney A25 (5)	
Kidney A20 (15)	Kidney A20 (10)	Kidney A20 (5)	
Kidney A15 (15)	Kidney A15 (10)	Kidney A15 (5)	
Kidney A7.5 (15)	Kidney A7.5 (10)	Kidney A7.5 (5)	
Liver E25 (15)	Liver E25 (10)	Liver E25 (5)	Liver control
Liver E20 (15)	Liver E20 (10)	Liver E20 (5)	Liver control
Liver E15 (15)	Liver E15 (10)	Liver E15 (5)	
Liver E7.5 (15)	Liver E7.5 (10)	Liver E7.5 (5)	
Liver M25 (15)	Liver M25 (10)	Liver M25 (5)	
Liver M20 (15)	Liver M20 (10)	Liver M20 (5)	
Liver M15 (15)	Liver M15 (10)	Liver M15 (5)	
Liver M7.5 (15)	Liver M7.5 (10)	Liver M7.5 (5)	
Liver A25 (15)	Liver A25 (10)	Liver A25 (5)	
Liver A20 (15)	Liver A20 (10)	Liver A20 (5)	
Liver A15 (15)	Liver A15 (10)	Liver A15 (5)	
Liver A7.5 (15)	Liver A7.5 (10)	Liver A7.5 (5)	
Spleen E25 (15)	Spleen E25 (10)	Spleen E25 (5)	Spleen control
Spleen E20 (15)	Spleen E20 (10)	Spleen E20 (5)	Spleen control
Spleen E15 (15)	Spleen E15 (10)	Spleen E15 (5)	
Spleen E7.5 (15)	Spleen E7.5 (10)	Spleen E7.5 (5)	

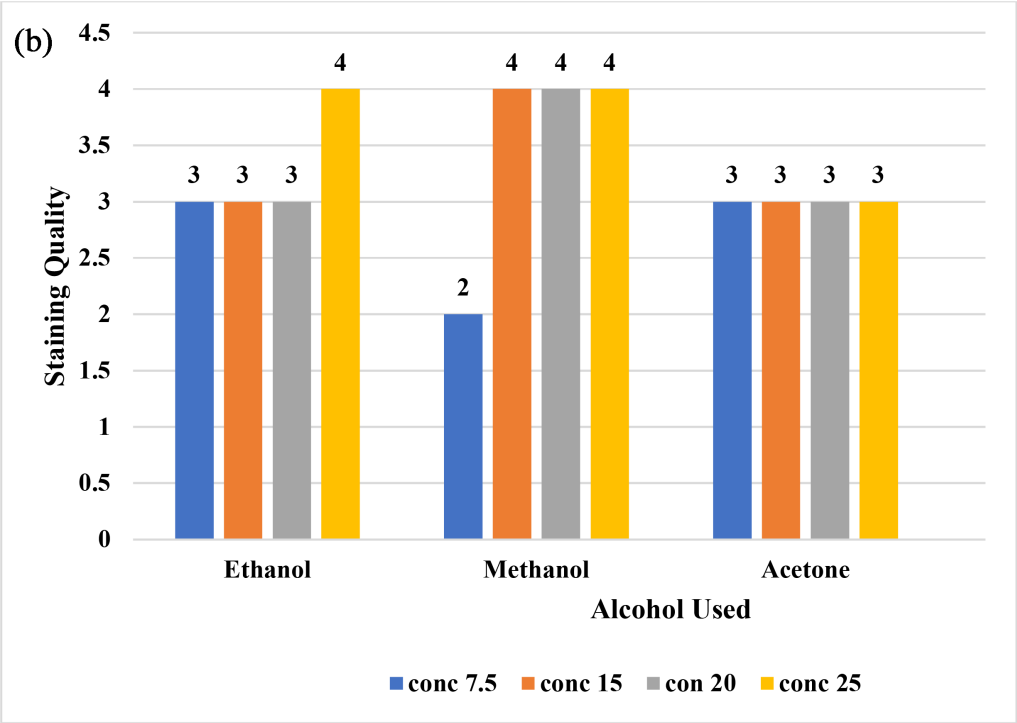
Continued

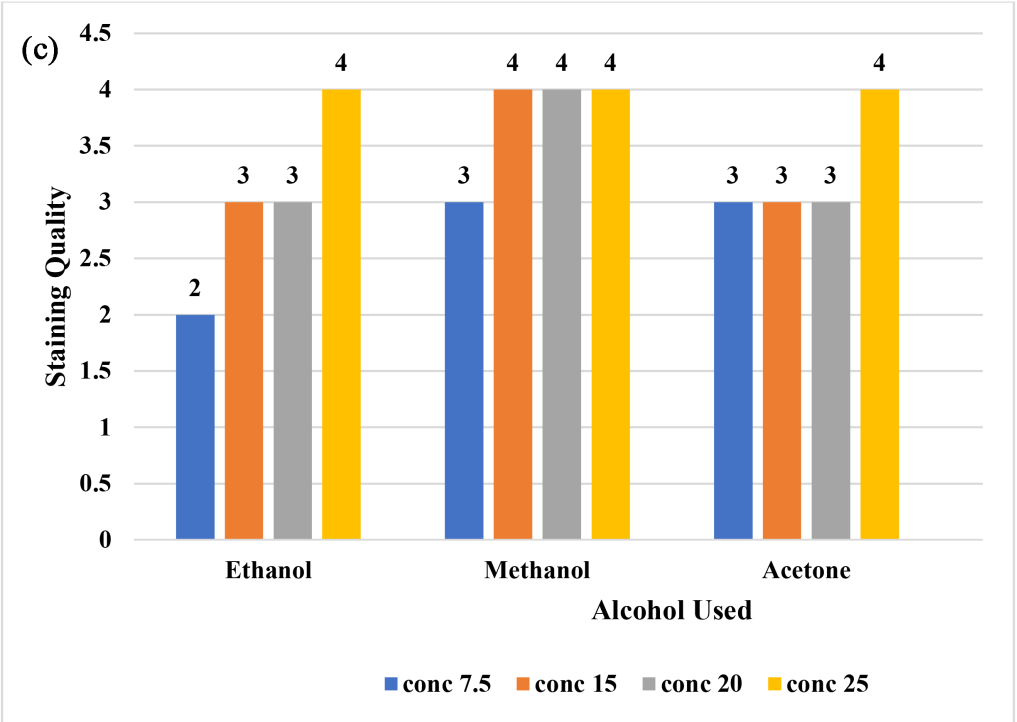
Spleen M25 (15)	Spleen M25 (10)	Spleen M25 (5)
Spleen M20 (15)	Spleen M20 (10)	Spleen M20 (5)
Spleen M15 (15)	Spleen M15 (10)	Spleen M15 (5)
Spleen M7.5 (15)	Spleen M7.5 (10)	Spleen M7.5 (5)
Spleen A25 (15)	Spleen A25 (10)	Spleen A25 (5)
Spleen A20 (15)	Spleen A20 (10)	Spleen A20 (5)
Spleen A15 (15)	Spleen A15 (10)	Spleen A15 (5)
Spleen A7.5 (15)	Spleen A7.5 (10)	Spleen A7.5 (5)

Key: kidney E25(15)—tissue, extract concentration (staining time).

Supplementary Data 2: Scores of Micrographs of the Different Extracts, Concentrations and Staining Time







*Organ; "extract concentration; #Duration of staining; conc—concentration per weight of the powder in grams.

Figure S1. (a) Graph of various alcohol concentrations and their staining quality for an average of 10 minutes on liver tissue; (b) Graph of alcohol concentrations against staining quality for an average of 10 minutes on kidney tissue; (c) Graph of alcohol concentrations against staining quality for an average of 10 minutes on spleen tissue.

Supplementary Data 3: List of Figures

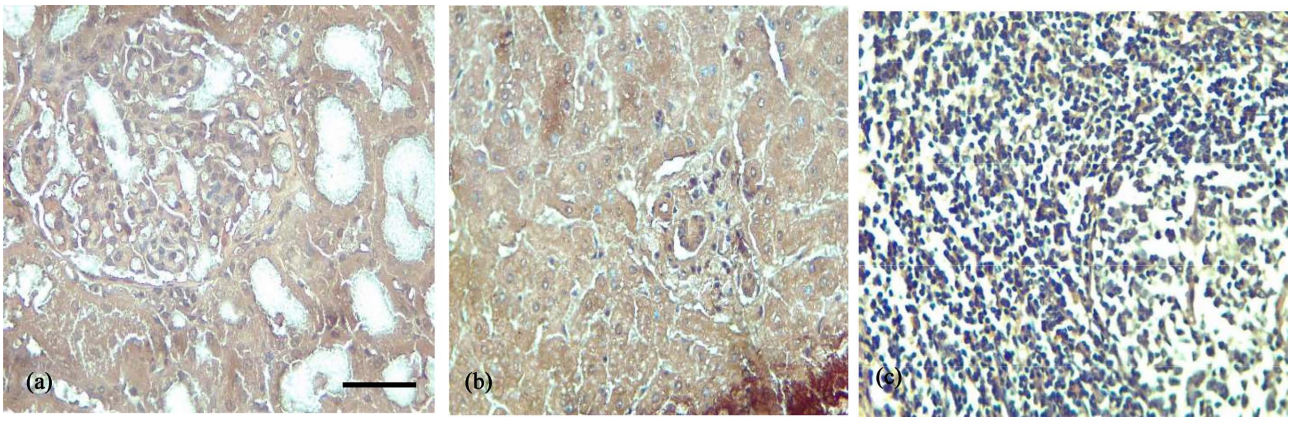


Figure S2. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 25 g/100mls for 5 minutes (magnification 400×).

