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HISTOPATHOLOGICAL CHANGES IN KIDNEYS AND LIVER OF SHEEP FED WITH DIFFERENT LEVELS OF *LEUCAENA LEUCOCEPHALA*

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Abstract: **Six male sheep with similar age (3-5 months) and body weight ranging from 16 kg to 20 kg were used in this study. Each animal was kept in individual cage and the animals were fed rice straw and sesame cake for one month and provided fresh water *ad libitum*. The experimental animals were fed 3% of body weight and it was formulated isonitrogenously not less than 21% of crude protein. Animals were randomly divided into three groups (A, B, C) and each group contained two animals. Group A was kept as control, and group B and C were fed 30% and 60% of total diet with *Leucaena leucocephala* leaves, respectively. The experimental period was taken two months. Histopathologically, the kidneys of sheep from group B and C revealed degeneration of glomerulus and renal tubules, cellular debris and RBC in the lumen of renal tubules. Destruction of blood vessels with perivascular fibrosis, hyperemic artery and proteinaceous fluid in the interstitial of renal tubules were also found. Histopathological changes in liver were degeneration of hepatocytes and central vein, wider sinusoidal spaces than the normal. In the portal area, connective tissues proliferation, congestion in the portal vein and degeneration of bile duct were observed. All of the above histopathological changes were more severe in the animals of group C than those of group B.**

Keywords: **histopathological changes, kidney, *Leucaena leucocephala*, liver, mimosine, sheep.**

I. INTRODUCTION

Leucaena leucocephala is widely spread through most tropical and sub-tropical regions of the world and it provides an important source of feed for ruminant due to leucaena is high in crude protein (Jones, 1979), highly palatable (Hess *et al.*, 2000), long-lived, and tolerant of frequent severe defoliation and drought.

Although leucaena has many positive nutritional benefits, it possesses the toxic non-protein free amino acid mimosine in relatively high concentrations in leaves and young pods (Hegarty *et al.*, 1964). According to Dalzell *et al.* (2006), mimosine stops cell division and caused severe toxicity and can often be fatal. The symptoms of mimosine toxicity are low serum thyroxine levels due to inhibit iodine uptake, poor appetite, ulceration of esophagus and reticulo-rumen, excessive salivation, raw coronet above the hooves, lameness, low live weight gains, foetal death and resorption

especially when the diet contains more than 30% leucaena in ruminants (Hegarty *et al.*, 1964). In sheep fed with 60% of leucaena leaves in the diets, average body weight gain of those animals were numerically declined and toxic effects of mimosine were more severe in skin, thyroid glands and testes than those of 30% after two months of experiment (Si Thu Hein, 2015).

There is very limited information on the histopathological changes of leucaena mimosine effect on kidneys and liver of local sheep in Myanmar. This research is intended to study the histopathological changes of kidneys and liver in experimentally induced with leucaena mimosine in sheep of Myanmar.

II. METHODOLOGY

Experimental Animals and Design

Six male sheep with similar age (3-5 months) of approximately 16.8 ± 20.2 kg of body weights were used in this experiment. Initially, the sheep were fed on basal diet (rice straw and sesame cake) for one month as a preliminary period. Fresh water was given freely throughout the experimental period. The experimental animals were fed 3% of body weight and it was formulated isonitrogenously not less than 21% of crude protein. After preliminary period, the sheep were randomly divided into 3 groups (A, B and C) containing two animals in each group. Group A which fed with basal diet was kept as a control group and group B and C were fed 30% and 60% of total diet with *Leucaena leucocephala* leaves, respectively.

Microscopic Examination

After two months of experimental period, sheep of all groups were sacrificed. The target organs such as the kidneys and liver were collected for tissue sections. The kidneys and liver of sheep from each group were fixed in 10% formalin. Afterwards, tissue processing and staining was carried out. The samples were dehydrated in the series of ascending grades of alcohol followed by clearing in changes of xylene, and then the tissues were infiltrated with different grades of melted paraffin in the oven. The tissues were then embedded in paraffin and finally the wax embedded specimens were sectioned at 4-5 µm thickness. The sections were floated on Luke-warm water in a floatation bath at 37°C for stretching and then they were mounted on clean slides using an adhesive. Egg albumins were used as adhesive and then glass slides were dried on a slide warmer at 37°C. Tissue section slides were stained with Haematoxylin and Eosin (H & E). Histopathological slides were examined under the light microscope (Digisystem) and microphotographed with camera attached microscope (DB2-180M).

III. RESULTS

Histopathological Changes of Kidneys

In the kidneys of sheep in both group B and C, the glomerular tuft became lobulated and atrophy and urinary space was wider than the normal structure. Degeneration of Bowman's capsule was revealed by the desquamation of parietal epithelium. Renal tubules were also degenerated and cellular debris was found in urinary space and renal tubules (Figure 1, 2, 3 and 4). RBCs were leaked into the lumen of renal tubules (Figure 5 and 6). Proliferation of connective tissue around the vein was noticed as perivascular fibrosis of renal venule in only group B (Figure 7). Blood vessel became degenerated (Figure 8) and connective tissue infiltration in the renal tubular wall and proteinaceous fluid was also seen in the interstitial tissue of renal tubules (Figure 9).

Histopathological Changes of Liver

Histopathological changes of liver of sheep in both group B and C exhibited that the degeneration of hepatocytes. The sinusoidal spaces became wider than the normal (Figure 10 and 11). In figure 11, hepatocytes revealed disappearance of nucleus and homogenous appearance. Moreover, connective tissues proliferated in the portal area (Figure 12 and 13). Invagination and degeneration of epithelium of bile duct was also found (Figure 12 and 14). Congestion in the portal vein was also observed (Figure 10, 12 and 14). In group C, central vein became degenerated and also the surrounding hepatocytes (Figure 15). The haemorrhages among the liver cell cords were also noticed (Figure 16).

