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Effect of herbal powder supplementation in protecting histopathological changes associated with Caecal coccidiosis

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Abstract
An experiment was conducted on one day old, 120 broiler chicks to study the effect of madar (*Calotropis procera*) leaf powder and amprolium supplementation on caecal histology of broiler chicks during coccidiosis. Broiler chicks were divided into five groups (I-V) each with two replicates of 12 chicks. Group I and II were provided unsupplemented diet, Group III was supplemented with 0.125% amprolium, group IV and V were supplemented with 0.2% and 0.4% madar leaf powder supplementation respectively. On 15th day of experiment, groups II, III, IV and V were infected with 50,000 sporulated oocysts containing 80% *Eimeria tenella*. Histopathological changes were studied on 5th, 10th and 15th days post infection from two birds of each replicate. Histopathological changes were minimum in group III. In madar leaf powder supplemented groups (IV and V) protection from histopathological changes was observed from lesions associated with coccidiosis and effect was concentration dependent.

Keywords: Histopathological changes, *Eimeria tenella*, madar leaf powder, Amprolium

Introduction
Coccidiosis is nightmare of the poultry industry causing considerable economic loss. Study conducted on the economic losses due to chicken coccidiosis was estimated to be around Rs. 1.5 billion in India (Berra *et al.*, 2010) [8]. Protozoan parasites of Genus *Eimeria* are known to cause coccidiosis in poultry. These are obligatory intracellular parasites with complex life cycles including both sexual and asexual stages. Nine *Eimeria* species (*Eimeria tenella*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria mivati*, *Eimeria hagani* and *Eimeria praecox*) infect chicken. Among these species *Eimeria tenella* is most pathogenic and responsible for caecal coccidiosis. Caecal coccidiosis is an acute disease characterised by diarrhoea and massive caecal haemorrhages. Caecal coccidiosis may produce bloody droppings and anaemia. Currently, coccidiosis control programmes largely rely on chemotherapy and immuno-prophylaxis (Abbas *et al.*, 2010; Allen and Fetterer, 2002) [2, 5], but development of drug resistance as well as the withdrawal period for these drugs prior to slaughter and chances of reversal of pathogenicity of vaccines necessitate the exploration of alternative methods for controlling coccidiosis (Chandrakesan *et al.*, 2009) [11]. Plant products could provide an alternative means of coccidia control to which resistance has not yet developed (Abbas *et al*, 2012) [1] and are cheaper than either drugs or vaccines.

Natural herbs have many pharmacological properties to reduce the effect of coccidiosis (Adhikari *et al.*, 2020) [4]. In a study El-Khtam *et al*. (2014) [14] found that allicin in garlic inhibits sporulation of *Eimeria tenella*. Another study by Hassan *et al.* (2008) [15] also demonstrated that dietary supplementation of guar beans (*Cyamopsis tetragonoloba*) suppressed coccidiosis in chickens, which is due to its saponin compound. Similarly, Molan *et al.* (2009) [22] also noticed that extract from the bark of pine tree, which is rich in condensed tannins reduced coccidiosis in chickens. Another researcher Khalafalla *et al.* (2011) [19] also observed that Curcumin (Phenol) in turmeric (*Curcuma longa*) destroyed sporozoites of *Eimeria tenella*. In this experiment effect of 0.4 % as well as 0.2 % *Calotropis procera* (madar) leaf powder and 0.0125% amprolium supplementation on caecal histology of broiler chicks during coccidiosis was observed.

Materials and Methods

The experiment was conducted on 120, one day old broiler chicks for 30 days after necessary permission from Institute animal ethical committee.

On arrival, chicks were randomly allocated to five groups. Each group had two replicates with 12 chicks each. Chicks of different replicates were kept in separate cages and maintained under similar managemental conditions. Broilers of group I and II were provided standard control diet without any supplement. In broilers of group III, standard feed was supplemented with 0.0125% amprolium and broilers of group IV were provided with 0.2% madar leaf powder supplementation. Broilers of group V were provided standard diet supplemented with 0.4% madar leaf powder. On 15th day of experiment, broilers of group II, III, IV and V were provided with 1 ml suspension of 50,000 sporulated oocysts containing 80% *Eimeria tenella*. To isolate the oocysts of *Eimeria sp.*, method described by Holdsworth *et al.* (2004) [16] was used with few modifications and then the number of oocysts were counted in McMaster chamber (Davies *et al.*, 1963) [12].

