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Effect of *Leucaena Leucocephala* Leaves on Microscopic Structure of Thyroid Gland of Sheep in Myanmar

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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 experiment. Each animal was kept in individual cages and the animals were fed rice straw and sesame cake for one month and provided clean water *ad-libitum*. The experimental animals were fed 3% of body weight with and it was formulated isonitrogenously not less than 21% of crude protein. Animals were divided randomly into three groups (A, B, C) each having two animals. Group A was kept as a control group and group B and C were fed 30% and 60% of total diet with *Leucaenaleucocephala* leaves, respectively. The experimental period was two months. In the histopathological study, the thyroid gland, there were the colloidal goiter and interfollicular congestion. Severe degeneration of follicles and follicular cells, shrinkage and changes in morphology in some follicles were found. Vacuolization of follicles were replaced with massive infiltration of connective tissue. All of the above lesions were more severe lesions in the animals of group C than those of group B. It was concluded that the toxic effects of mimosine presented in the *L. leucocephala* were more severe lesions in those animals fed with 60% of LL in the diet than those fed with 30% LL in the diet.**

Keywords: **Leucaena, Mimosine, thyroid gland, Interfollicular congestion, sheep, Myanmar.**

I. INTRODUCTION

Leucaena (*Leucaena* spp., especially *Leucaena leucocephala*) is a pantropical, arboreal legume which has used as forage in ruminant production (Jones, 1979; Brewbaker et al., 1985). Leucaena is used as a livestock forage in tropical and subtropical regions. However, leucaena leaves contain a toxic non-protein amino acid, called mimosine. Mimosine stops cell division, and it is severely toxic to animals and can often be fatal (Dalzell et al., 2006).

In livestock, as well as in experimental animals, mimosine is believed to induce alopecia, growth retardation, cataract, decreased fertility and mortality. In addition, it can cause fleece sheeding, excessive salivation, and even loss of hooves in sheep (Hegarty et al., 1964; Reis et al., 1975). However, the leucaena leaves could be used up to 45% of the diets without adverse effects on body weight gain (Yami et al., 2000) and rumen fermentation in goats (Dutta et al., 2002).

Similarly, Myanmar sheep consumed 3.5% DM of live weight and the sheep tolerate to leucaena up to 30% of total diet (Aung, 2007). The sheep fed 40% leucaena showed toxic symptoms after one week of experiment. Therefore, the objective of this experiment was to observe the effect of *L. leucocephala* on microscopic structure of thyroid gland of local sheep in Myanmar.

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II. METHODOLOGY

Experimental animals and study design:

The experiment was carried out at the Department of Anatomy, University of Veterinary Science, Yezin, Nay Pyi Taw, Myanmar. Six male sheep with similar age (3-5 months) and body weight ranging from 16 kg to 20 kg were used in this experiment. Each animal was kept into individual cages; the animals were fed rice straw and sesame cake for one month. They were also provided clean water *ad-libitum*. The experimental animals were fed 3% of body weight of the diet and it was formulated isonitrogenously not less than 21% of crude protein. Sheep were divided randomly into three groups (A, B, C) each having two animals. Group A was kept as a control group and group B and C were fed 30% and 60% of total diet with *Leucaena leucocephala* leaves respectively. The experimental period was two months.

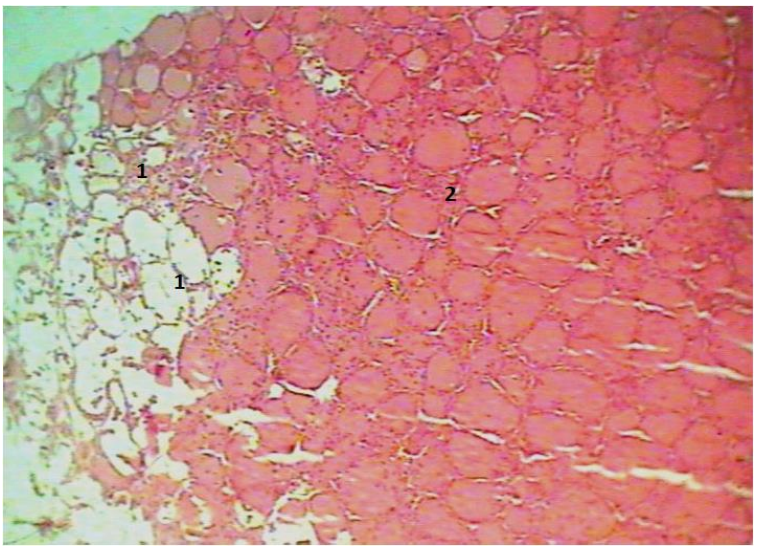
Histopathological examination:

All animals from group A, B and C were sacrificed at the end of experiment and collected the thyroid glands. For histopathological examination, the thyroid glands were collected. Tissues were fixed in 10% formalin solution for 72 hours and then histopathological processing was carried out. The tissue samples were dehydrated in the series of ascending grades of alcohol followed by cleaning in changes of xylene. Then, the tissues were embedded in paraffin and the wax embedded specimens were sectioned at 3-4 µm. All sections were stained with haematoxylin and eosin (H & E). Histopathological slides were examined under the light microscope (Digisystem) and microphotographs were taking by camera attached microscope (DB2-180M).

III. RESULTS

Histopathological changes of thyroid glands in group B were shown in Figure 1 to 3, thyroid follicles were enlarged and filled with copious amount of colloid (colloidal goitre). There were infiltrations of connective tissue between follicles. Most of the follicles were observed as non-active stage (Figure 2). The discrimination of follicular epithelium was observed. Sloughing of follicular cells from the basement membrane and mild to moderate interfollicular congestion was observed (Figure 5). Severe degeneration of follicles and follicular cells, shrinkage and changes in morphology in some follicles were found (Figure 6).

In group C, most of the follicles were lost and sizes were reduced. The interfollicular spaces were wide (Figure 7). There were compressive atrophy of follicular cells and rupture of follicles due to increase of the colloid and marked deposition of collagen in the interfollicular spaces. Severe interfollicular congestion was observed in most area. Vacuolization of follicles were replaced with massive infiltration of connective tissue (Figure 8). Connective tissue (collagen fibre) infiltrated from the capsule of the gland to the interlobular space were found (Figure 9). Ruptures of thyroid follicles due to degeneration of the follicular cells and vacuolization of follicles were observed (Figure 10).



(H&E × 100)

Figure 1: Microphotograph of thyroid gland of sheep in group B showingdestruction of follicles (1), Mild to moderate interfollicular congestion (2).

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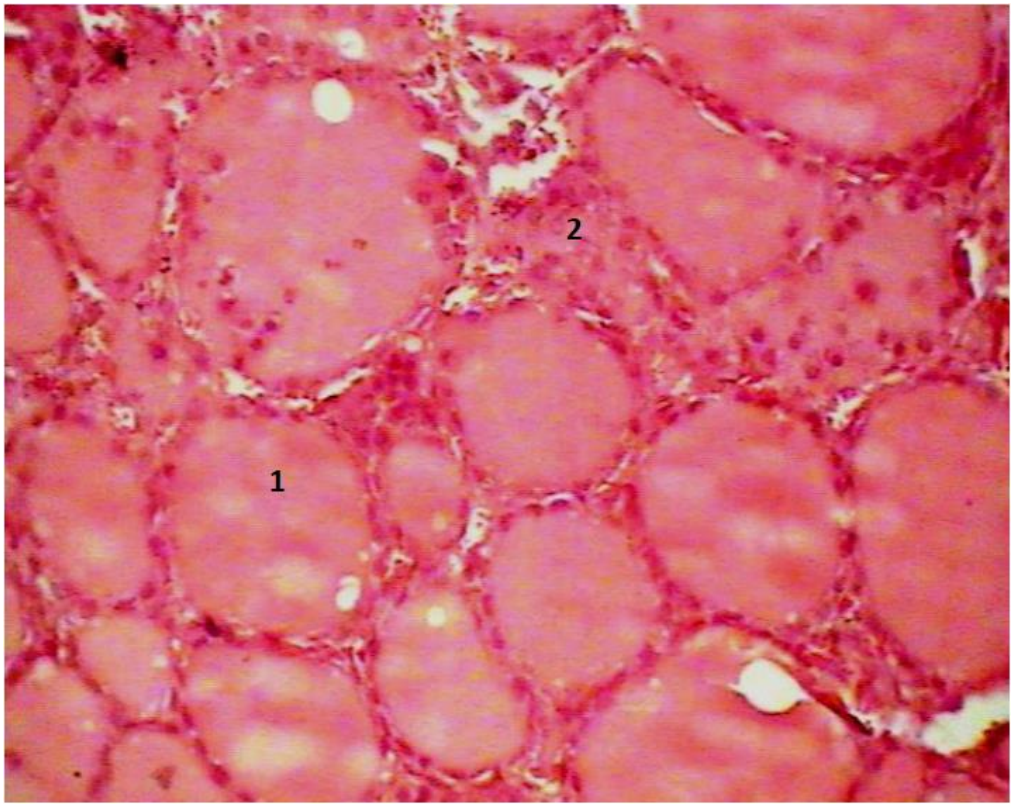