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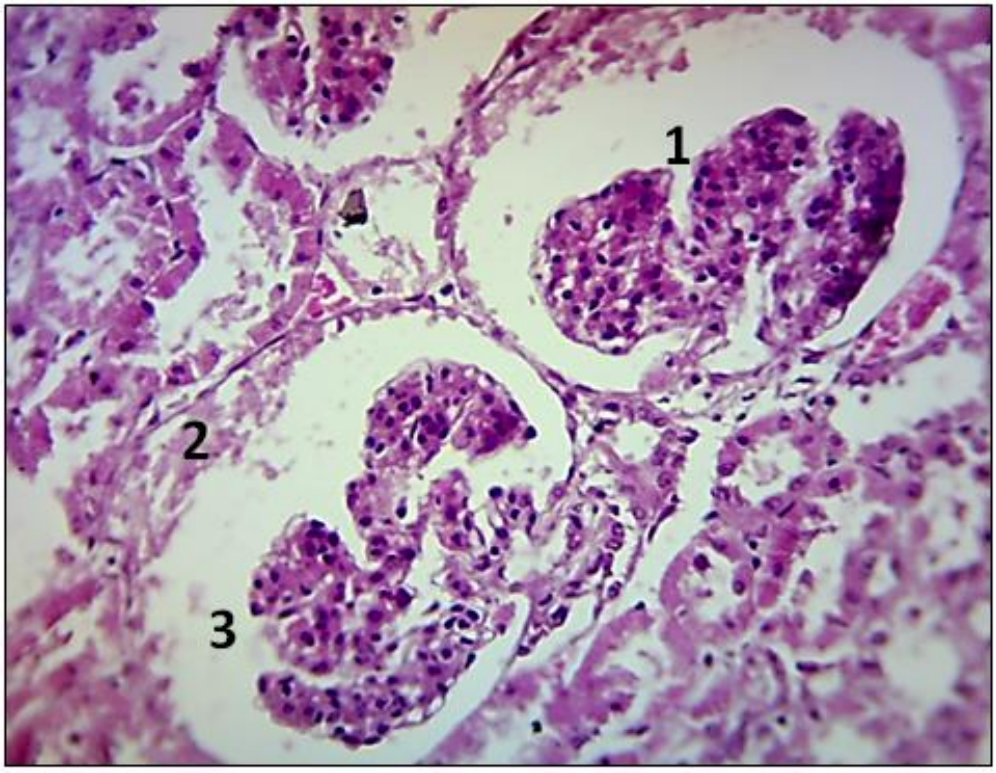

(H&E ×400)

Figure 1: Microphotograph of kidney of sheep in group B showing lobulation of glomerular tuft (1), degeneration and desquamation of parietal epithelium in the Bowman's capsule (2) and wider urinary space (3)

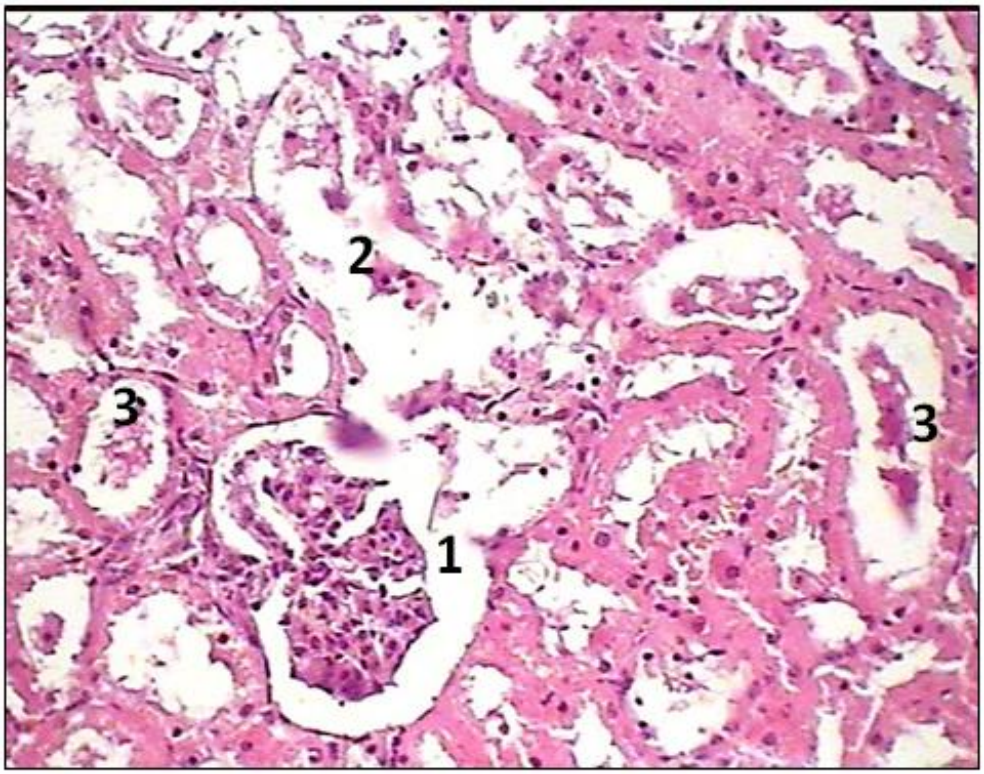

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Figure 2: Microphotograph of kidney of sheep in group C showing lobulation of the glomerular tuft and degeneration of Bowman's capsule (1), degenerated renal tubule (2) and cellular debris in renal tubule (3)

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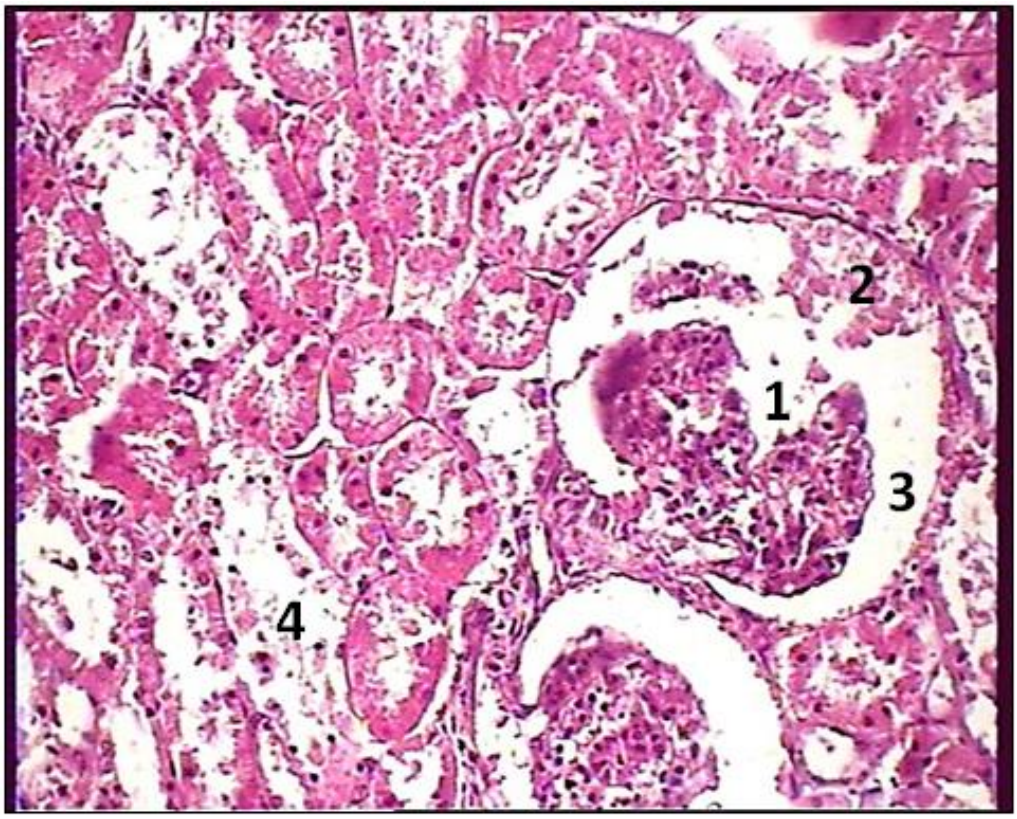