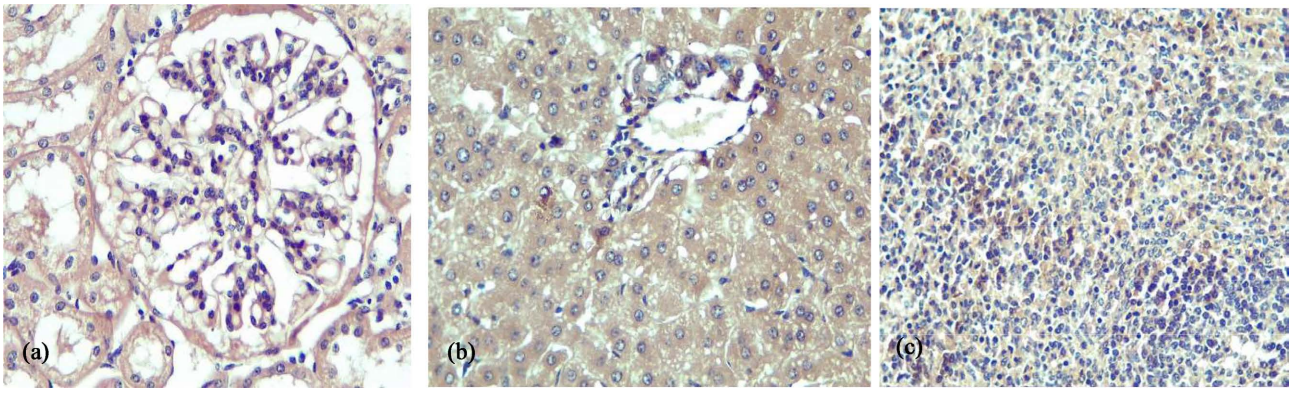


Figure S3. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 25 g/100mls for 10 minutes (magnification 400×).

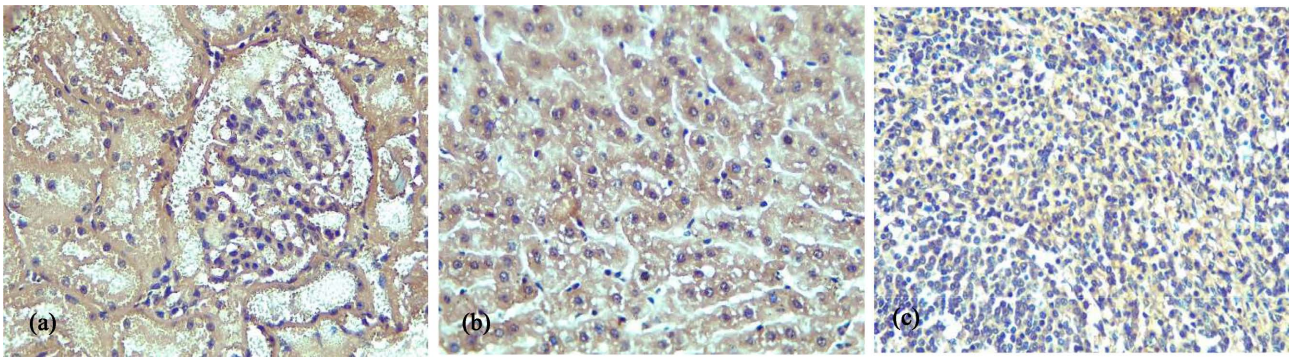


Figure S4. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 25 g/100mls for 15 minutes (magnification 400×).

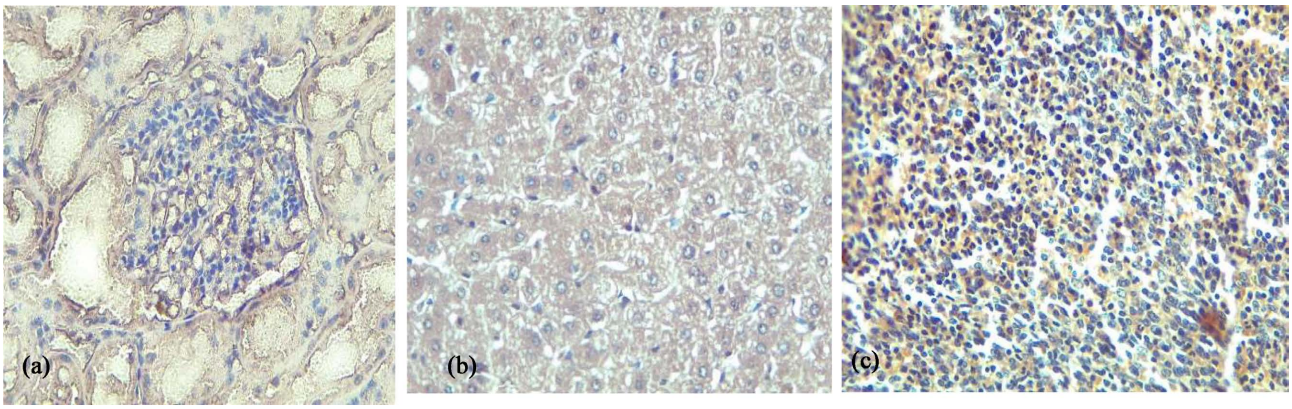


Figure S5. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 20 g/100mls for 5 minutes (magnification 400×).

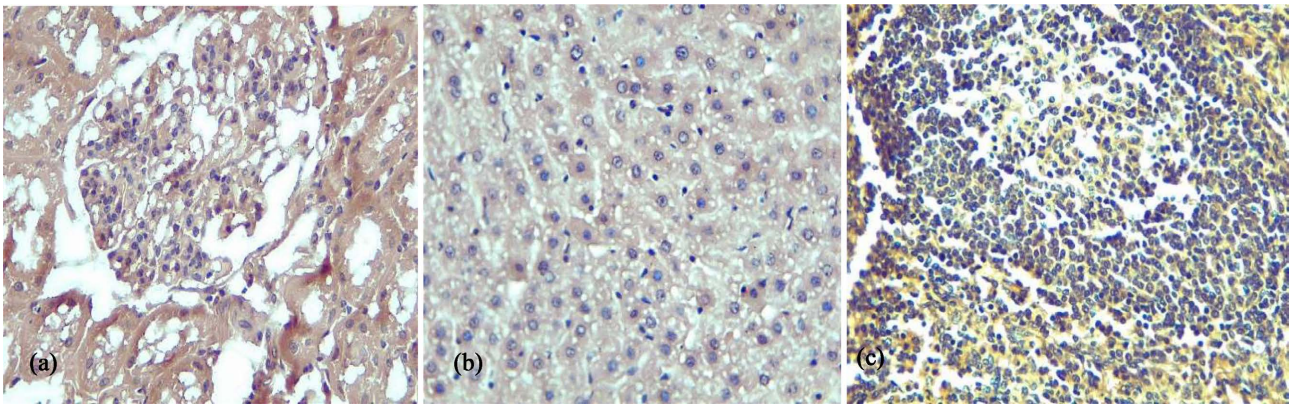


Figure S6. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 20 g/100mls for 10 minutes (magnification 400×).

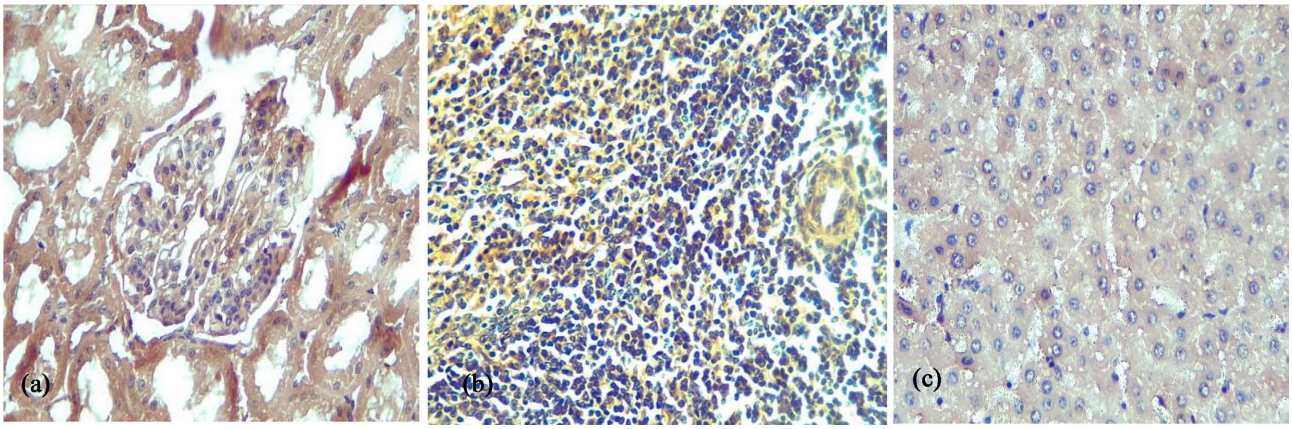


Figure S7. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 20 g/100mls for 15 minutes (magnification 400×).