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Figure 2: Microphotograph of thyroid gland of sheep in group B showing enlarged and non- active thyroid follicles (1), infiltration of connective tissue in interfollicular spaces (2).

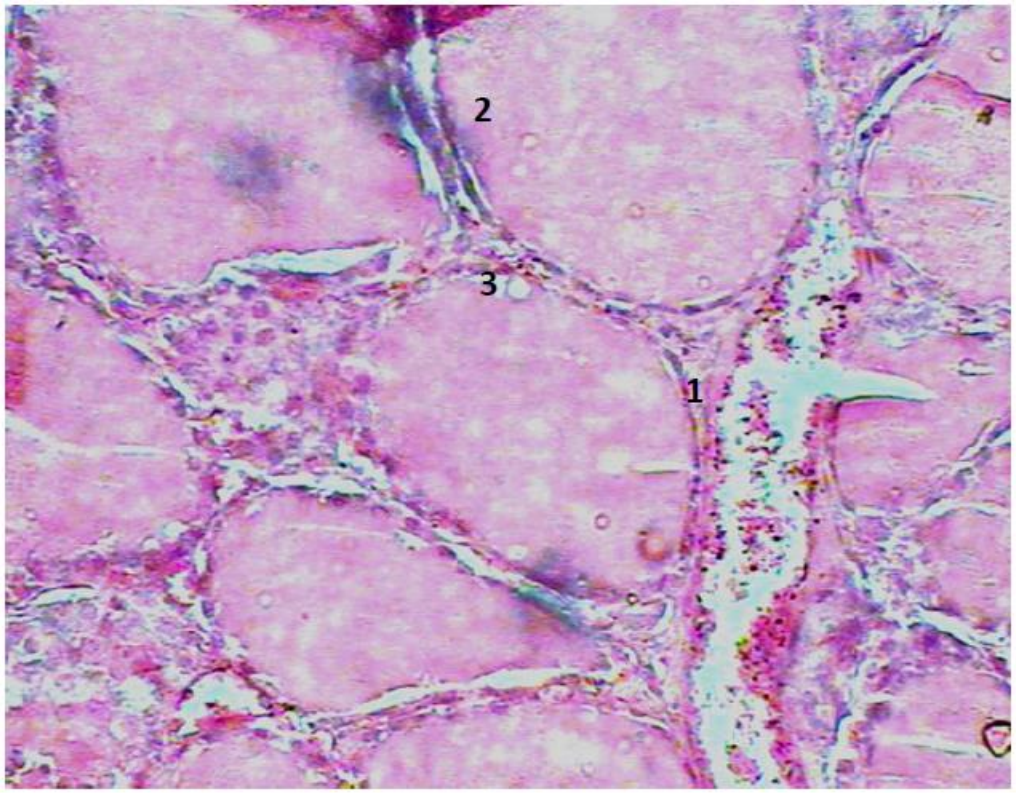

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Figure 3 Microphotograph of thyroid gland of sheep in group B Showing congested blood vessel (1), colloid in the follicles (2) and squamous shape follicular cells (3).