(H&E ×400)

Figure 3: Microphotograph of kidney of sheep in group C showing lobulation and atrophy of glomerular tuft (1), cellular debris in urinary space (2) wider urinary space (3) and degenerated renal tubules (4)

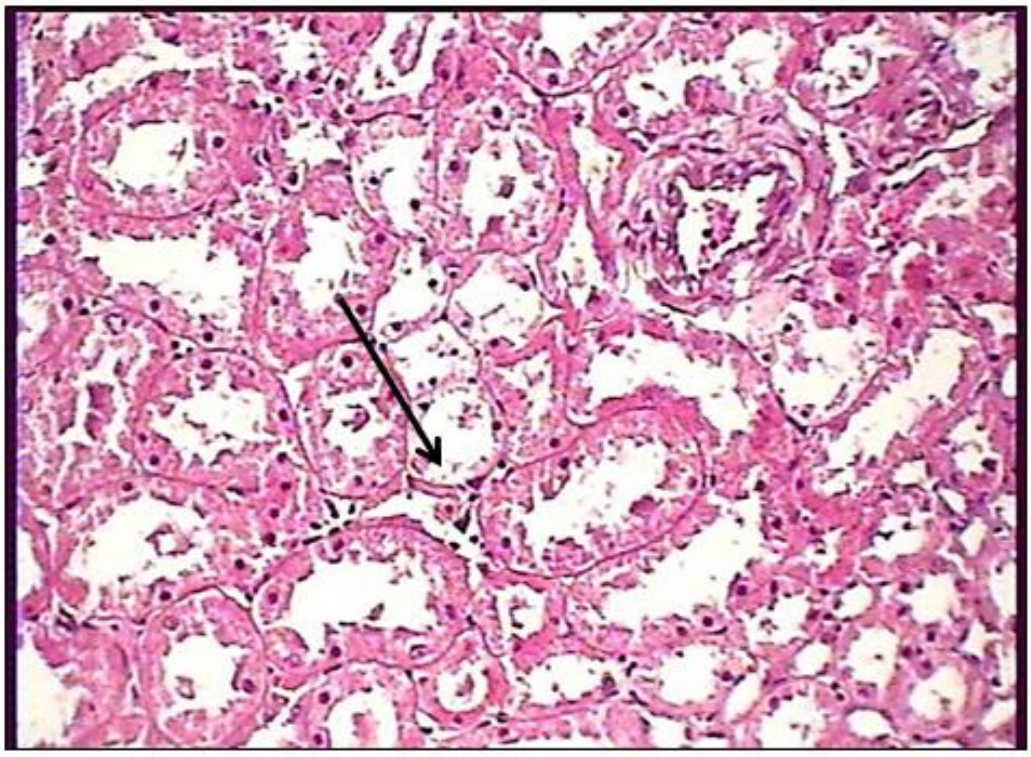

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Figure 4: Microphotograph of kidney of sheep in group C showing degenerated renal tubular epithelium (arrow)

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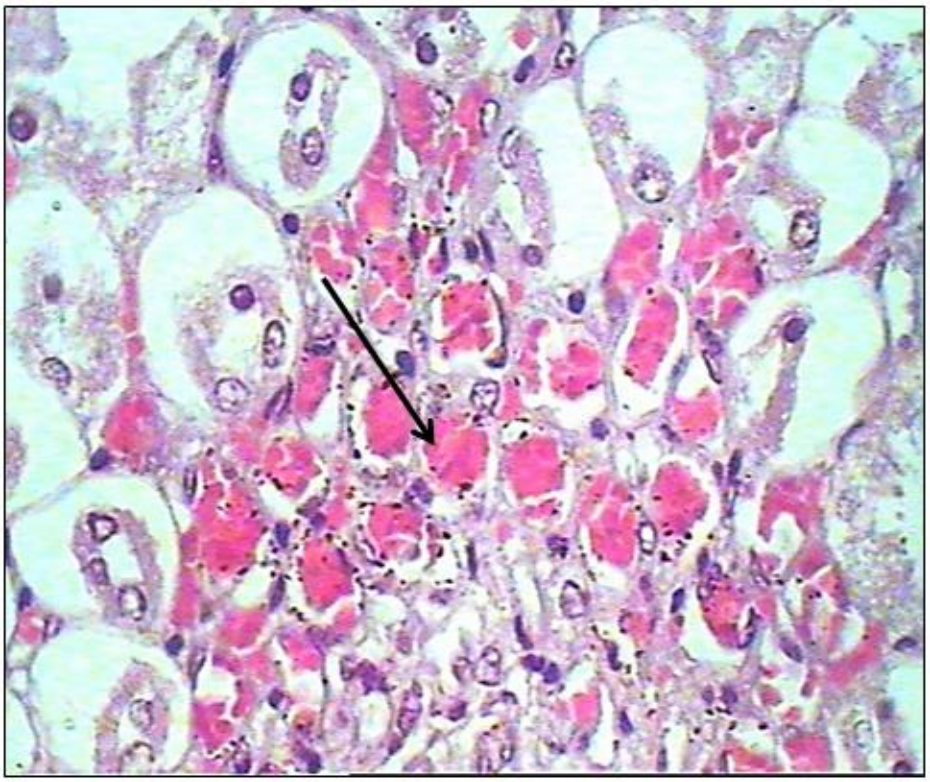