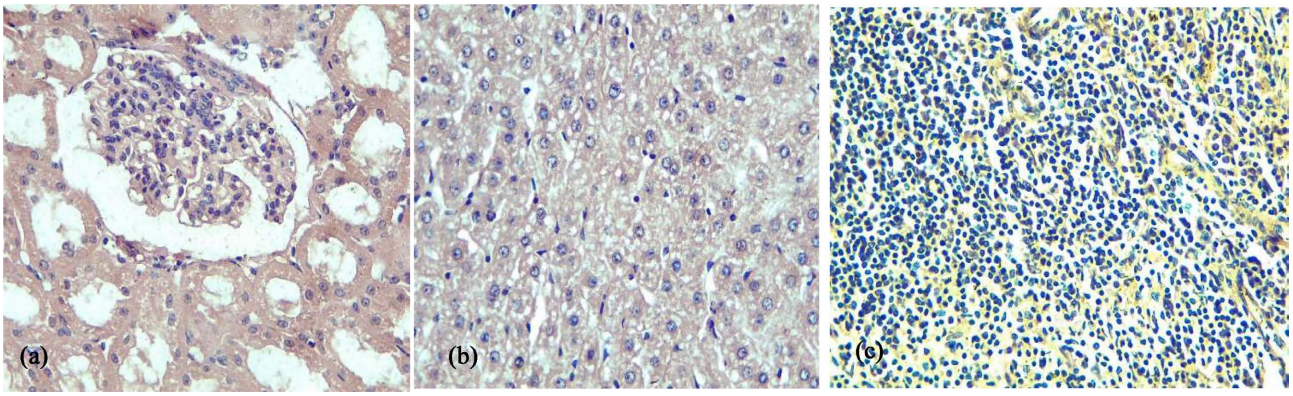


Figure S8. Micrographs of bovine kidney (a), liver (a) and spleen (c) sections stained with the acetone extract of concentration 15 g/100mls for 5 minutes (magnification 400×).

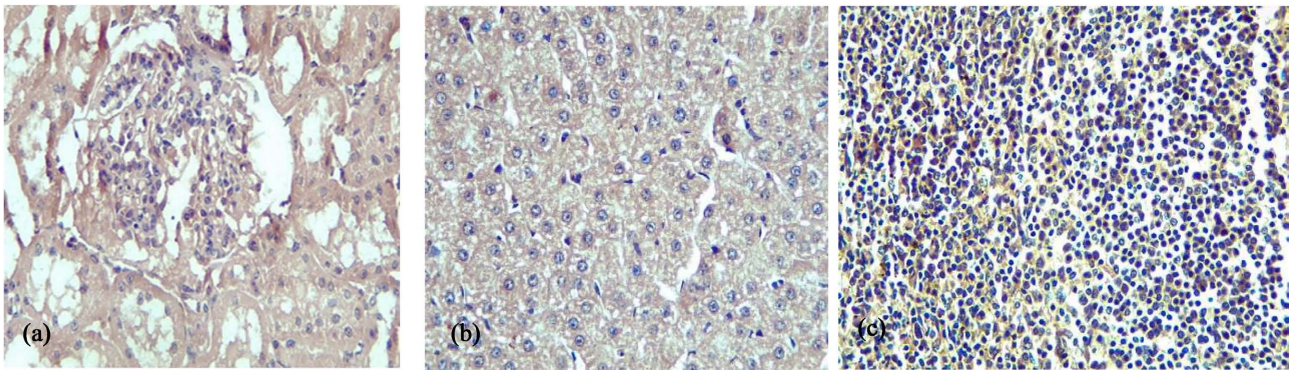


Figure S9. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 15 g/100mls for 10 minutes (magnification 400×).

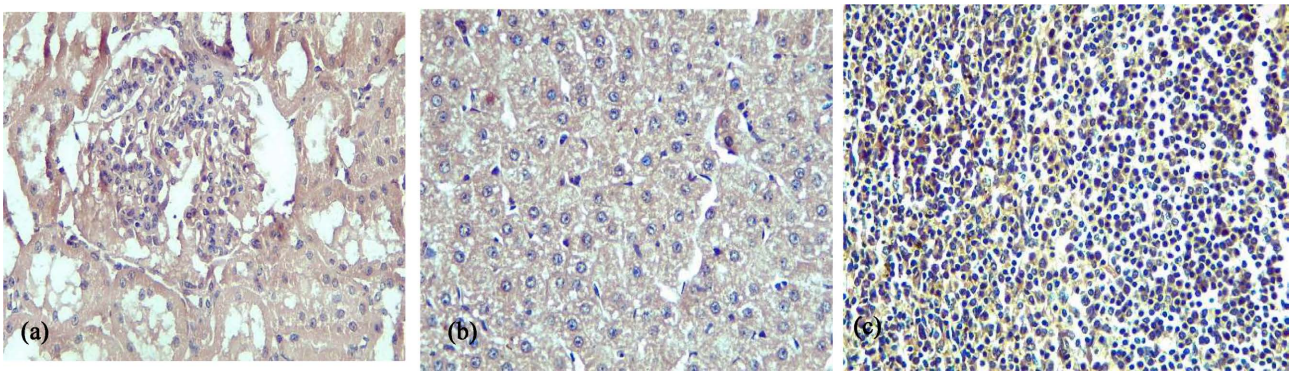


Figure S10. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 15 g/100mls for 15 minutes (magnification 400×).

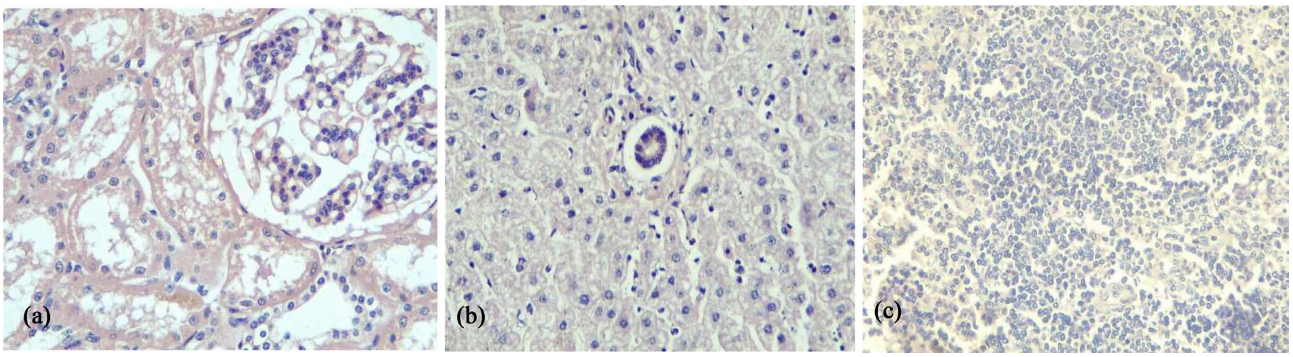


Figure S11. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 7.5 g/100mls for 5 minutes (magnification 400×).

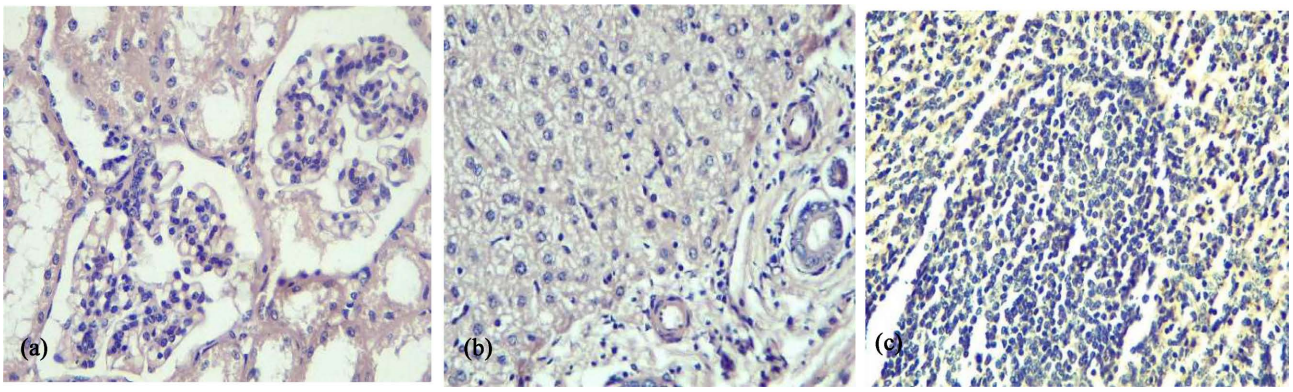


Figure S12. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 7.5 g/100mls for 10 minutes (magnification 400×).

