Gross and histopathological effects of *Gmelina arborea* in the internal organs of Goats

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Journal of Livestock Science (ISSN online 2277-6214) 16: 644-652
Received on 18/3/25; Accepted on 20/11/25; Published on 1/12/25
doi. 10.33259/JLivestSci.2025.644-652

Abstract

This study was conducted to determine the toxic effects of *Gmelina arborea* in goats through gross and histopathological examination of internal organs. Effects were determined through feeding experiment in full confinement system in a monitor type goat house. Results revealed diffuse black coloration in the ruminal mucosa with erosion and ulceration of the papillae in goats fed with 100% *G. arborea*. In combination with basal forage, kidneys had also multifocal ecchymotic hemorrhages of the serosa. There were multifocal white spots observed in the liver serosa. Smooth papillae structure was also observed in the forestomach. Histopathological examination also supports these results as collapsed renal tubule, necrosis of the hepatocytes, and erosion and excoriation of the rumen was also observed. The results of gross and histopathological examination revealed that *G. arborea* fed 100% or in combination with basal forages can induced toxicity to goats. These effects can also be attributed to the plant secondary products present in the plant that have induced these effects

Keywords: histopathology; toxicity; internal organs; *Gmelina arborea*; goat

Introduction

Naturally, goats are active foragers and browsers. Free-foraging goats are able to cover a wide area in search for food. The morphology of a goat's mouth enables them to pick small plant parts (Mkhize et al., 2014). As browsers, goats can stand and stretch on their hind legs which allow them to reach the leaves and twigs of shrubs and trees. They can also throw their bodies against saplings. Schlect at al. (1999) observed that goats can climb trees or shrubs to consume preferred forage. Goats have developed a unique feeding behavior compared to other ruminants like sheep and cattle (Dias-Silva & Abdalla Filho, 2020). They tend to (1) select young grass over clover; (2) browse than graze pastures; (3) consume wide range of plant species; (4) feed on rough and steep lands; (5) graze along fences before grazing at the center of pasture; and (6) travel longer distance in search of preferred forages (Fernandes et al., 2016).

On the other hand, in some small-scale production system, goat raisers are practicing tethering wherein goats are tied in a specific location particularly in open vegetated areas. Naturally vegetated areas are composed of different species of plants which can be of poor quality and may contain toxic compounds (Salvaña et al., 2021). Goat raisers had no option but to feed their animals with plant species present in the area wherein availability is greatly affected by climatic conditions. Environmental extremes like drought are known to increase the number of toxic compounds (da Silva et al., 2013). In the study of Sanchez et al. (2022), two of the common problems associated to goats include diarrhea and bloat. These two are commonly attributed to the consumption of *Gmelina arborea, Chromolaena odorata* and *Manihot esculenta*.

Thus, this study will provide information of the effect of *G. arborea* as consumed by goats particularly of the gross morphology and histology of the internal organs. Manifestation of toxicity, aside from the signs observed in the animal and death, can also be observed in the structures of internal organs especially those that are involved in the digestion, absorption and metabolism of feed. Implications of this study can be an important consideration as some studies have elaborated the potential of using this plant as a component in the feed formulation for goats.

Materials and Methods

Experimental feeding was conducted in local goat farm in Brgy. Pedtad, Kabacan, North Cotabato (7.1527 N, 124.8341 E). There were 3 treatments in the study: T1- 100% Basal forage; T2- 100% *G. arborea*; T3- 50% *G. arborea* + 50% Basal forage. Plants are collected in the field near the experimental site (Fig. 1). The experiment was conducted in a monitor type goat house. It is an open roof type wherein there is an open vertical space at the center of the roofing to allow air circulation. The house was built on poles with a height of the floor at 1.5- 2 meters from the ground (Fig. 2). Full confinement or cut-and-carry system was employed in the experimental feeding. A total of 9 upgraded goats, 3 individuals per treatment, with age ranges from 7-8 months and weigh 10-11 kilograms were selected as test animals for the feeding experiment. Preliminary feeding was done in few goats to check forage palatability. Freshly cut forage and combinations were fed on goats three times a day: (1) 7:00 AM; (2) 11:00 AM; and (3) 4:00 PM. The amount fed was calculated using the reference gram (g) forage per kg body weight. Water was provided *ad libitum*. Experimental feeding was done in 120 days. Proximate analysis of forages used was also conducted (Table 1). In the examination of the gross morphology of the internal organs, 1 goat per treatment was randomly selected and was slaughtered following proper slaughtering protocol. Gross pathological examination of essential parts, such as the liver, kidney, and rumen, was conducted with a veterinarian. These parts were fixed with 10% neutral phosphate-buffered formalin. A subjective scoring system was arbitrarily assigned for microscopic evaluation of lesions in kidney, liver and rumen using the following criteria: (-) no visible lesion; (+) mild; (++) moderate; and (+++) severe. Morphological and anatomical changes in tissues were observed and compared to normal morpho-anatomical features.