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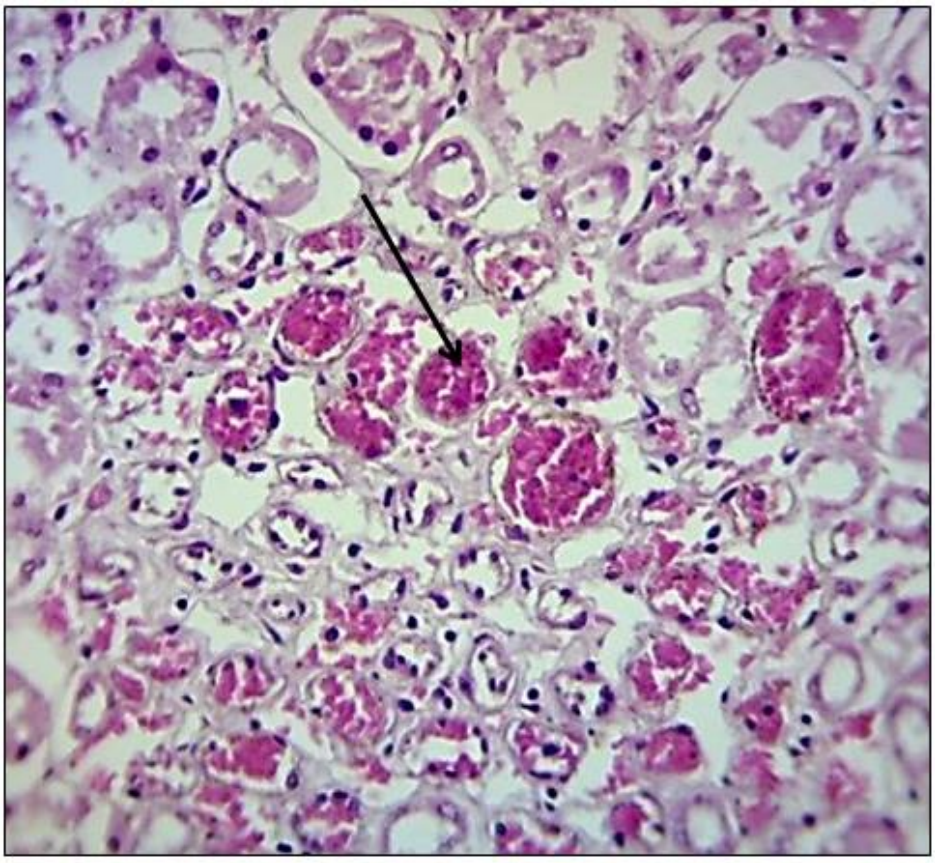
Figure 5: Microphotograph of kidney of sheep in group B showing RBC in the lumen of renal tubules (arrow)



(H&E ×400)

Figure 6: Microphotograph of kidney of sheep in group C showing RBC in renal tubules (arrow)

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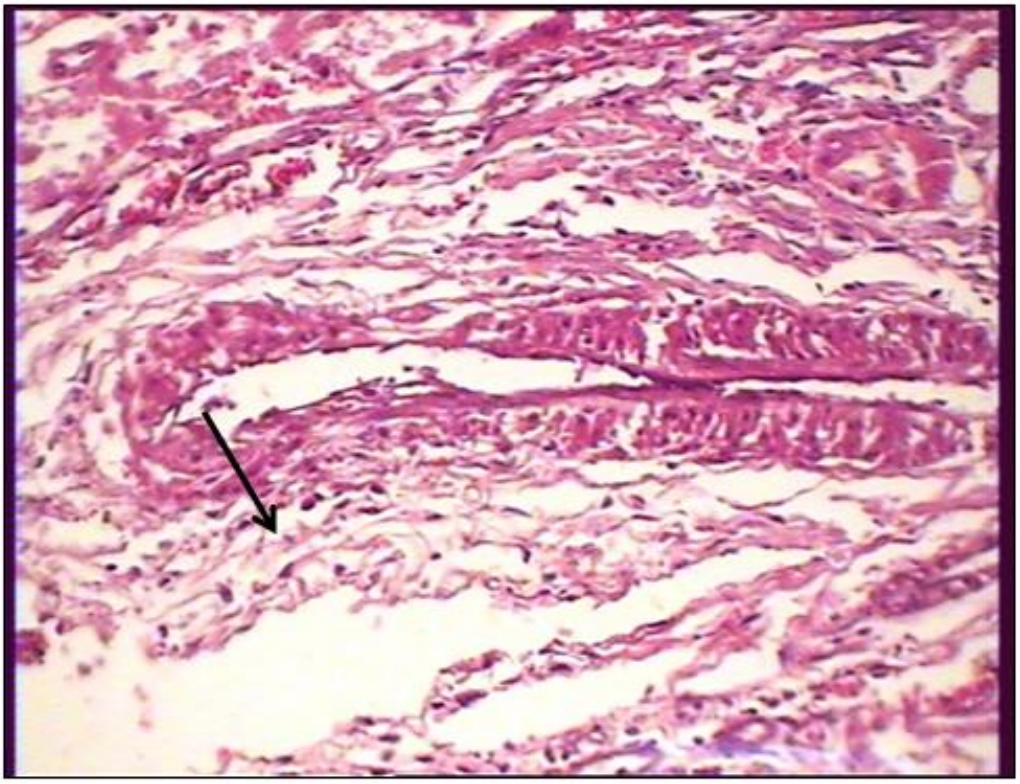

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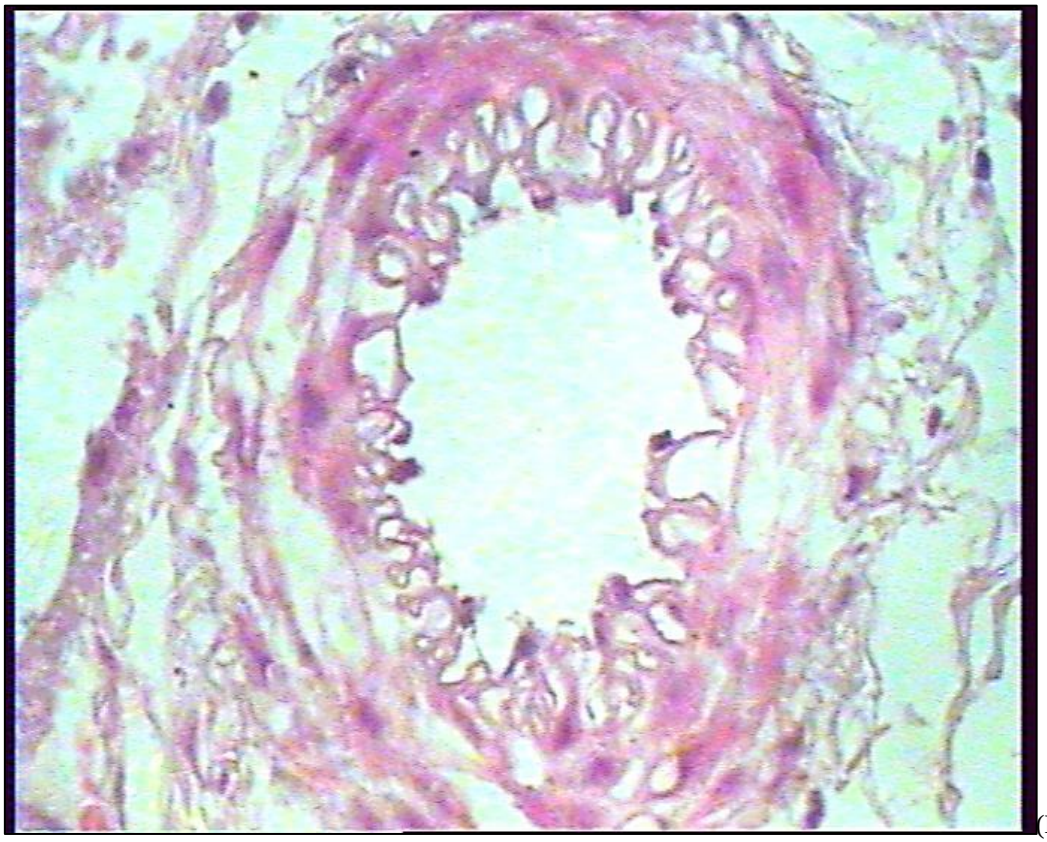
Figure 7: Microphotograph of kidney of sheep in group B showing perivascular fibrosis of renal veule (arrow)