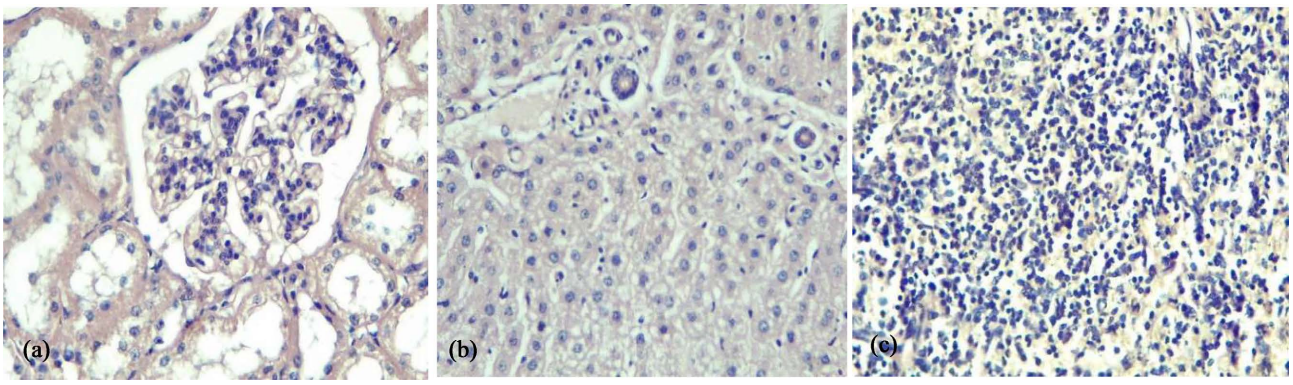


Figure S13. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the acetone extract of concentration 7.5 g/100mls for 15 minutes (magnification 400×).

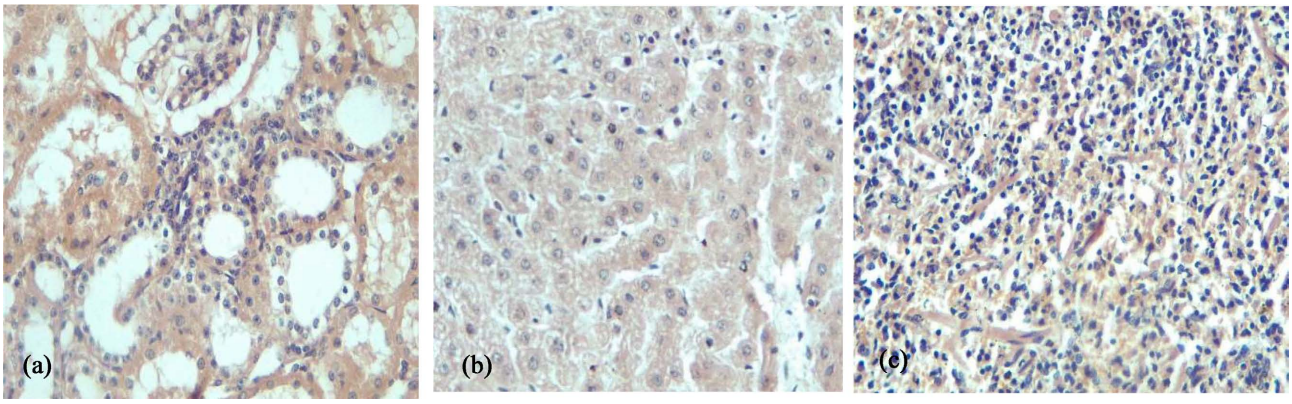


Figure S14. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 25 g/100mls for 5 minutes (magnification 400×).

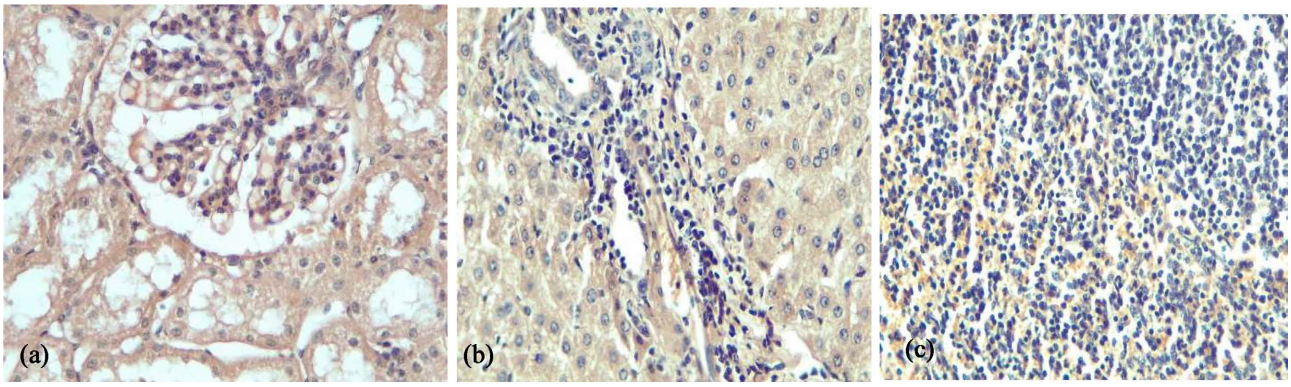


Figure S15. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 25 g/100mls for 10 minutes (magnification 400×).

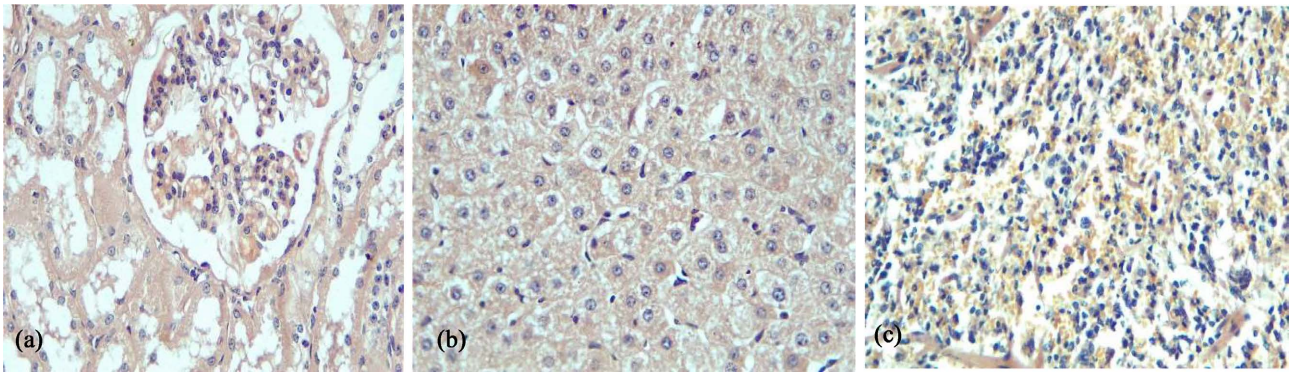


Figure S16. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 25 g/100mls for 15 minutes (magnification 400×).

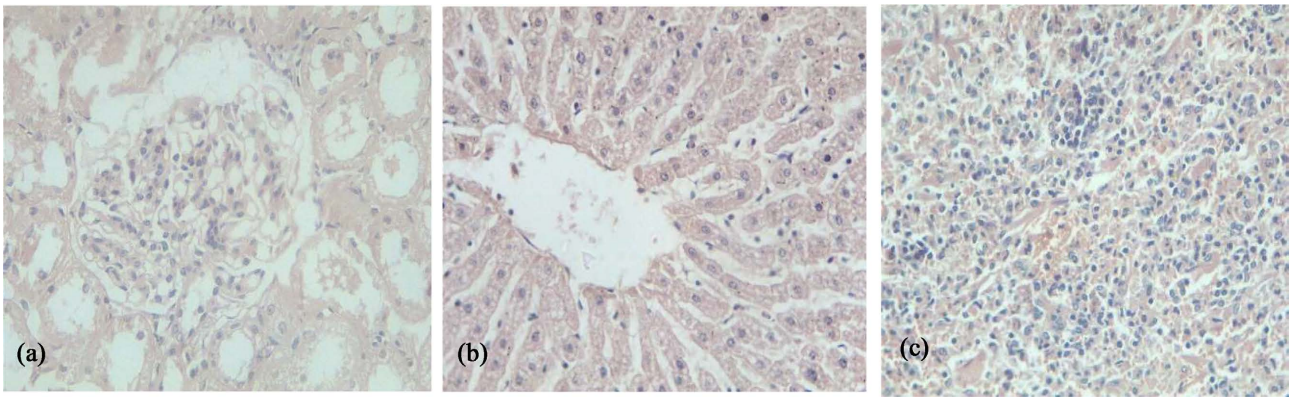


Figure S17. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 20 g/100mls for 5 minutes (magnification 400×).