Figure 1. (A) Field where plants are collected and (B) a *Gmelina arborea* plant

Table 1. Results of proximate analysis of forages used in the feeding experiment.

Samples	Crude Protein (%)	Crude Fiber (%)	Ash (%)	Crude Fat (%)	Moisture (%)	Dry Matter (%)	Nitrogen Free Extract (%)
Basal Forage	8.67	37.30	11.92	5.67	13.98	86.02	38.49
Gmelina arborea	13.04	8.41	9.04	5.34	9.52	90.48	64.52
50% basal forage + 50% *Gmelina arborea*	9.63	19.82	9.67	4.41	11.28	88.72	57.72

Figure 2. Housing facility used for experimental feeding.

Results

Goats fed with basal forage (T1) and in combination with *G. arborea* (T3) have high ADG and low FCR values compared to other treatments. Signs of bloat was observed in all goats fed with 100% *G. arborea* (T2). Gross pathological examination results revealed that goat fed with basal forage (T1) showed no visible lesions in the heart, kidney and liver. It was also observed that the forestomach had no eroded and excoriated portions with smooth papillae as shown in Figure 3. On the other hand, the goat fed with *G. arborea* (100%) showed no visible lesions in the heart, liver and kidney. Although, kidneys were smaller as compared to the control (T1). It was also observed that there was diffuse black coloration in the ruminal mucosa with erosion and ulceration of the papillae (Fig.4). The goat fed with combined *G. arborea* (50%) and basal forage (50%) (T3) showed no visible lesions in the heart. Kidneys had also multifocal ecchymotic hemorrhages of the serosa. There were multifocal white spots observed in the liver serosa. Smooth papillae structure was also observed in the forestomach (Fig. 5).

Results of histopathological examination revealed that kidney sections of all samples had: aggregates of inflammatory cells predominantly neutrophils, few lymphocytes and plasma cells in the interstitium surrounding distal and proximal convoluted tubules and some glomeruli; atrophy of glomeruli and mild to moderate diffuse tubular necrosis (Table 2). In T3, congested blood vessels were observed. In T2 kidney section, random numbers of collapsed distal and proximal tubules was also observed. Sections of the liver from T2 showed mild lysis of the cytoplasm of hepatocytes (Table 3). Mild coagulative and multifocal necrosis in hepatocytes was observed in T3 while portal fibrosis was observed in both T2 and T3. Large parenchyma abscess was observed in T3 liver sections. Rumen sections of T1 and T3 have no visible lesions. In T2 (100% *G. arborea*) rumen section, severe necrosis of keratinized stratified epithelium of rumen mucosa was observed (Table 4).

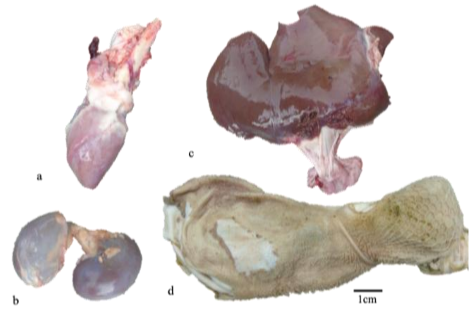


Figure 3. Internal organs of goats fed with 100% basal forage (T1). (a- heart; b- kidney; c- liver; d- forestomach).