(H&E ×1000)

Figure 8: Microphotograph of kidney of sheep in group C showing degeneration of blood vessel.

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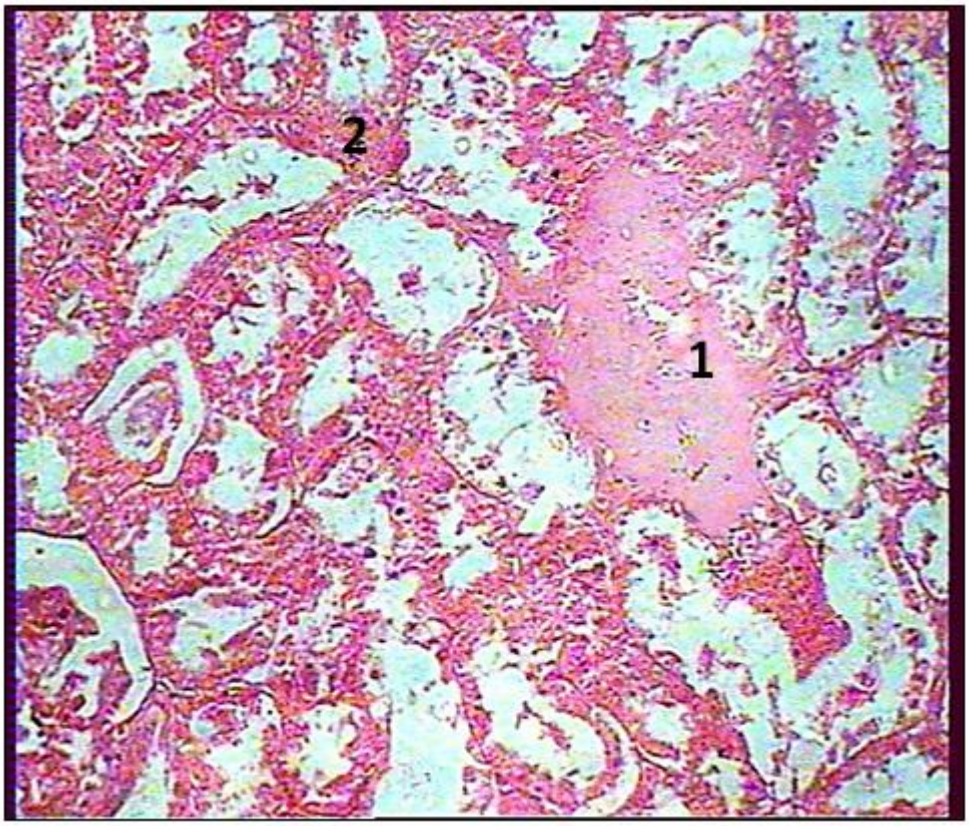

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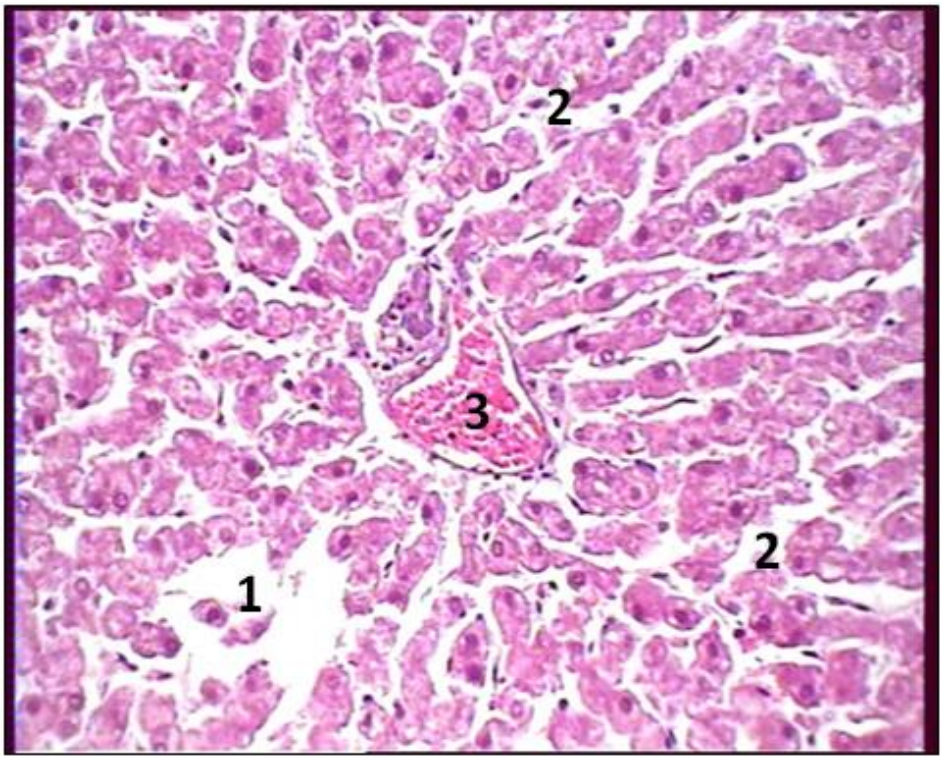
Figure 9: Microphotograph of kidney of sheep in group C showing proteinaceous fluid in the interstitial of renal tubules (1) and thickening of tubular wall with connective tissue infiltration (2)