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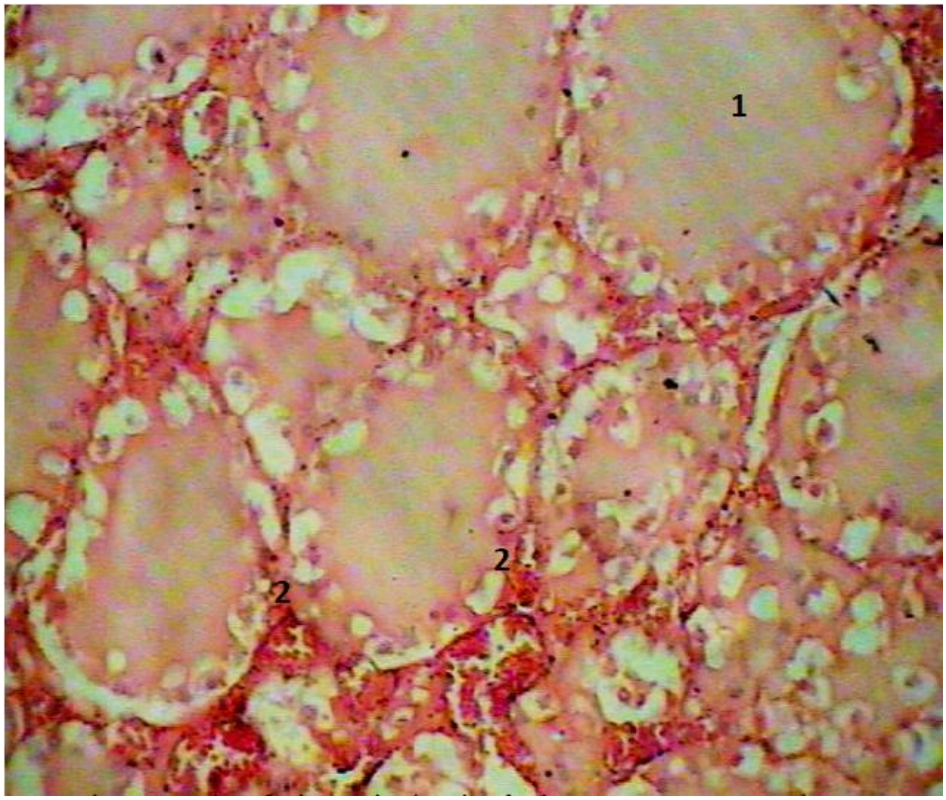

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Figure 4: Microphotograph of thyroid gland of sheep in group B showing colloid in the follicles (1) and interfollicular congestion (2).

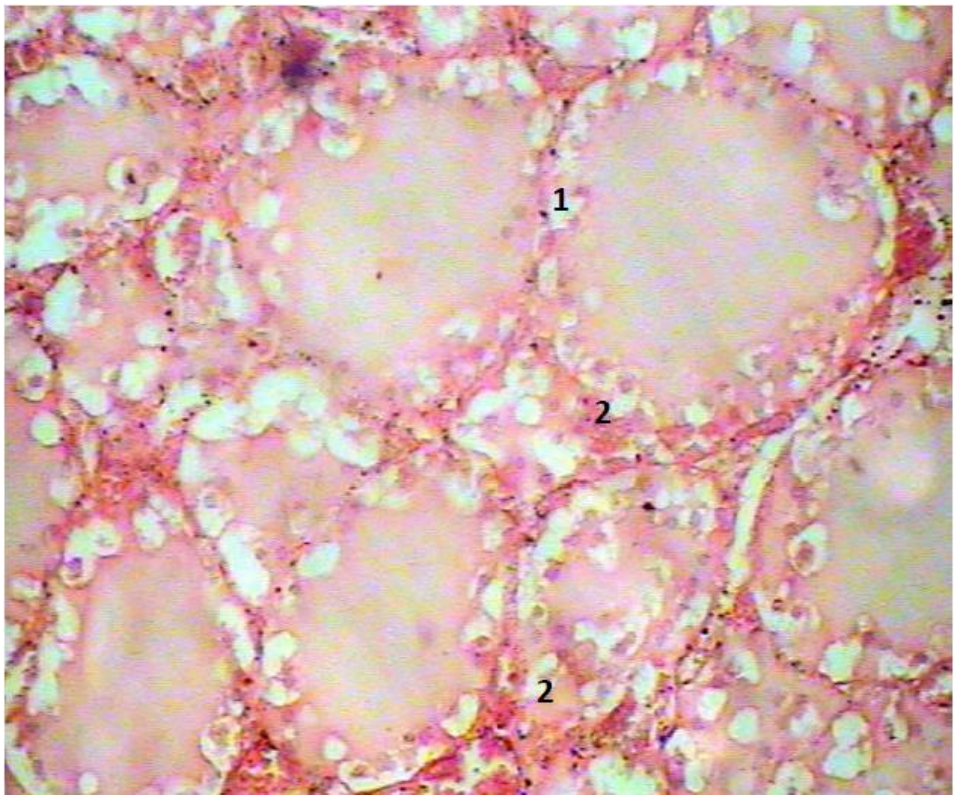

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Figure 5: Microphotograph of thyroid gland of sheep in group B showing sloughing of follicular cells from the basement membrane (1) and interfollicular congestion (2).