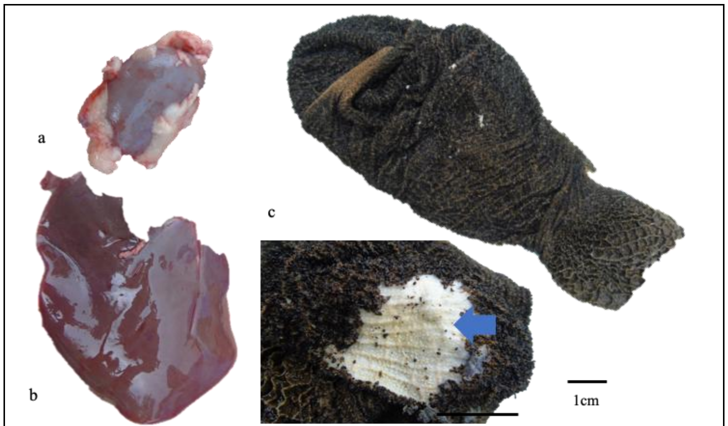


Figure 4. Figure 35. Internal organs of goats fed with 100% *G. arborea* (T2). (a- kidney; b- liver; c- forestomach with excoriated part; blue arrow).

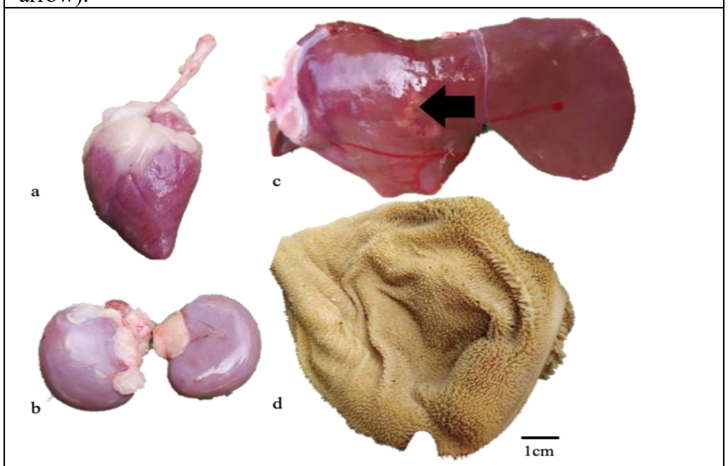
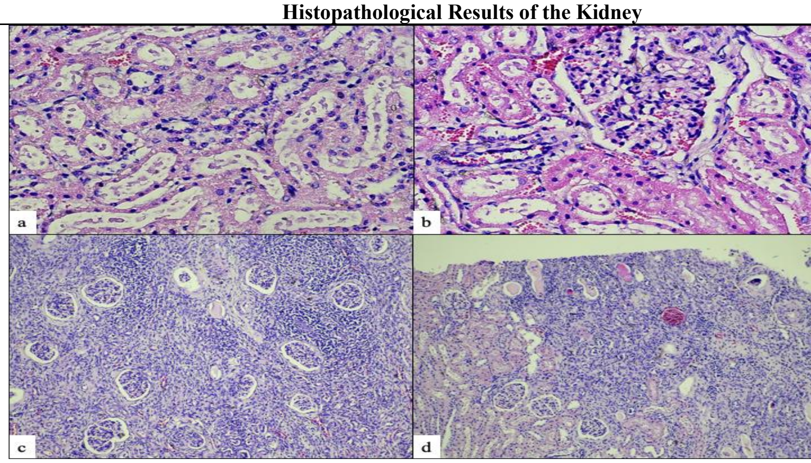
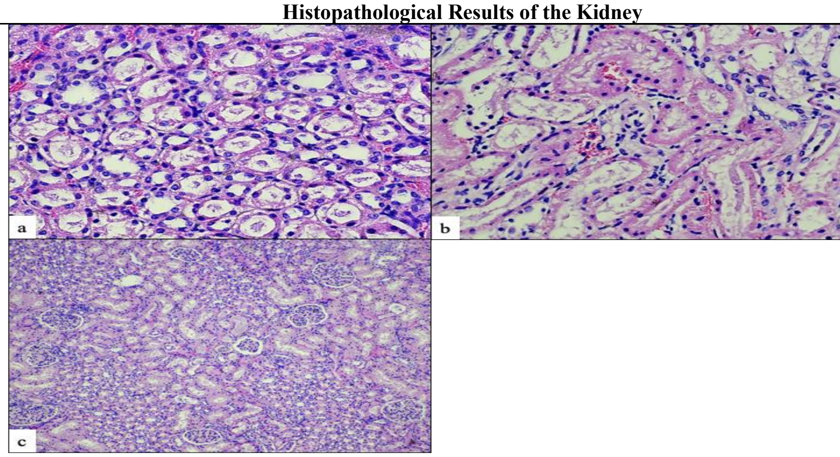
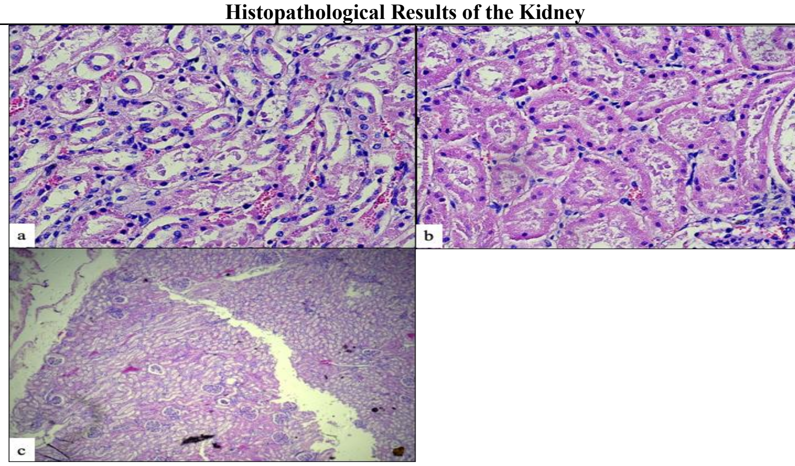