Histopathological changes were studied on 5th, 10th and 15th days post infection in caeca from two birds of each replicate. For histopathological examination, after slaughter of broilers, the caecal tissue samples were fixed in 10% formalin for histopathological examination. Tissues were trimmed to 3 to 5 µm thickness, placed in tissue capsules and thereafter washed overnight under running water. Then they were dehydrated by placing in chambers containing different alcohol concentrations (50, 70, 80, 90, absolute I and absolute II), for 1 hour in each, consecutively. The processed tissues were cleared in xylene I and xylene II for 1 hour each and then embedded in paraffin for preparation of fine blocks. Blocks were sectioned with a microtome to a size of 5 µm, and tissue sections were sticked to the slide with egg albumin and glycerine, afterward tissue sections on slides were dewaxed on hot plate and placed in xylene II and xylene I for 2 minutes in each, then rehydrated using decreasing grade of alcohols for 2 minutes in each grade. The tissues sections were then stained using haematoxylin and eosin (H and E) stain as described by Bancraft *et al.* (1990) [7] with few modifications. According to them after 50% alcohol, tissue sections were dipped in water for 1-2 minutes and then placed in haematoxylin for 4 minutes followed by washing in water, then they were dipped in acid alcohol twice, thereafter placed in eosin yellow for 3 minutes. After that slides were dehydrated by using different alcohol concentration from 50% to 100% for 1 minute each. Clearing of slides was done by treating them with xylene for 1-2 minutes twice. The slides were mounted with distrene plasticizer xylene (DPX) and allowed to dry before examination under oil immersion lens (Adamu *et al.*, 2013) [3] and histopathological changes as well as presence of parasitic stages were examined in the caecum tissue of broilers of different groups.

Results and Discussion

Histopathological findings for the various experimental groups are explained below.

(a) Group I or uninfected non supplemented group

In broilers of uninfected group, all the layers (mucosa, submucosa, muscularis and serosa) of caecum did not reveal any change. The mucosal layer was intact with its epithelium (Plate. 1).

(b) Group II or infected non supplemented group

On 5 days post infection in broilers of infected non supplemented group, caecal mucosal epithelium showed degenerative and necrotic change. Infiltration of mononuclear cells was present. There were haemorrhages in caecum and in between caecal mucosal glands which were enlarged. Second generation schizonts and merozoites were present in large number (Plate. 2 and 3). There was also deposition of fibrinous exudate containing fibrin network and inflammatory cells. The microscopic findings were in accordance with that of Manafi (2011) [21] who observed numerous intracellular schizonts of *Eimeria tenella* liberating merozoites and severe haemorrhages along with extensive sloughing of mucosa on 5 DPI. You (2014) [27] also observed that on 120 hours post infection/5 DPI, intracellular schizonts containing merozoites and severe inflammatory cells were observed in lamina propria and among crypts on histopathological examination of *Eimeria tenella* infected caeca. Kadhim (2014) [18] also noticed disappeared morphology of crypt cells of *Eimeria tenella* infected caeca on 5 days post infection due to invasion of them by schizonts. Olabode *et al.* (2020) [23] also found loss of epithelial tissue, severe mucosal oedema and haemorrhage in caecal form of coccidiosis.

On 10 days post infection in broilers of control infected group, caecal epithelium showed release of numerous *Eimeria* oocysts in the lumen and macrogametes throughout the caecum and there was infiltration of mononuclear cells in the mucosa and submucosa (Plate. 4). Similar to our study, Adamu *et al*. (2013) [3] and Zulpo *et al.* (2007) [29] also noticed inflammatory infiltrate and foci of discrete haemorrhages associated with various intralesional form of parasite.

On 15 days post infection in broilers of control infected group, caecal epithelium showed few coccidial oocysts in the caecum and there was regeneration of caecal epithelium and proliferation of fibrous tissue. Catarrhal inflammation along with few goblet cells were also seen and there was infiltration of mononuclear cells. (Plate. 5)

(c) Group III or Infected 0.0125% amprolium supplemented group

On 5 days post infection in broilers of infected amprolium supplemented group, caecum showed no significant change in architecture of the mucosal epithelium, but catarrhal inflammation and very few coccidial stages were present. There was mild infiltration of mononuclear cells along with few RBCs (Plate. 6). Other study by Amer *et al.* (2010) [6] also observed that in caecal coccidiosis amprolium supplemented groups showed few parasitic stages on 5 days post infection. Its mode of action is that it affects the second generation schizonts in the life cycle of *Eimeria sp.* due to its thiamine antagonist property (Bozkurt *et al.*, 2013) [10].