(H&E ×400)

Figure 10: Microphotograph of liver of sheep in group B showing degeneration of hepatocytes (1), wider sinusoidal spaces (2) and congestion in central vein (3)

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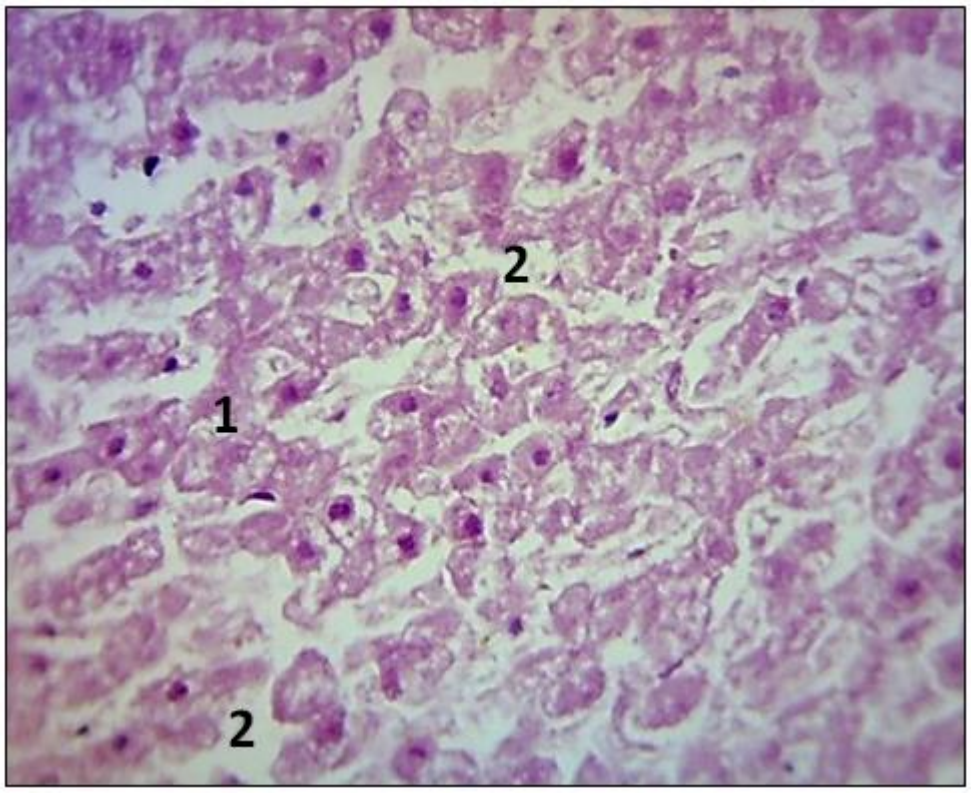
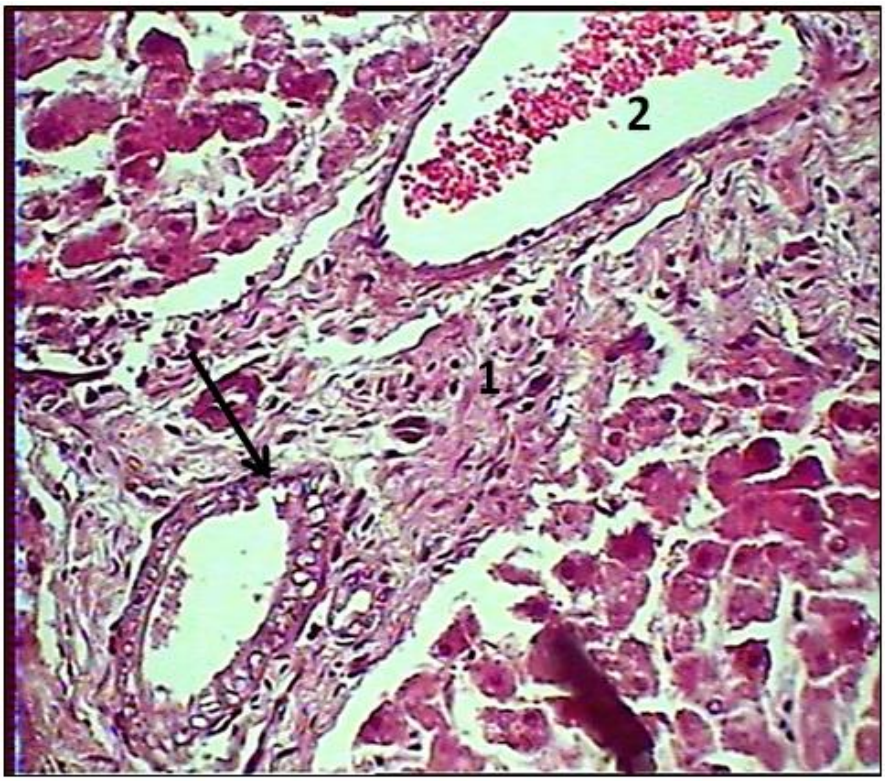


(H&E ×400)

Figure 11: Microphotograph of liver of sheep in group C showing degeneration and necrosis of liver cell cords with disappearance of nucleus and homogenous appearance (1) and wider sinusoidal spaces (2)



(H&E ×400)

Figure 12: Microphotograph of liver of sheep in group B showing perivascular fibrosis in the portal area (1), dilation of portal vein containing RBCs (2) and invagination of epithelium of bile duct (arrow)