Figure 5. Internal organs of goats fed with combined 50% Gmelina arborea + 50% basal forage (T6). (a- heart; b- kidney; c- liver- showing location of milky spot (arrow); d- forestomach-showing good papillae structure).

Discussion

The effects of feeding *G. arborea* to goats was observed in the kidney, liver and rumen samples. One of the highlighted result was observed in the rumen of a goat fed with 100% *G. arborea*. There was diffuse black discoloration of the ruminal mucosa with erosion and ulceration of the papillae. It was indicated by Patra and Saxena (2011) that damages in rumen tissues is associated to large amount of tannins in the diet goats. These compounds also change the morphology of microvilli (Crozier et al., 2009). It was indicated in the review of Hossain et al. (2021) that hydrolysable tannins (HT) from some plant species cause severe necrosis and ulceration of the epithelium of the esophagus, stomach, intestines, and proximal renal tubules. The cause of the injury is the binding of tannins to mucosal epithelium, endothelium, and renal tubular epithelium (Maugeri et al., 2022). Hydrolyzable tannins (HTs) are enzymatically depolymerised in the rumen to gallic acid. Gallic acid is further metabolized by microbes to pyrogallol and other low molecular weight phenols which are absorbed and responsible for cellular damage (Yang et al., 2020). Another possible reason for the excoriation of the rumen observed in T2 goat is mechanical abrasion. During feeding experiment, these goats were observed to consume parts of the stem and bark. This contributed to the dark coloration of the rumen.

Table 2. Histopathological results of kidneys from goats fed with individual and combined forages. A subjective scoring system was arbitrarily assigned for microscopic evaluation of lesions in kidney using the following criteria: (-) no visible lesion; (+) mild; (++) moderate; (+++) severe.