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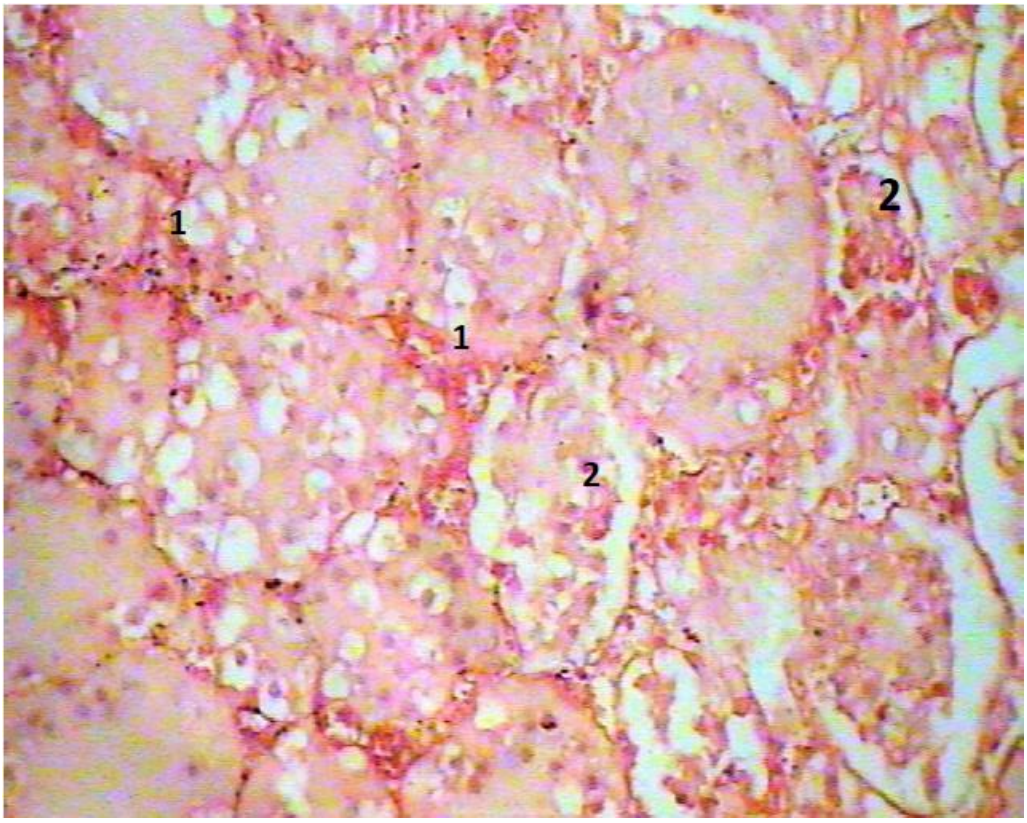