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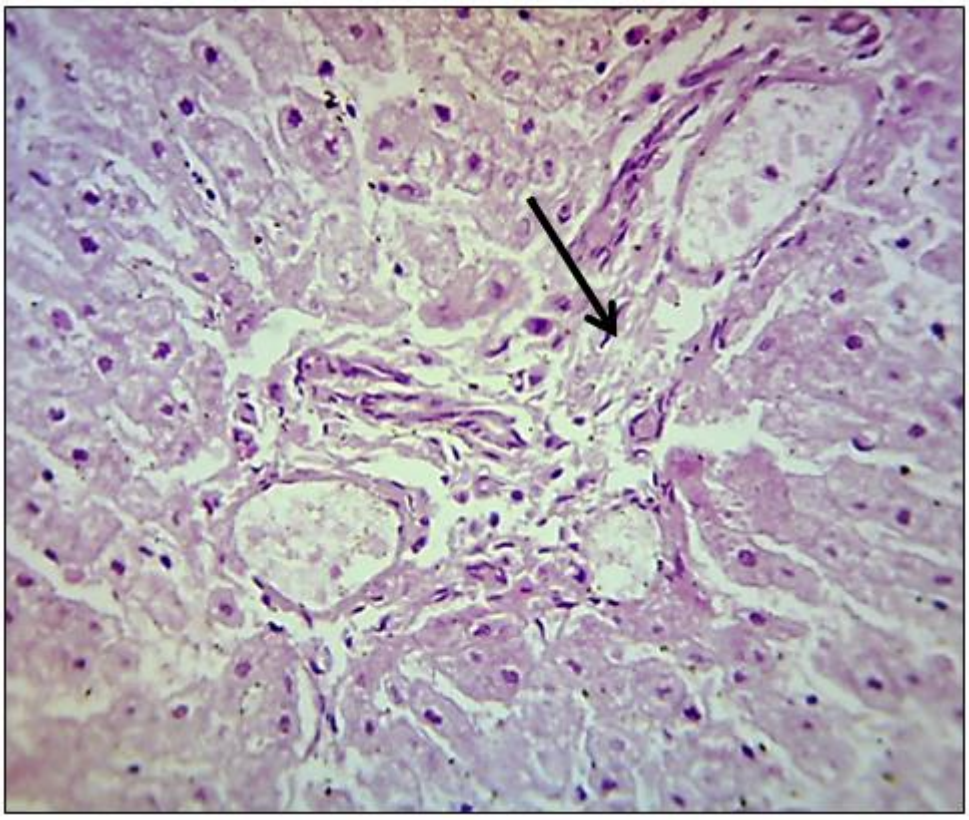

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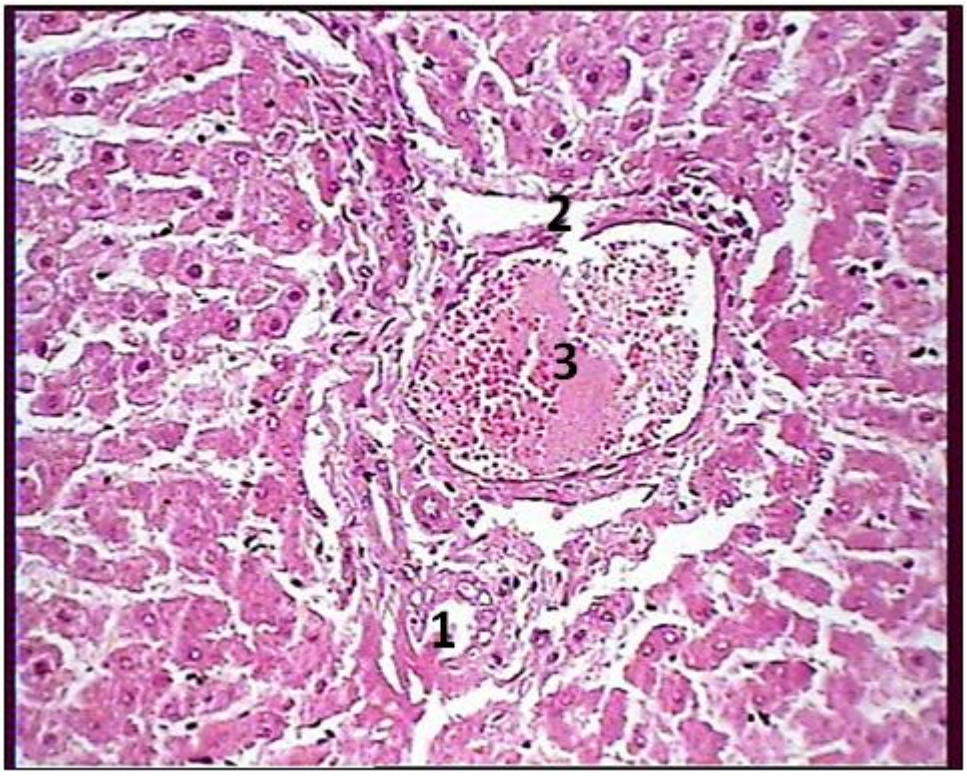
Figure 13: Microphotograph of liver of sheep in group C showing perivascular fibrosis in the portal area (arrow)



(H&E ×400)

Figure 14: Microphotograph of liver of sheep in group C showing degeneration of bile duct (1), degeneration of portal vein (2) and congestion in the portal vein (3)