Treatment	Histopathological Results of the Kidney
1	 a) (++) diffuse tubular necrosis with moderate amount of cellular debris in most of the tubular lumina (Mag= 800x); b) aggregates of inflammatory cells predominantly neutrophils, few lymphocytes and plasma cells in the interstitium and some glomeruli (Mag= 800x); c) atrophy of few glomeruli due to fibrosis (Mag=400x); d) foci of hyalinization around glomeruli (Mag=400x)
Treatment	**Histopathological Results of the Kidney**
2	
	a)(++) diffuse tubular necrosis (Mag=800x); b) random numbers of collapsed distal and proximal tubules; aggregates of inflammatory cells predominantly neutrophils, few lymphocytes and plasma cells in the interstitium surrounding distal and proximal convoluted tubules (Mag=800x); c) atrophy of few glomeruli. (Mag=400x)
Treatment	**Histopathological Results of the Kidney**
3	

	a) (++) diffuse tubular necrosis with moderate to scant amount of cellular debris in most of the tubular lumina (Mag=800x); b) aggregates of inflammatory cells predominantly neutrophils, few lymphocytes and plasma cells in the interstitium surrounding distal and proximal convoluted tubules and some glomeruli (Mag=800x); c) atrophy of few glomeruli; blood vessels are congested elsewhere (Mag=400x)

Table 3. Histopathological results of liver from goats fed with individual and combined forages. A subjective scoring system was arbitrarily assigned for microscopic evaluation of lesions in the liver using the following criteria: (-) no visible lesion; (+) mild; (++) moderate; (+++) severe.

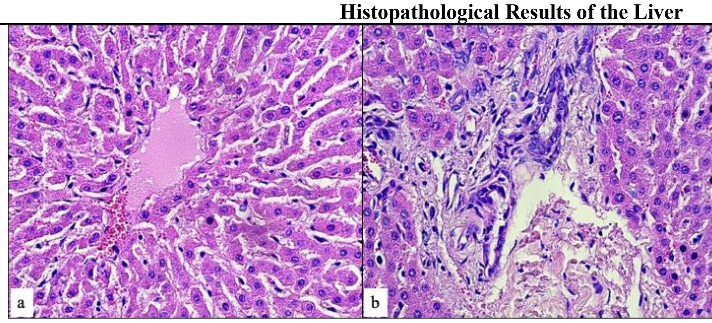
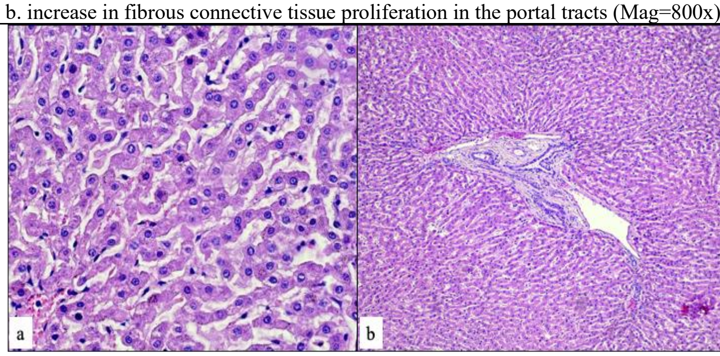
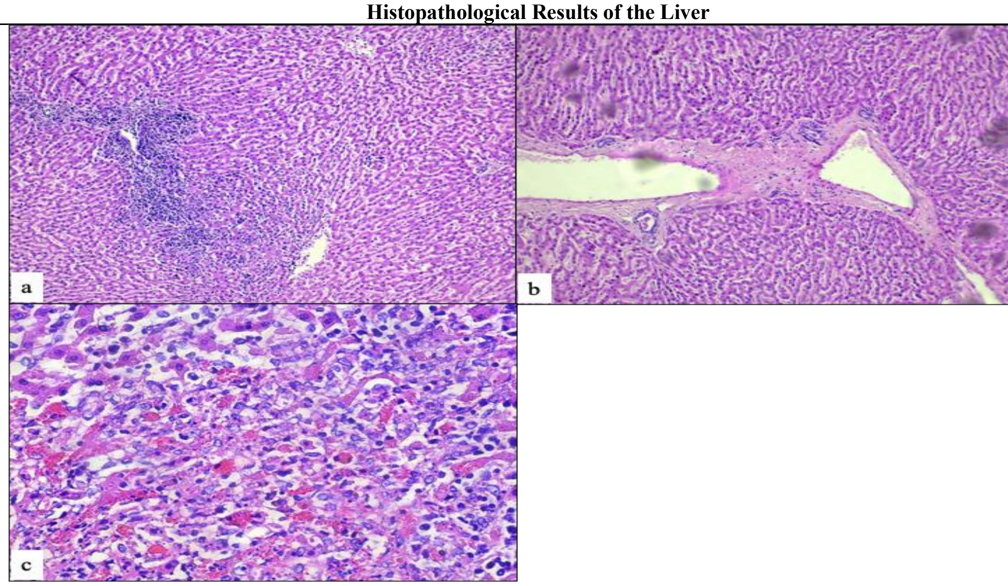
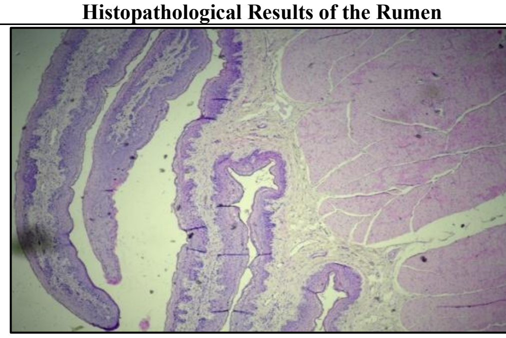
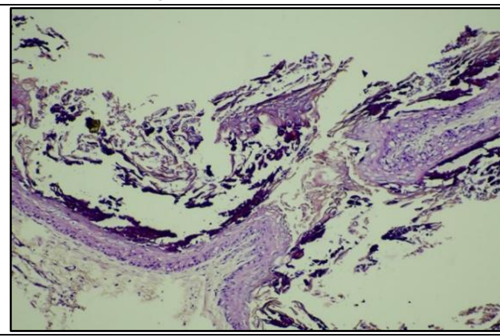
Treatment	Histopathological Results of the Liver
1	
	a. (+) mild lysis of the cytoplasm of hepatocytes (Mag= 800x); b. increase in fibrous connective tissue proliferation in the portal tracts (Mag=800x)
2	 a) (+) lysis of the cytoplasm of hepatocytes (Mag=800x) b) (+) fibrosis of the portal tract (Mag=400x)
Treatment	**Histopathological Results of the Liver**
3	
	a) multifocal hepatocellular necrosis and inflammatory infiltrates composed of neutrophils and lymphocytes (Mag=400x); b) prominent portal fibrosis (Mag=400x); c) parenchyma are large abscesses, sometimes confluent (Mag=800x)