On 10 days post infection in broilers of infected amprolium supplemented group, caecum showed the presence of normal architecture of the mucosal epithelium and catarrhal inflammation. There was infiltration of mononuclear cells along with few RBCs. Coccidial stages were absent in the caecum. (Plate. 7)

On 15 days post infection in broilers of infected amprolium supplemented group, caecum showed the presence of normal architecture of the mucosal epithelium and few goblet cells at places. There was infiltration of mononuclear cells and coccidial stages were absent in caecum. (Plate. 8)

(d) Group IV or Infected 0.2% madar leaf powder supplemented groups

On 5 days post infection in broilers of infected 0.2% madar leaf powder supplemented group, caecum showed mild degenerative and necrotic changes in the mucosal epithelium with the presence of few coccidial stages and infiltration of mononuclear cells in the mucosa and submucosa (Plate. 9). The protection from lesions in madar leaf powder supplemented groups may be due to its saponin, phenols and flavonoids contents (Joshi *et al.*, 2009; Zaman *et al.*, 2011) [17, 28]. Saponin act on the protozoan development by interacting with cholesterol present on the parasitic cell membrane and resulting into parasitic death (Wang *et al.*, 1998) [26]. Phenols and flavonoids present in madar leaf powder contribute to its antioxidant property and they limit *Eimeria* induced damage to the intestinal wall during pro-inflammation reaction and resulting in less damage to the gut (Bozkurt *et al.*, 2010) [10]. Anti-inflammatory and antiulcer property of madar leaf powder also contribute to its anticoccidial property which may be due to the inhibition of COX-2 pathway of prostaglandin synthesis, its procoagulant activity and also due to its direct protective effect on gastric mucosa (Kshirsagar *et al.*, 2008; Patil *et al.*, 2011) [20, 24].

On 10 days post infection in broilers of infected 0.2% madar leaf powder supplemented group, caecum did not reveal any significant structural alterations in mucosal epithelium. However, there was mild infiltration of mononuclear cells and proliferation of fibroblasts. Very few coccidial oocysts and macrogametocytes were present (Plate. 10). Deyab and Laji (2007) [13] also found mild histopathological lesions in *Curcuma longa* treated groups than infected showing effectiveness of its phenol content curcumin as anticoccidial.

On 15 days post infection in broilers of infected 0.2% madar leaf powder supplemented group, caecum showed normal architectural details of mucosal epithelium with mild infiltration of mononuclear cells and proliferation of fibroblasts at places. (Plate. 11)

(e)Group V or Infected 0.4% madar leaf powder supplemented groups

On 5 days post infection in broilers of infected 0.4% madar leaf powder supplemented group caecum showed the presence of goblet cell, few coccidial stages as schizonts and infiltration of mononuclear cells along with mild degenerative and necrotic changes in the mucosal epithelium (Plate. 12). Degenerative changes were milder than 0.2 % madar leaf powder supplemented group, showing concentration dependent anticoccidial effect. Many other researchers as Abbas *et al.* (2010) [2], Biu *et al.* (2006) [9] and Tipu *et al.* (2002) [25] also observed this concentration dependent anticoccidial effect of herbs.

On 10 days post infection in broilers of infected 0.4% madar leaf powder supplemented group, caecum did not show any abnormal architectural details of epithelium and showed presence of very few coccidial oocysts and macrogametocytes. Infiltration of mononuclear cells in mucosa and submucosa and proliferation of fibroblasts in submucosa were also seen (Plate. 13). Manafi (2011) [21] also found reduced intensity of histopathological lesions and early recovery in herbal anticoccidial treated group than control infected group.

On 15 days post infection in broilers of infected 0.4% madar leaf powder supplemented group, caecum did not show abnormal structural details of epithelium. There was infiltration of mononuclear cells in mucosa and submucosa and proliferation of fibroblasts in submucosa (Plate. 14).