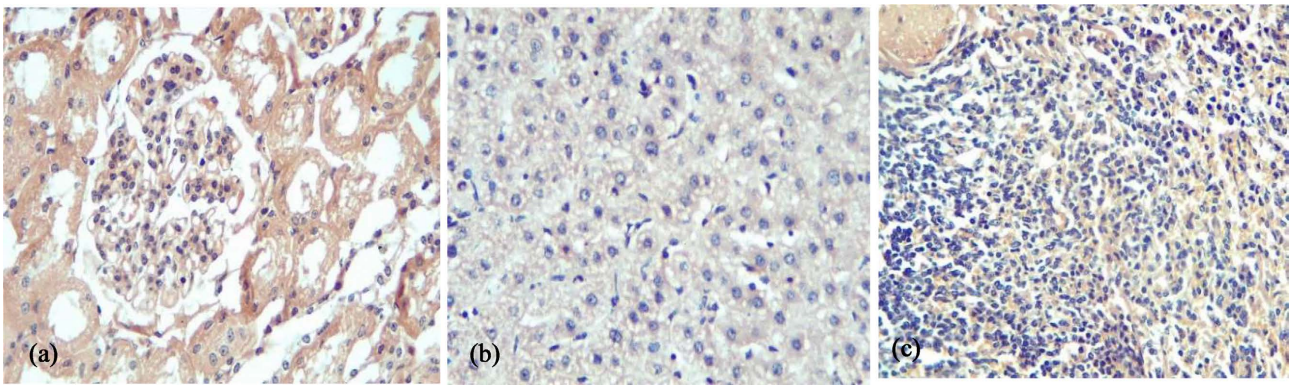


Figure S18. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 20 g/100mls for 10 minutes (magnification 400×).

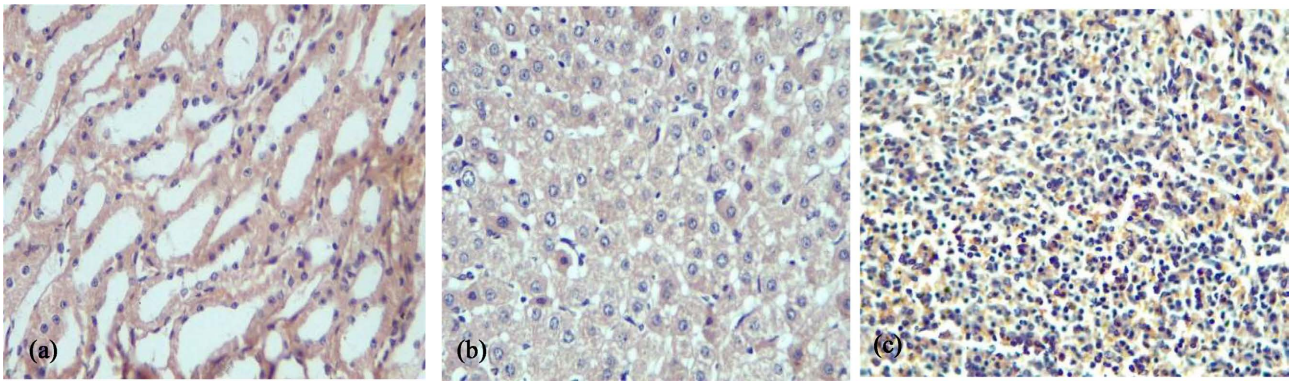


Figure S19. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 20 g/100mls for 15 minutes (magnification 400×).

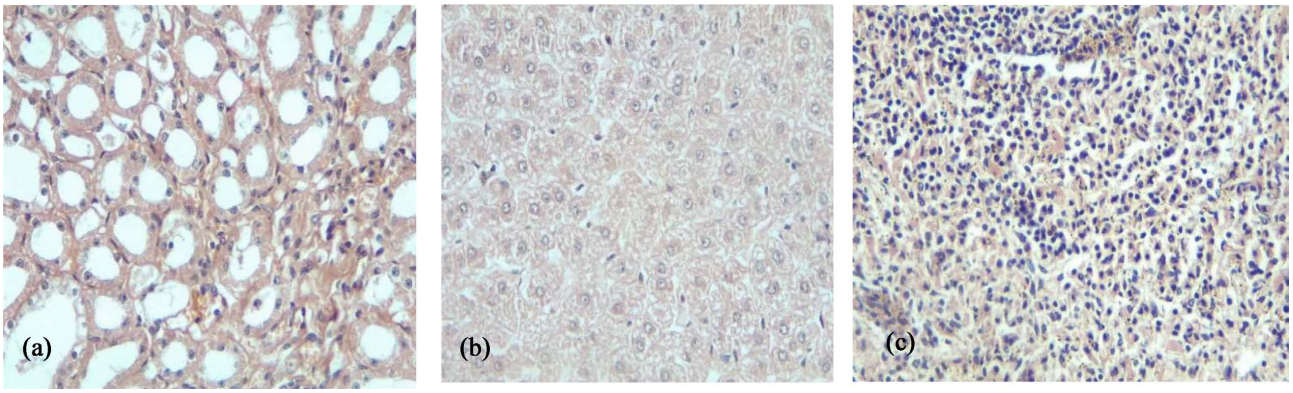


Figure S20. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 15 g/100mls for 5 minutes (magnification 400×).

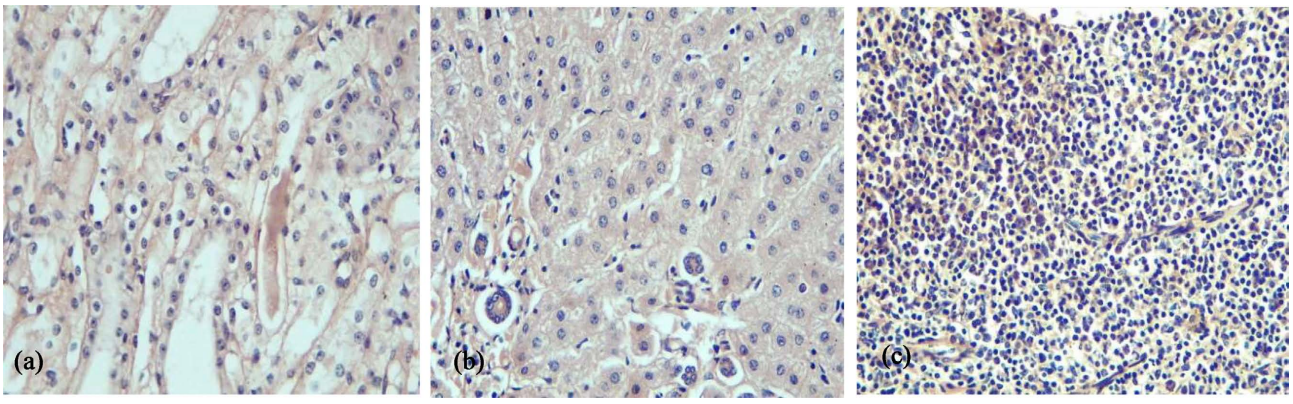


Figure S21. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 15 g/100mls for 10 minutes (magnification 400×).

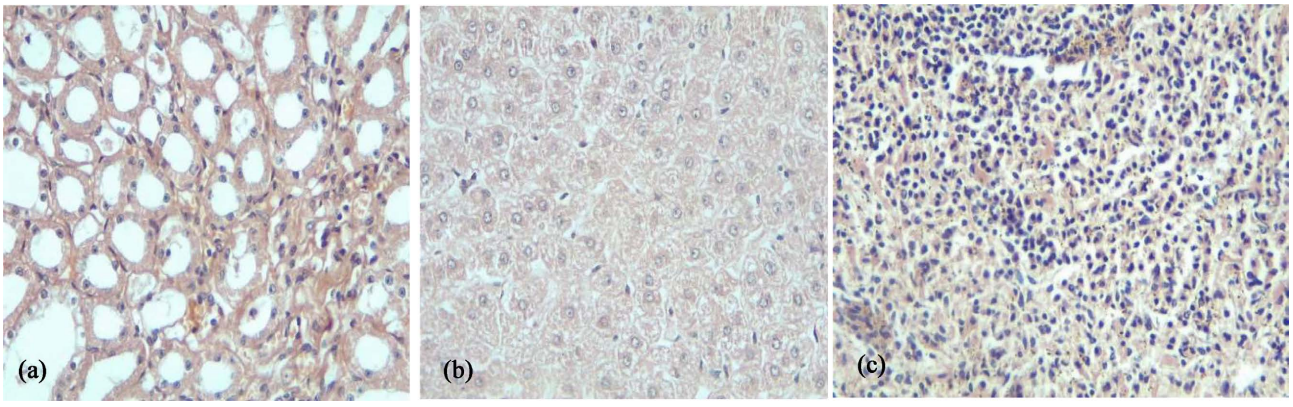


Figure S22. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 15 g/100mls for 15 minutes (magnification 400×).