Table 4. Histopathological results of rumen from goats fed with individual and combined forages. A subjective scoring system was arbitrarily assigned for microscopic evaluation of lesions in the rumen using the following criteria: (-) no visible lesion; (+) mild; (++) moderate; (+++) severe.

Treatment	Histopathological Results of the Rumen
1	
	(-) no visible lesion (Mag=100x)
2	
	(+++) necrosis of keratinized stratified epithelium of rumen mucosa (Mag=100x)
Treatment	**Histopathological Results of the Rumen**
3	
	(-) no visible lesion (Mag=100x)

Discussion

The effects of feeding *G. arborea* to goats was observed in the kidney, liver and rumen samples. One of the highlighted result was observed in the rumen of a goat fed with 100% *G. arborea*. There was diffuse black discoloration of the ruminal mucosa with erosion and ulceration of the papillae. It was indicated by Patra and Saxena (2011) that damages in rumen tissues is associated to large amount of tannins in the diet goats. These compounds also change the morphology of microvilli (Crozier et al., 2009). It was indicated in the review of Hossain et al. (2021) that hydrolysable tannins (HT) from some plant species cause severe necrosis and ulceration of the epithelium of the esophagus, stomach, intestines, and proximal renal tubules. The cause of the injury is the binding of tannins to mucosal epithelium, endothelium, and renal tubular epithelium (Maugeri et al., 2022). Hydrolyzable tannins (HTs) are enzymatically depolymerised in the rumen to gallic acid. Gallic acid is further metabolized by microbes to pyrogallol and other low molecular weight phenols which are absorbed and responsible for cellular damage (Yang et al., 2020). Another possible reason for the excoriation of the rumen observed in T2 goat is mechanical abrasion. During feeding experiment, these goats were observed to consume parts of the stem and bark. This contributed to the dark coloration of the rumen.