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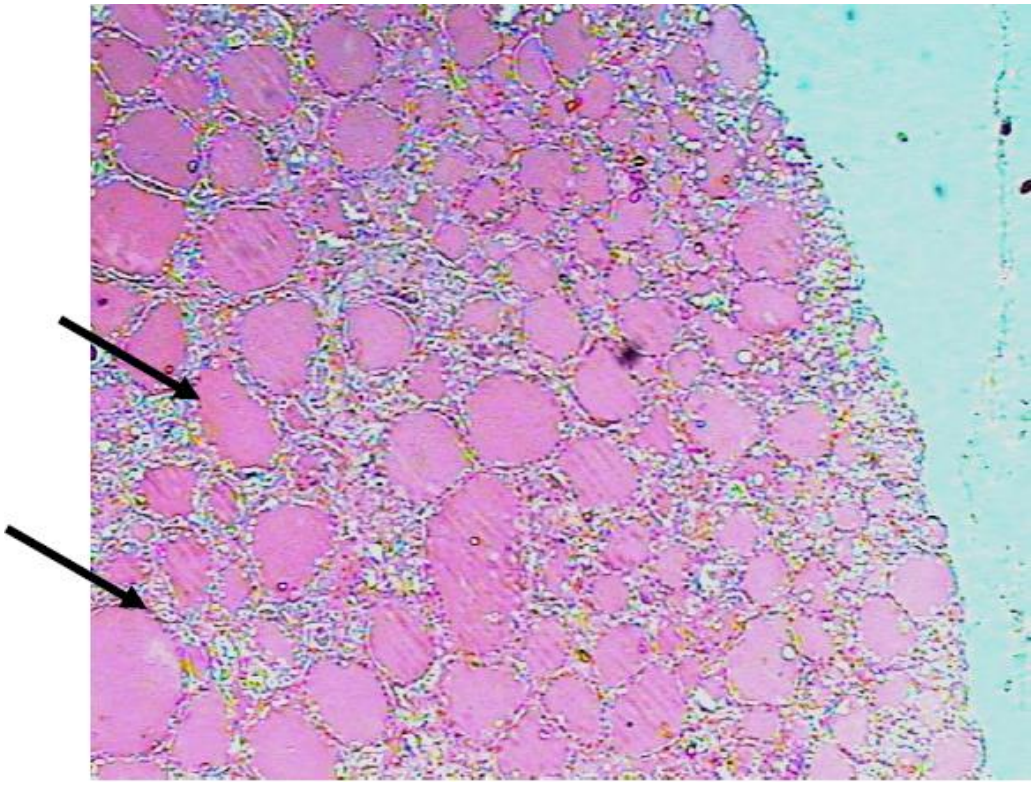
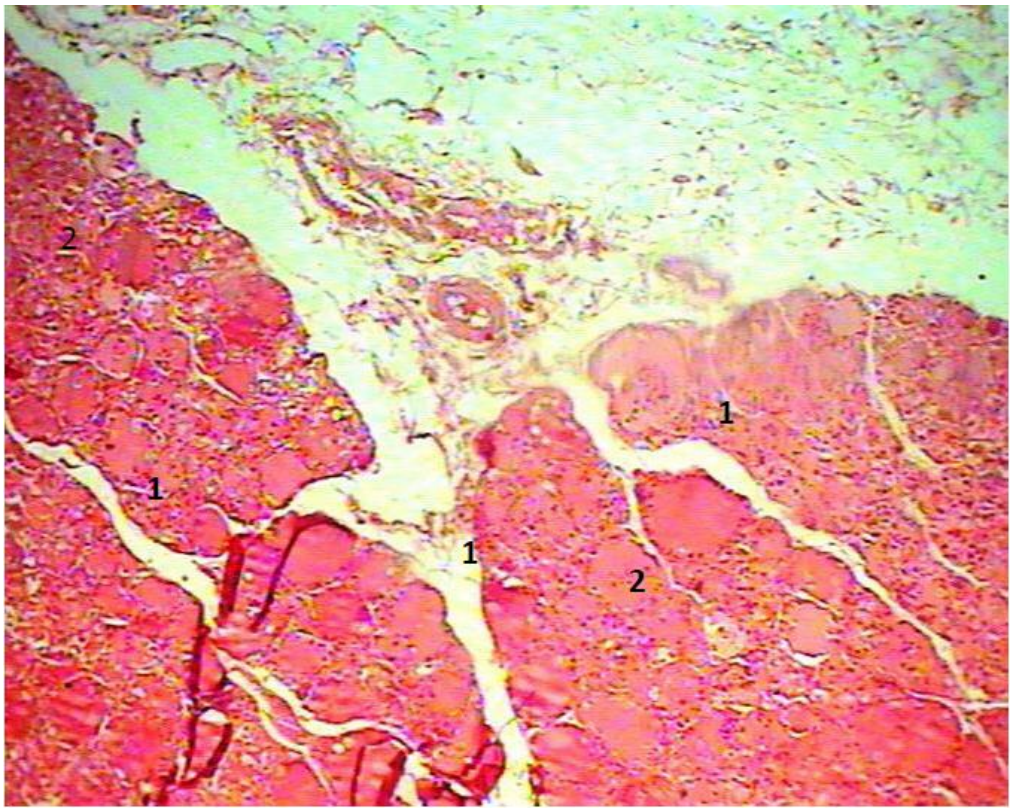
Figure 6: Microphotograph of thyroid gland of sheep in group B showing severe congestion (1), degeneration and shrinkage of follicle (2).