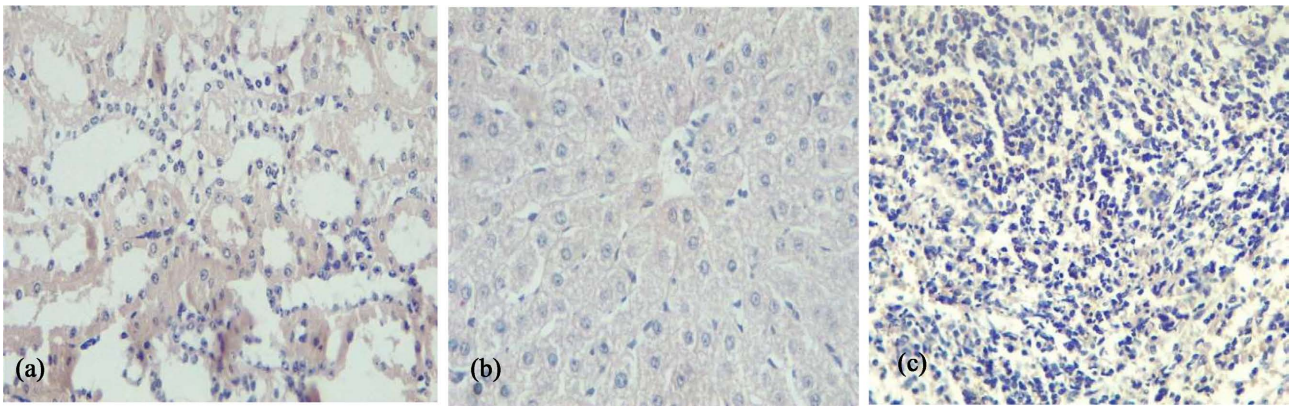


Figure S23. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 7.4 g/100mls for 5 minutes (magnification 400×).

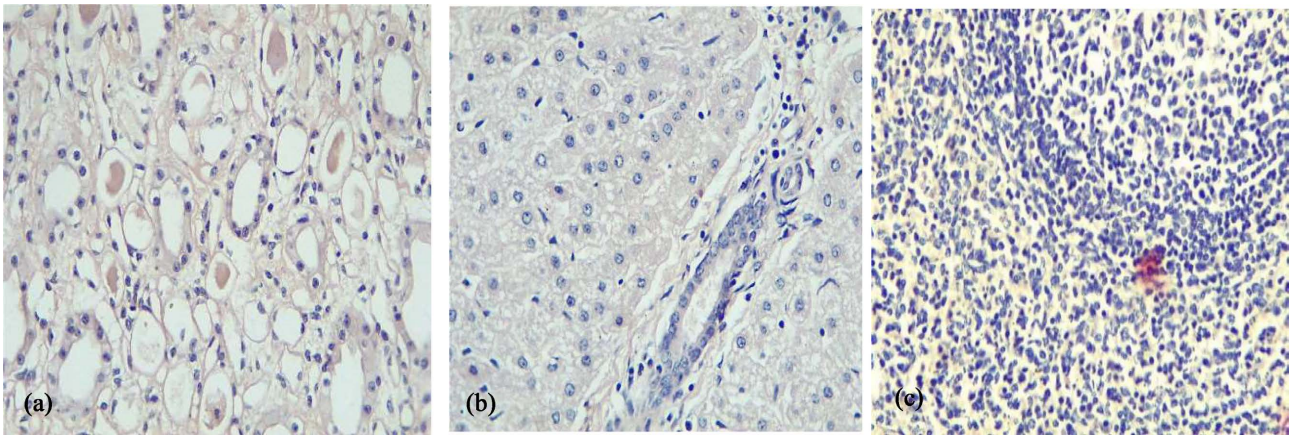


Figure S24. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 7.5 g/100mls for 5 minutes (magnification 400×).

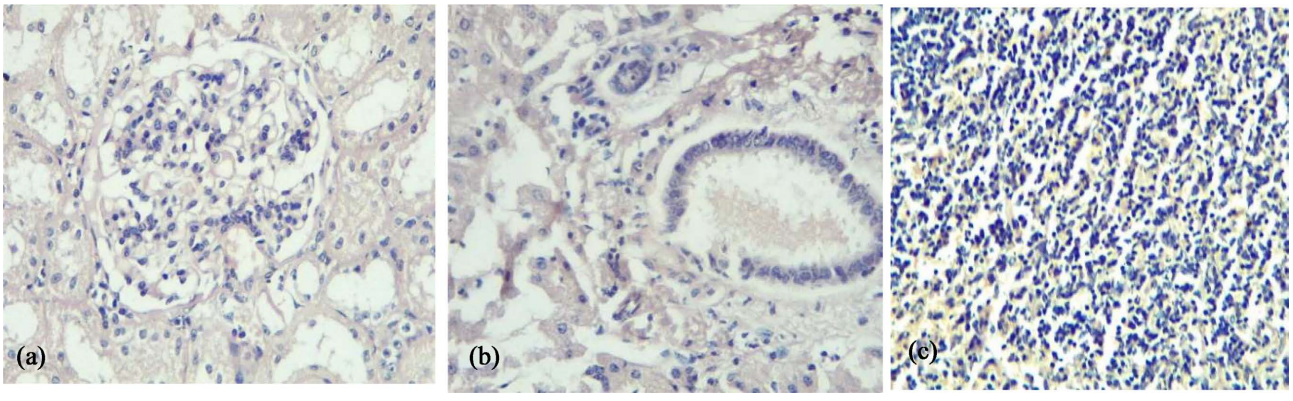


Figure S25. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the methanol extract of concentration 7.5 g/100mls for 15 minutes (magnification 400×).

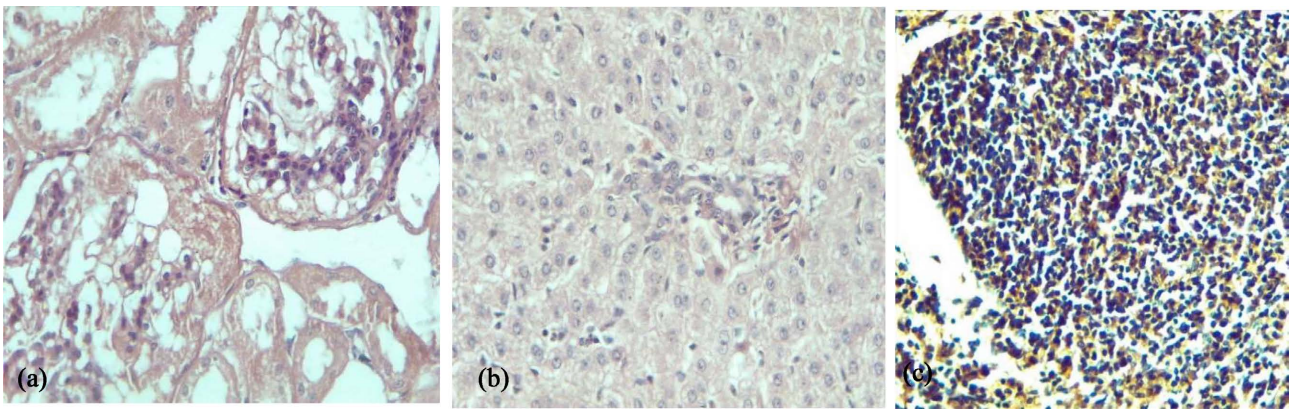


Figure S26. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 25 g/100mls for 5 minutes (magnification 400×).

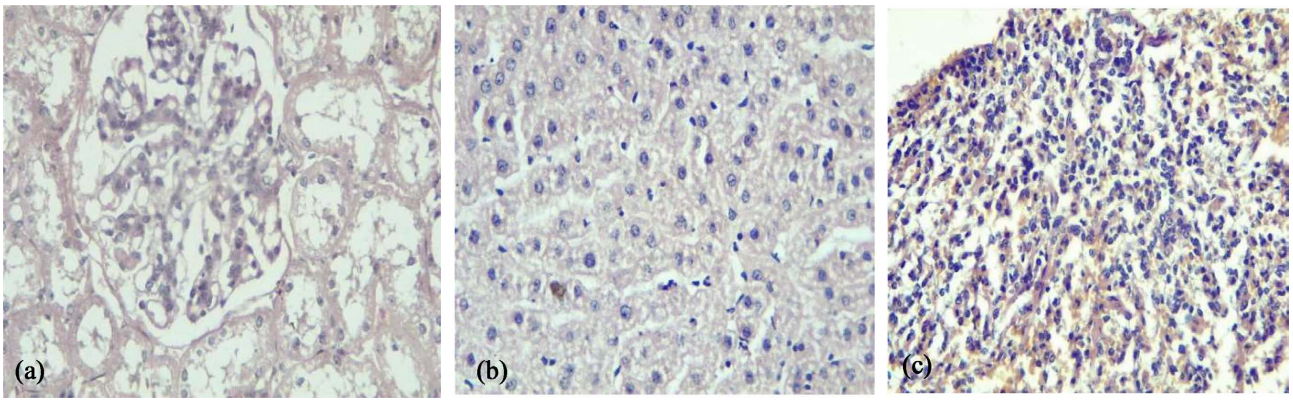


Figure S27. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 25 g/100mls for 10 minutes (magnification 400×).