Results of the histopathological examination of kidney, liver and rumen can be indicators of the toxic effects of plant secondary products in individual and combined forages used in the feeding experiment. It was observed that goats fed with

100% *G. arborea* (T2) had random numbers of collapsed distal and proximal tubules in the kidney due to high tannin content. Severe necrosis and ulceration of the proximal and distal renal tubules can be observed in animals fed with considerable amount of hydrolysable tannins (HTs) (Maugeri et al., 2022). Collapse of these tubules is due to the binding of HTs in the tubular epithelium (Spier et al., 1987). Toxic effects of tannins, particularly hydrolysable tannins, is also associated with the depolymerization of these compounds in the rumen forming gallic acid. Gallic acid is further metabolized by microbes to pyrogallol and other phenols with low molecular weight. These are absorbed and induce cellular damage (Murdiarti et al., 1992). In addition to tannins, saponins are also noted to induce necrosis in the epithelial cells of the renal proximal and distal convoluted tubules. Total saponins were high in the examined parts of 100 % *G. arborea* (T2). The effects of saponins in the renal tubules is also supported by the study of Medonca et al. (2013) wherein goats fed with *Stryphnodendron fissuratum* (Fabaceae) at 20- 40 g/ kg BW showed mild tubular epithelium necrosis and renal glomeruli atrophy. *Stryphnodendron fissuratum* is noted to contain considerable amount of triterpenoid saponins (Haraguchi et al., 2006; Yokosuka et al., 2008). The pathogenesis of renal tubule necrosis can be attributed to the conversion of saponins to sapogenins by rumen microflora and absorbed in the gastrointestinal tract (Kholif, 2023). Hepatic portal fibrosis is a generic response to hepatic injury (Driemeier et al., 1998). Bridging fibrosis characterized by portal tracts distended by fibrous connective tissue extending from the portal triad to another portal area was observed in the liver of goats fed with *Brachiaria decumbens* (Rosa et al., 2016) which has a considerable amount of lithogenic steroidal saponins. Saponins are hydrolyzed in the gastrointestinal tract forming sapogenins and diosgenin. These metabolites are reduced and undergo subsequent glucoronidation in the liver. These glucuronidated sapogenins precipitate as calcium salts resulting to mechanical occlusion and damage of the biliary system and adjacent hepatocytes (Haschek et al., 2013).

In the rumen, necrosis of the keratinized stratified epithelium was observed in T2 (100% *G. arborea*) can be due high value of total saponin and tannin contents. In addition, the effect of saponins in the rumen include prominent detachment of the epithelium together with redness and adherence of rumen papilla and congestion of lamina propria (Brito et al., 2001). In addition, these compounds also cause degeneration and diffuse necrosis of the ruminal mucosa and mild spongiosis of the rumen epithelium (Medonca et al., 2013). High amount of tannins in forages can also affect rumen epithelium. Condensed tannins induce flatness and erosion of the rumen epithelial tissues of goats. Higher amount (200 g/ kg) of these compounds causes partial to total denudation of sunken microvilli in some patches of the epithelium (Mbatha et al., 2002).

Conclusion

The results of gross and histopathological examination revealed that *G. arborea* fed 100% or in combination with basal forages can induced toxicity to goats which may have both short and long terms effects in the goat production system. These effects can also be attributed to the plant secondary products present in the plant that have induced these effects. The use of this plant as pure feed or in high concentration may be prohibited and further treatments, like fermentation, can be applied to reduce its toxic effects.

Acknowledgement

Heartfelt gratitude is due to the following as their support and guidance lead to the completion of this study: Dr. Lourdes B. Cardenas, Dr. Percival P. Sangel, Dr. Michelle Grace V. Paraso, Dr. Antonio C. Laurena, Dr. Carlito B. Sanchez, Prof. Elma G. Sepelagio, Dr. Lillian Lumbao, Livestock Research Division and Institutional Development Division of the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARD).

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