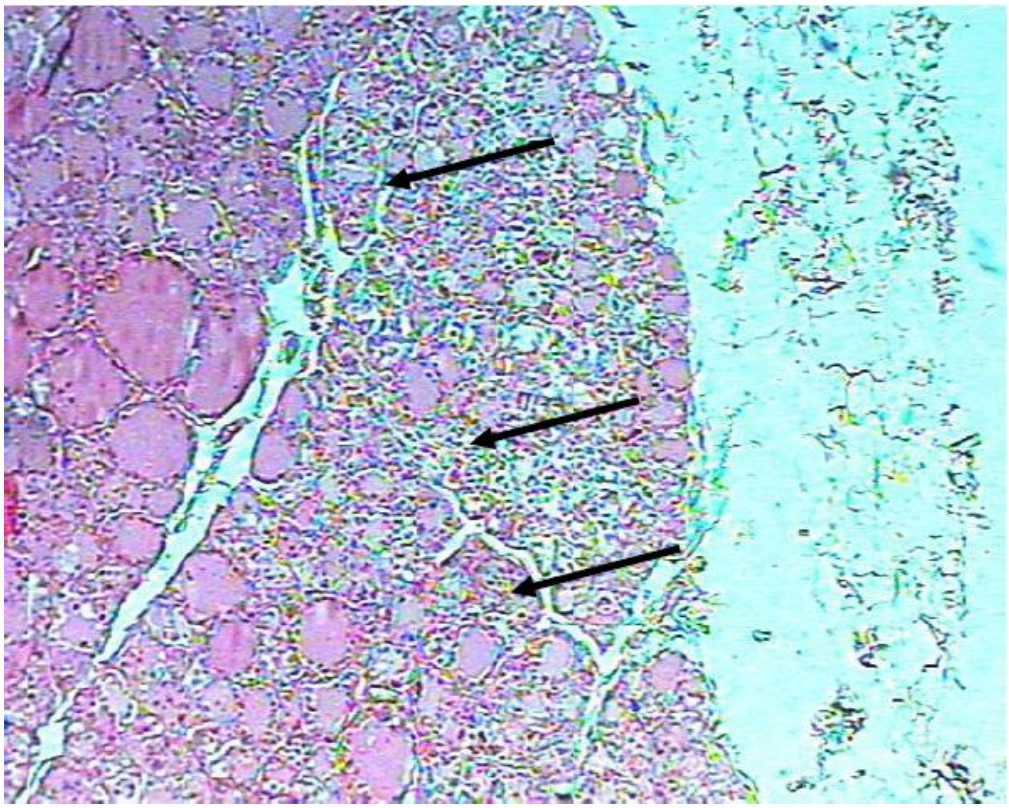
(H&E ×100)

Figure 7: Microphotograph of thyroid gland of sheep in group C showing reduce numbers of follicle, wide interfollicular spaces and infiltration of collagen fiber between follicles (arrow).



(H&E ×100)

Figure 8: Microphotograph of thyroid gland of sheep in group C showing the wider interlobular space (1) and interfollicular space (2) due to massive infiltration of collagen fibre from the capsule.



(H&E × 100)

Figure 9: Microphotograph of thyroid gland of sheep in group C showing the collagen fibre from the capsule of the gland infiltrated in interfollicular spaces (arrow).