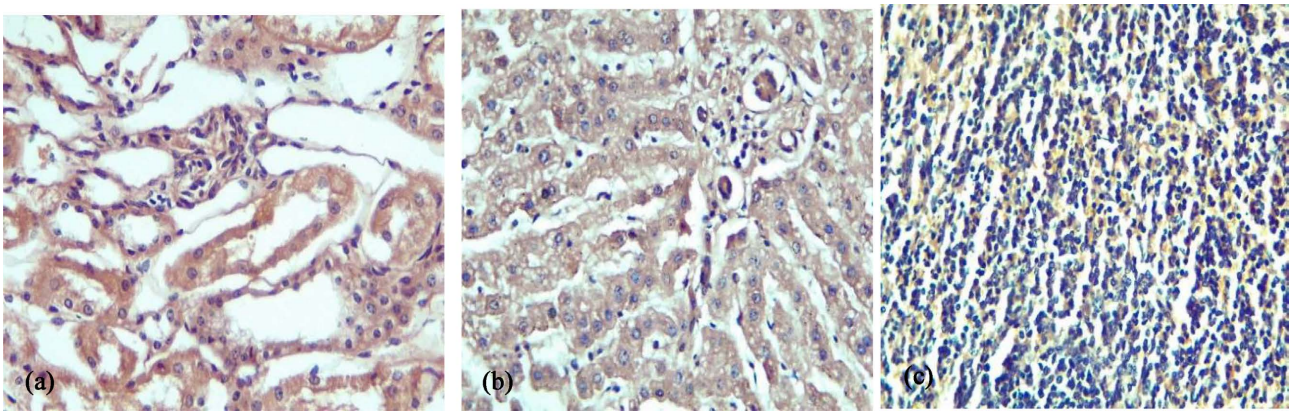


Figure S28. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 25 g/100mls for 15 minutes (magnification 400×).

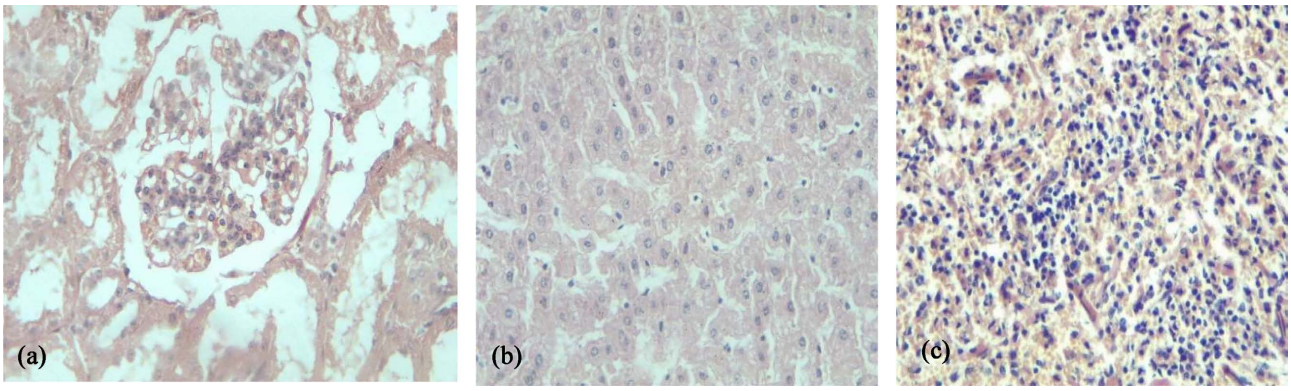


Figure S29. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 20 g/100mls for 5 minutes (magnification 400×).

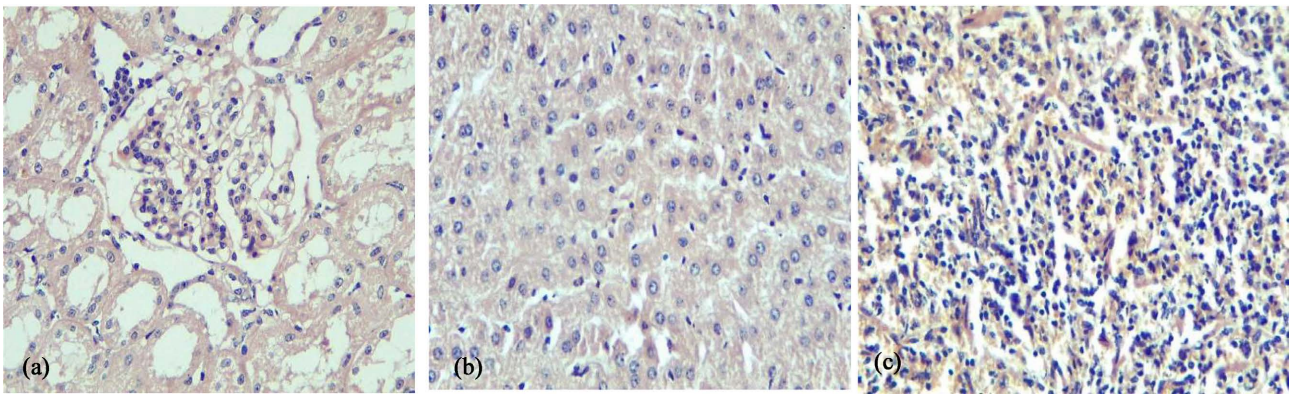


Figure S30. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 20 g/100mls for 10 minutes (magnification 400×).

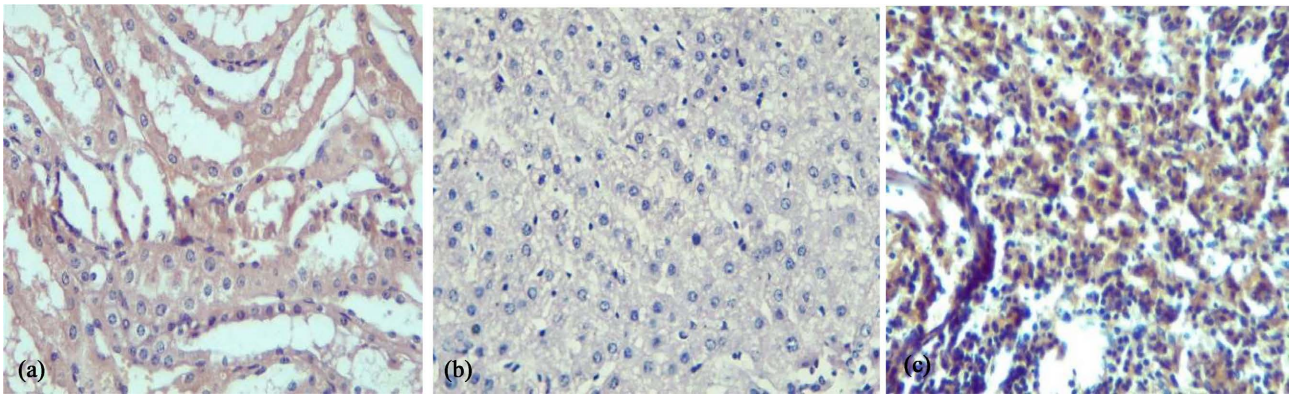


Figure S31. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 20 g/100mls for 15 minutes (magnification 400×).

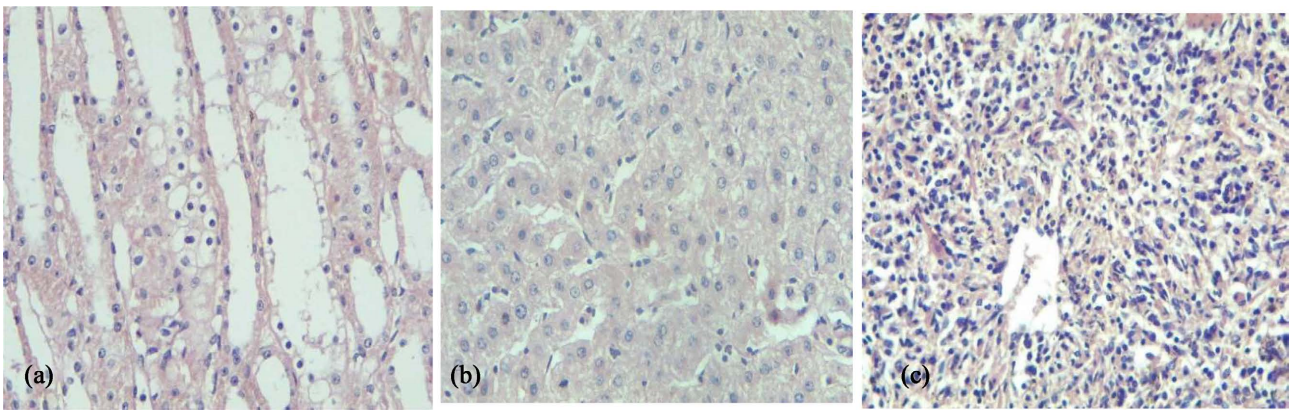


Figure S32. Micrographs of cow kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 15 g/100mls for 5 minutes (magnification 400×).