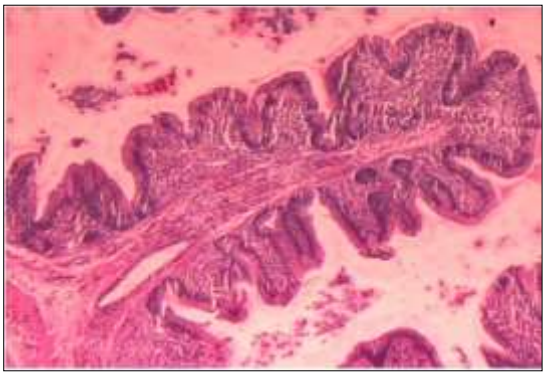

Plate 1: Photomicrograph of caecumof control uninfected groupshaving normal architectural details and intact mucosal surface [X100, H&E]

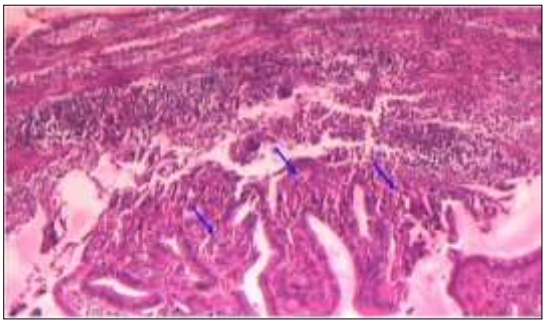

Plate 2: Photomicrograph of caecum of control infected group (5DPI) showing degenerative and necrotic changes in the mucosal epithelium, haemorrhages, schizonts (arrow) in the mucosal epithelium and infiltration of mononuclear cells in the mucosa [X100, H&E]

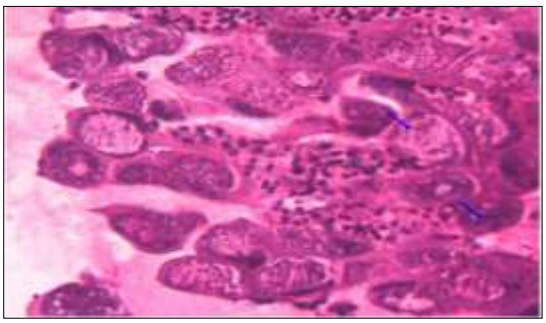

Plate 3: Photomicrograph of caecum of control infected group (5DPI) showing presence of coccidial second generation schizonts liberating merozoites (arrow) in the caecal epithelial cells and infiltration of mononuclear cells [X1000, H&E]

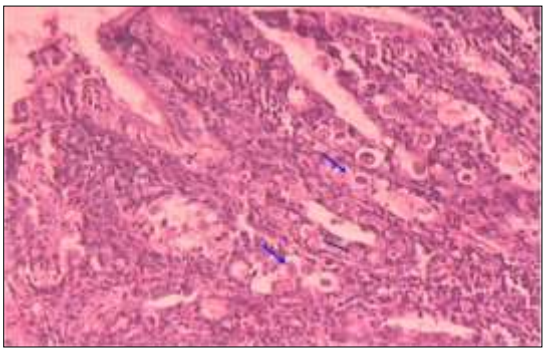

Plate 4: Photomicrograph of caecum of control infected group (10 DPI) showing presence of coccidial oocysts (arrow) and macrogametes (line) in the mucosal epithelium and infiltration of mononuclear cells in the mucosa and submucosa [X400, H&E]

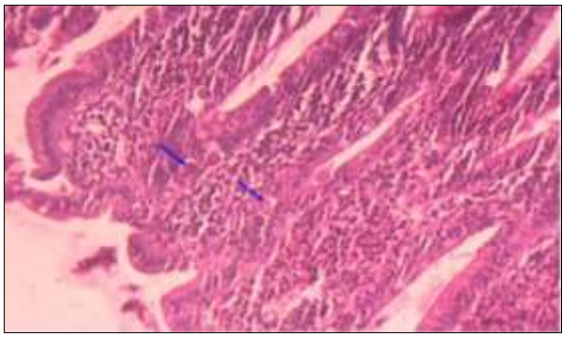

Plate 5: Photomicrograph of caecumof control infected group (15 DPI) showing the presence of few coccidial oocysts (arrow) in the mucosa and infiltration of mononuclear cells [X400, H&E]

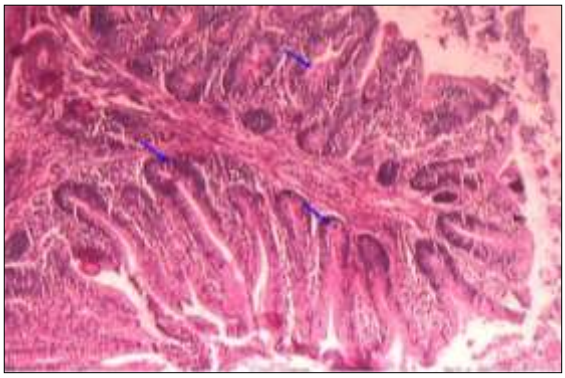

Plate 6: Photomicrograph of caecum of amprolium supplemented infected group (5 DPI) showing the presence of almost normal architecture of the mucosal epithelium, few coccidial schizonts (arrow) and infiltration of mononuclear cells [X100, H&E]