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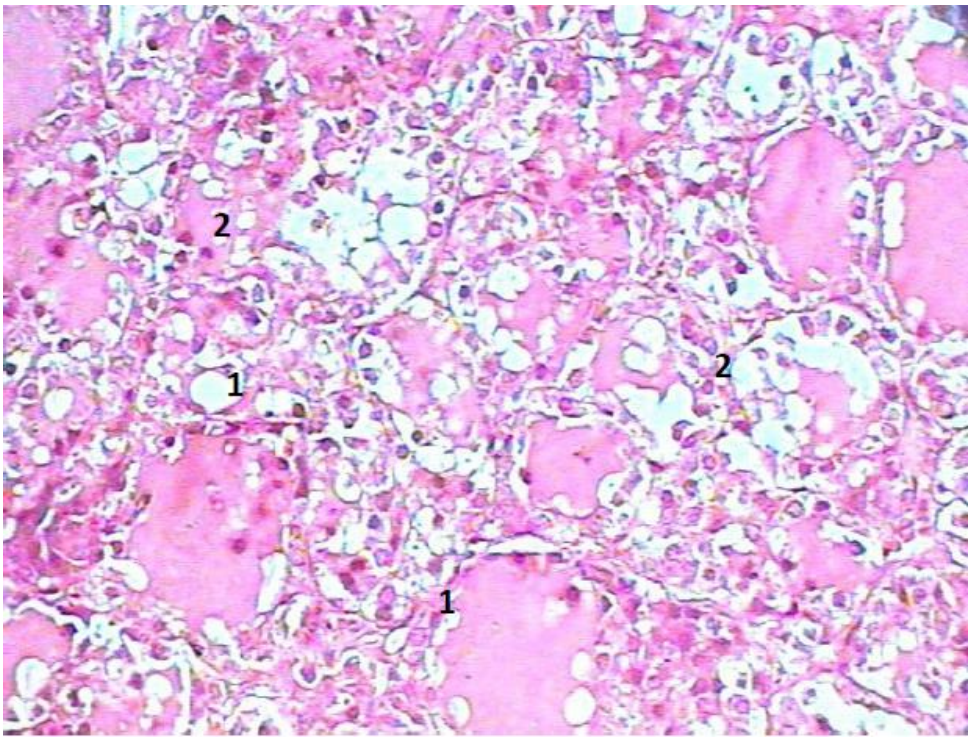

(H&E × 400)

Figure 10: Microphotograph of thyroid gland of sheep in group C showing ruptures of follicles due to degeneration of the follicular cells (1) and vacuolization of follicles (2).

IV. DISCUSSION

In the present study, compressive atrophy and rupture of the follicular cells of the thyroid gland was observed in animals in both group B and C. The incidence of these lesions was more severe in animals in group C. It might be due to increase amount of production of colloid (colloidal goiter), and that colloid was not secreted into the blood stream, marked deposition of the collagen between the follicles in some areas of thyroid gland.

Mimosine itself could not be responsible for the development of goitre in the cattle and sheep grazing leucaena (Jones, 1985). However, the colloidal goitre is due to the action of 3, 4-DHP that interferes in the intracellular process of iodine oxidation of the thyroid gland, which results in accumulation of colloid, poor in thymoglobulin, decrease of thyroxin.

In this experiment, there were infiltrations of connective tissue especially the collagen fiber between the follicles in both group B and C. This was similar lesions with the findings of (Peixoto et al., 2008) who reported that spontaneous poisoning was observed in goat fed with *L. leucocephala*.

In thyroid gland of the animals of group B and C revealed the severe interfollicular congestion as the mimosine-fed animals might induce the capillaries haemorrhage in various organs (Dewreede et al., 1970).

The typical signs of leucaena toxicosis in ruminants include alopecia, anorexia, reduced weight gain or weight loss, excessive salivation, esophageal lesions, enlarged thyroid, and low circulating concentrations of thyroid hormones (Jones, 1985). However, in present study, there were no obvious clinical signs of mimosine toxicity in all groups of animals. This is generally associated with the amount of the plant in the diet (Jones et al., 1976). Similarly, the effect of *L. leucocephala* depends on the variety of the tree, the amount of other fodder available and feed selection by the animal (Blood and Radostitis, 1989).

Nonetheless, if the sheep are slowly introduced to leucaena feeds, the rumen bacteria can adjust and the animals can feed on the plant (especially the low mimosine types) with the minimum depilatory effect (Ruskin, 1977).

According to Quirk et al., (1988), the effects of DHP toxicity in animals depend on both the amount of leucaena present in the diet and time of feeding of leucaena plant. Clinical symptoms may take up to 8 weeks to become evident.

The diagnosis of leucaena toxicity was based on the diffuse alopecia and on the histopathological alterations, typical for an animal that, for at least 4 months, has been ingesting exclusively *Leucaenaleucocephala* (Peixoto et al., 2008). However, in this experiment was only 2 months. Furthermore, the effects of these plants on gross and histopathological changes in sheep deserve further investigation.

Deficiencies of other microminerals also have been hypothesized to play a role in the pathogenesis of leucaena toxicosis. In steers fed only leucaena, a combination of iron and copper injections, and zinc supplementation increased feed intake and body weight gain but did not affect reduced concentrations of serum T_4 (Thyroxine) (Jones et al., 1978).

V. CONCLUSION

From this study, it was concluded that there were the colloidal goiter and interfollicular congestion in the thyroid gland. According to these lesions, group C was the most severe histopathological changes among the all groups.

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