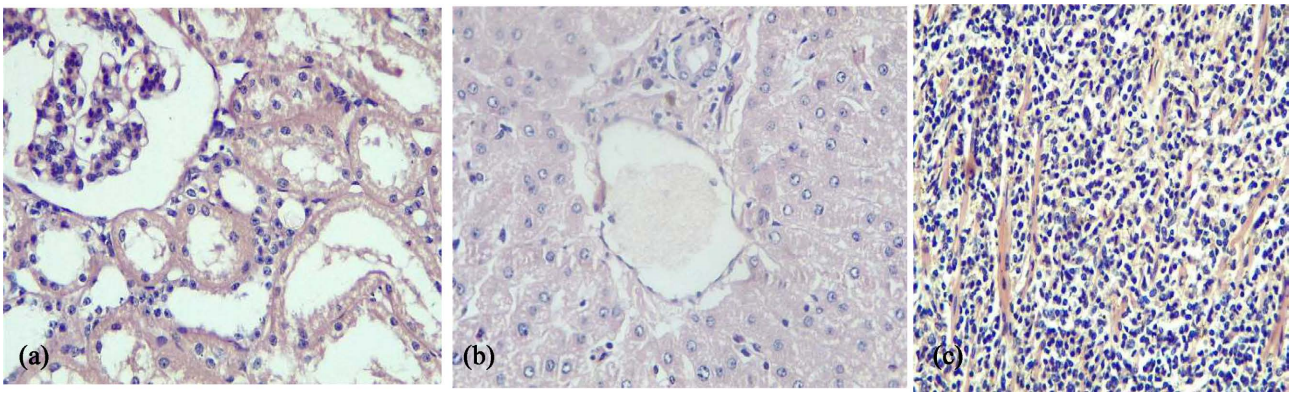


Figure S33. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 15 g/100mls for 10 minutes (magnification 400×).

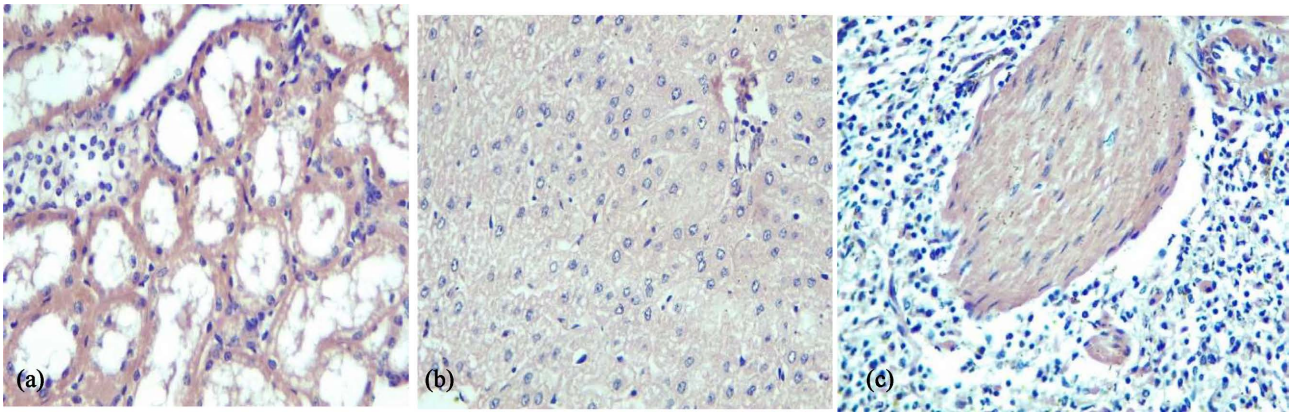


Figure S34. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 15 g/100mls for 15 minutes (magnification 400×).

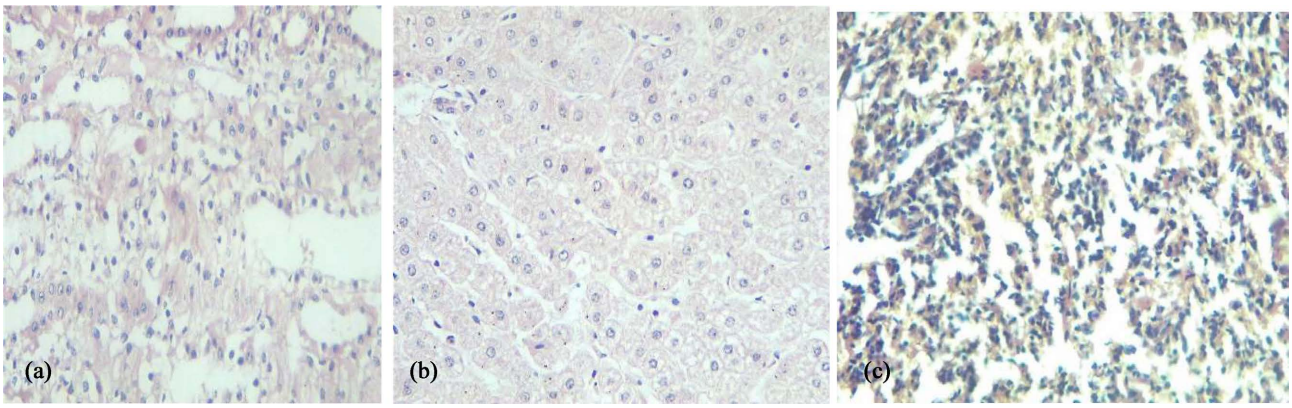


Figure S35. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 7.5 g/100mls for 5 minutes (magnification 400×).

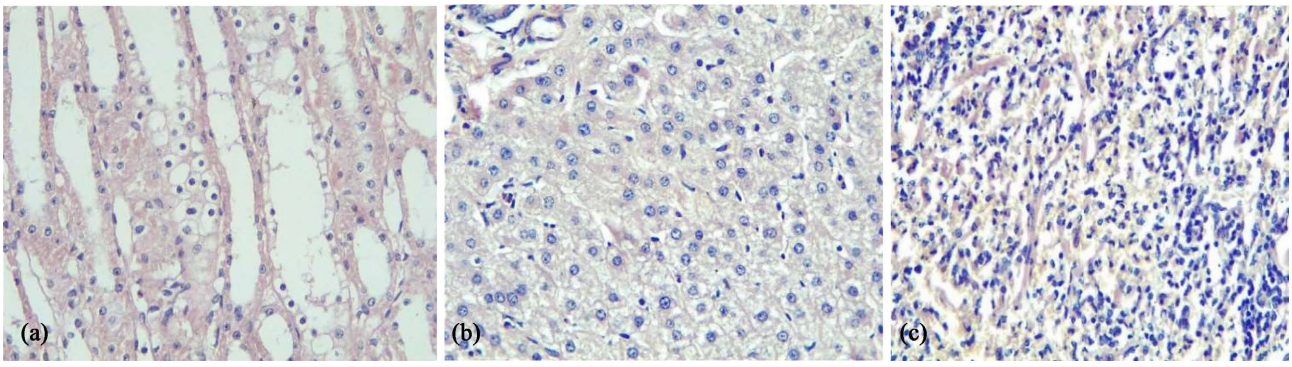


Figure S36. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 7.5 g/100mls for 10 minutes (magnification 400×).

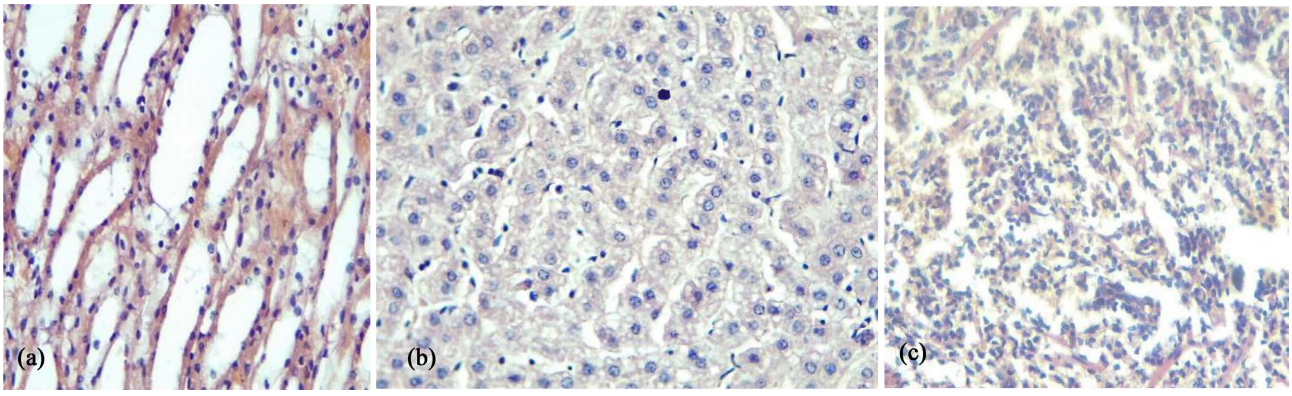


Figure S37. Micrographs of bovine kidney (a), liver (b) and spleen (c) sections stained with the ethanol extract of concentration 7.5 g/100mls for 15 minutes (magnification 400×).