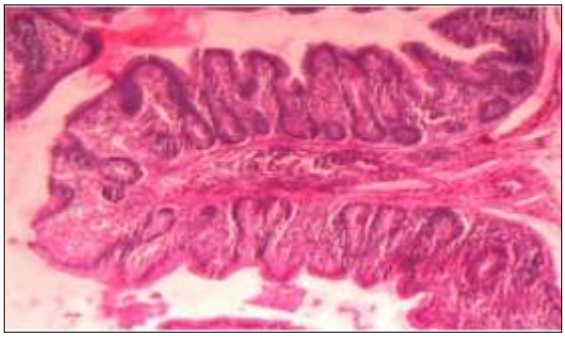

Plate 7: Photomicrograph of caecum of amprolium supplemented infected group (10 DPI) showing the presence of normal architecture of the mucosal epithelium, serofibrinous inflammation and infiltration of mononuclear cells [X100, H&E].

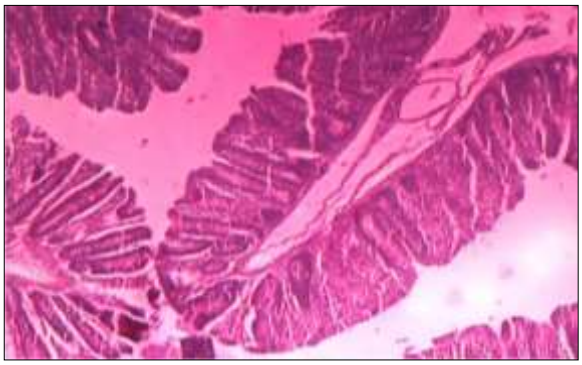

Plate 8: Photomicrograph of caecum of amprolium supplemented infected group (15 DPI) showing the presence of normal architecture of the mucosal epithelium, few goblet cells and infiltration of mononuclear cells [X100, H&E]

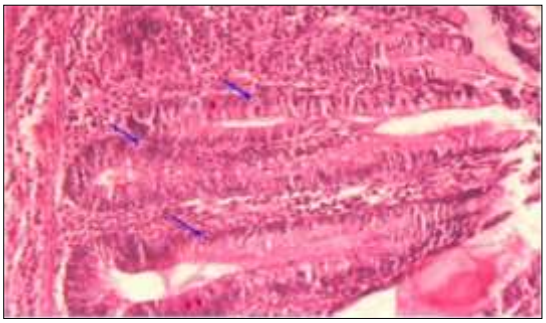

Plate 9: Photomicrograph of caecum of 0.2 % madar leaf powder supplemented infected group (5 DPI) showing mild degenerative and necrotic changes in the mucosal epithelium with the presence of few coccidial schizonts (arrow) and infiltration of mono nuclear cells in the mucosa and submucosa [X400, H&E]

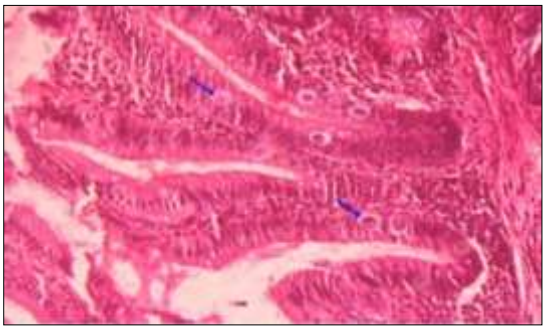

Plate 10: Photomicrograph of caecum of 0.2 % madar leaf powder supplemented infected group (10 DPI) showing normal architectural details of mucosal epithelium, presence of coccidial oocysts (arrow) and mild infiltration of mononuclear cells along with proliferation of fibroblasts [X400, H&E]

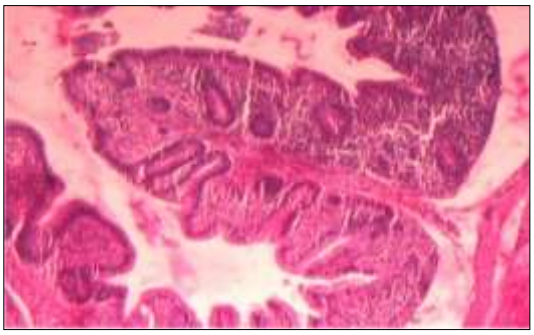

Plate 11: Photomicrograph of caecum of 0.2 % madar leaf powder supplemented infected group (15 DPI) showing normal architectural details of mucosal epithelium with mild infiltration of mononuclear cells and proliferation of fibroblasts [X100, H&E]