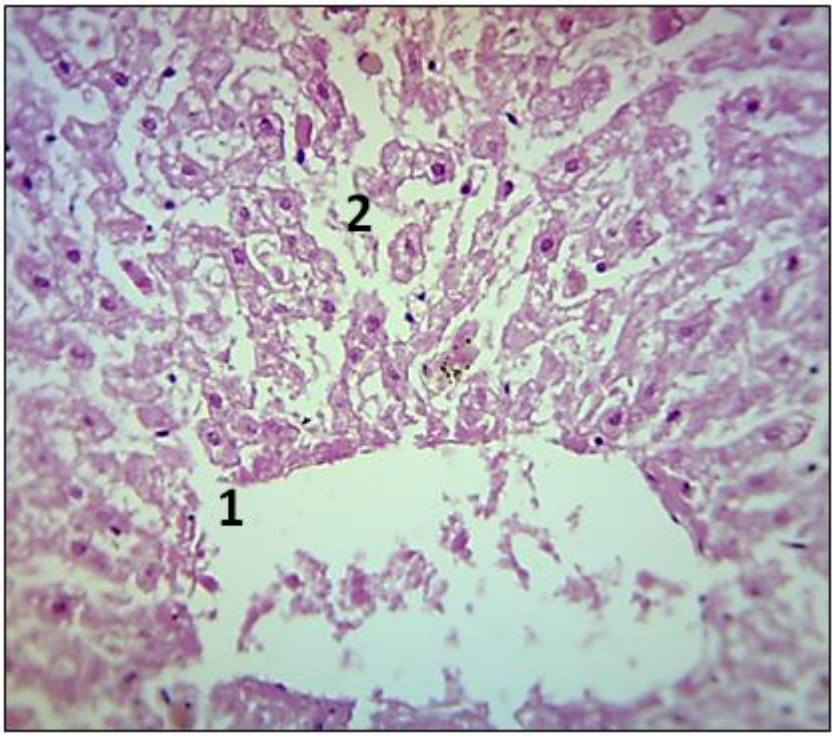


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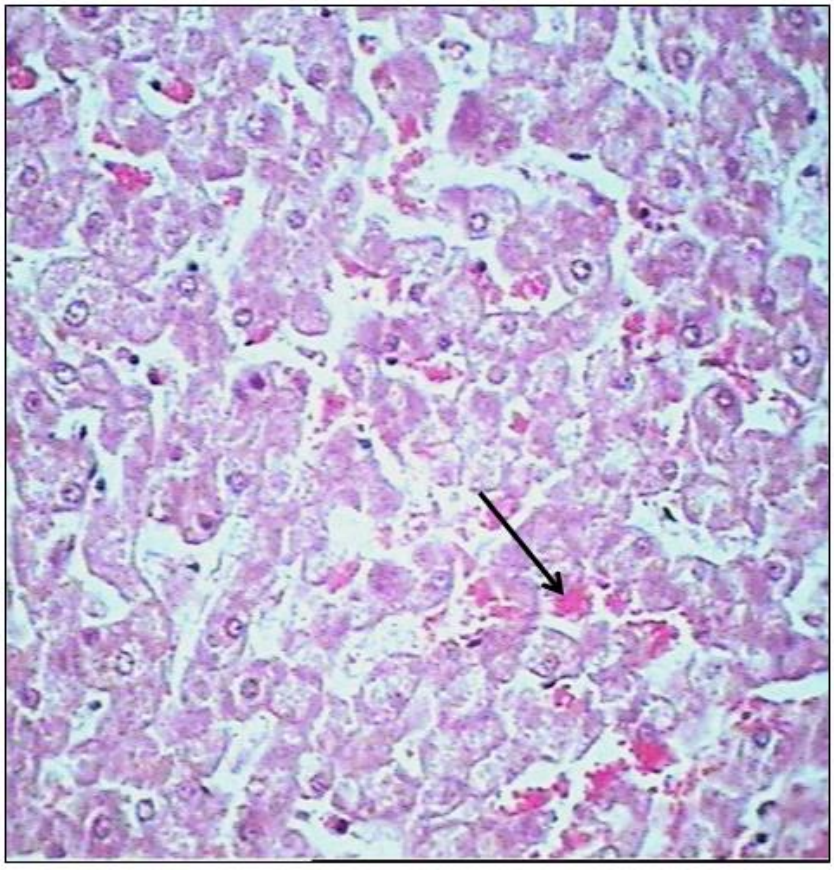
Figure 15: Microphotograph of liver of sheep in group C showing degeneration of central vein (1) and degeneration of hepatocytes (2)



(H&E ×400)

Figure 16: Microphotograph of liver of sheep in group C showing haemorrhage in liver cell cord (arrow)

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IV. DISCUSSION

Histopathological Changes of Kidneys

In the kidneys, the glomerulus has a basement membrane consisting mainly of laminins and collagen which are synthesized and secreted by both endothelial cells and podocytes (Barbara *et al.*, 2006). According to findings of Hashiguchi and Takahashi (1977), mimosine caused the deficiency of copper that was necessary for the collagen synthesis and maintenance. In the present study, the glomerular tuft lobulation, degeneration of Bowman's capsule, widening of the urinary space were observed in animals of both group B and C (Figure 1, 2 and 3). These histopathological changes indicated the atrophy of glomerular tuft and also defined as glomerular nephritis. These changes might be caused by copper deficiency due to mimosine.

According to the investigation of Brahmachari (2011), the membrane proteins were responsible for cell structure and originated from the alkaline phosphatase. Chang (1960) reported that the mimosine inhibited the enzyme alkaline phosphatase of cell membrane in the kidneys. In this study, degeneration of renal tubular epithelium and cellular debris were also found in both group B and C (Figure 1, 2, 3 and 4). Therefore, degeneration of renal tubular epithelium might be the result of damage to membranes of tubular epithelial cells due to mimosine present in leucaena. These lesions were indicative of interstitial nephritis and that was consistent with the findings of Prasad and Paliwal (1989).

Moreover, Peixoto *et al.* (2008) revealed that vascular damage lesions of medullary tubules of kidneys were observed in goat due to the toxic effect of mimosine contained in leucaena. In this study, the kidneys of sheep in both group B and C showed perivascular fibrosis of renal venule, degeneration of blood vessel, RBC in lumen of renal tubules and proteinaceous fluid in the interstitial tissue of renal tubules (Figure 5, 6, 7, 8 and 9). The finding from this study supports the observation by Peixoto *et al.* (2008).

Histopathological Changes of Liver

Lin *et al.* (1962) reported that mimosine inhibited the formation of glutamine which was the precursor of glutathione by inhibition of glutamate oxaloacetate transaminase. Sufficient depletion of cellular glutathione content allowed some reactive metabolites to react with cellular macromolecules leading to cellular injury and death (Docks and Krishna, 1976). In the present study, degeneration of hepatocytes with widening of sinusoidal spaces was observed in both groups B and C (Figure 10 and 11). Therefore, in the present study, degenerative changes of hepatocytes may be due to the effect of mimosine presented in leucaena. This result was also similar with the finding of Onwudike (1995) who mentioned that the leucaena-fed rabbits had serious degenerative changes in the liver.

Tang and Ling (1975) reported that mimosine in leucaena might cause vascular injury by inhibition of the synthesis of hydroxyproline, which was used, in the synthesis of elastin (Gorres and Raines, 2010). The sheep from both groups B and C showed infiltration of connective tissues around the blood vessels at the portal area which was termed as perivascular fibrosis (Figure 12 and 13). This is due to the destruction of the blood vessel walls and substitution of the damaged wall with the connective tissues (Sastry, 1983). Moreover, the sheep from group C showed haemorrhage among the hepatocytes (Figure 16). This lesion was associated with the vascular wall injury.

The liver of sheep in both groups B and C showed congestion in the portal vein (Figure 12 and 14) that referred to as passive hyperaemia of the liver. It is caused by hindrance to the flow of blood in the hepatic vein, posterior vena cava, heart or lungs (Runnell *et al.*, 1965). In addition, the liver revealed degeneration of central vein (Figure 15). These results may be associated with the direct toxic effect of mimosine and ischaemic changes resulting from vascular wall injury.

Furthermore, Lin and Tung (1964) mentioned that mimosine interfered the epithelial integrity by inhibition of protein biosynthesis in living body. Degeneration of bile duct was found in sheep of group B and C (Figure 12 and 14). Therefore, the degeneration of bile duct may be due to the loss of epithelial integrity of bile duct epithelium. The above lesion contrasted with the observation of Mostaghni *et al.* (2008) who mentioned that bile duct hyperplasia was presented in sheep induced hypothyroidism.

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V. CONCLUSION

From the present study, it could be concluded that the mimosine presented in leucaena caused the nephritis in kidney, degeneration and haemorrhage in liver and blood vessel destructions in both liver and kidney of all groups. According to these lesions, leucaena mimosine toxicity is more sever in sheep fed with 60% leucaena than sheep fed with 30% leucaena.

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