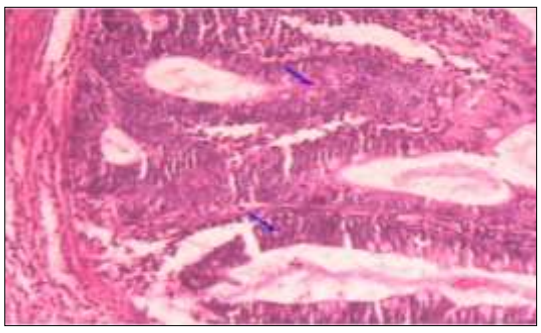

Plate 12: Photomicrograph of caecum of 0.4 % madar leaf powder supplemented infected group (5 DPI) showing the presence of few coccidial schizonts (arrow), infiltration of mononuclear cells along with mild degenerative and necrotic changes in the mucosal epithelium [X400, H&E]

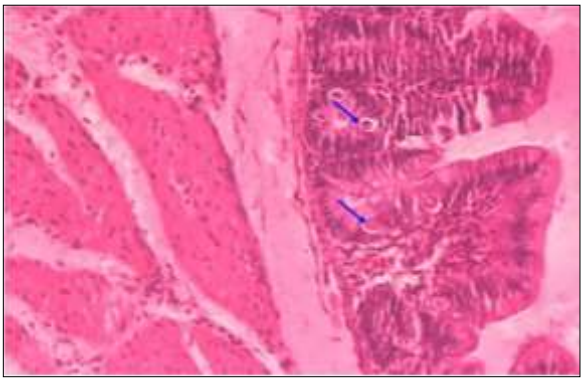

Plate 13: Photomicrograph of caecum of 0.4 % madar leaf powder supplemented infected group (10 DPI) showing normal architectural details of epithelium, presence of coccidial oocysts (arrow) infiltration of mononuclear cells in mucosa and submucosa and proliferation of fibroblasts in submucosa [X400, H&E]

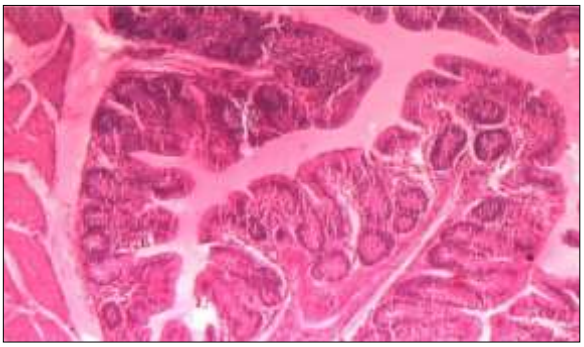

Plate 14: Photomicrograph of caecum of 0.4 % madar leaf powder supplemented infected group (15 DPI) showing normal architectural details of epithelium, infiltration of mononuclear cells in mucosa and submucosa along with proliferation of fibroblasts in submucosa [X100, H&E]

Conclusion

Histopathological changes due to coccidiosis among infected groups were minimum in amprolium supplemented group followed by 0.4% madar leaf powder supplemented and 0.2% madar leaf powder supplemented groups, whereas maximum changes were observed in infected unsupplemented group. In infected untreated group (group II), architectural details were completely lost and maximum parasitic stages as second generation schizonts, macrogametocyte and oocysts were present on 5, 10 and 15 DPI. In infected, amprolium supplemented group (group III) architectural details were almost normal and parasitic stages were absent on 10 and 15 DPI. Madar leaf powder supplemented groups showed low variations in architectural details of caeca and parasitic stages were absent on 15 DPI with restoration of normal architecture. Protection effect of madar leaf powder on histopathological changes associated with coccidia showed concentration dependent effect as higher concentration of madar leaf powder provide more protection. So, results of histopathological examination indicate that amprolium supplementation provide maximum protection against lesions produced due to caecal coccidiosis followed by 0.4% and 0.2% madar leaf powder supplementation.

From present study it was concluded that though amprolium offers best protection from *Eimeria tenella* induced histopathological changes, however madar leaf powder is also having good potential to prevent histopathological changes associated with ceacal coocidiosis.

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Conflict of interest

Authors declare that they have no conflict of interests.

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