

Effect of *Calotropis Procera* on Rabbits Coccidiosis

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Abstract

Medicinal plants being an effective source of both traditional and modern medicines. They are genuinely useful for primary health care so that the objective of the present study was to evaluate the effect of *Calotropis Procera* plant on rabbits coccidiosis. Fresh leaves of *C. Procera* were collected from Qena Province, and properly cleaned and dried. The powdered leaves were mixed with rabbit ration at ratio of 10 - 20- 50 and 100g / kg rabbit ration. A total of 36 white New-Zealand male rabbits at 8 weeks old were divided into 6 groups each of 6 rabbits. 1st group used as negative control (coccidian free) and fed on ration free of *C. Procera*, 2nd group used as positive control, naturally infested rabbits with coccidia and fed on ration free of *C. Procera*, 3rd, 4th, 5th and 6th groups naturally infested rabbits and fed on pelleted ration contained 10, 20, 50 and 100g of *C. Procera* / kg ration respectively for 15 days. Clinical signs and post-mortem lesions were recorded all over the 15 days of the experimental period. Fecal samples were collected for detection and counting of *Eimeria* oocysts. Blood samples were collected for estimation of RBCs count and Hemoglobin concentration and for biochemical parameters. Samples from livers and intestines were collected for histopathological examination at the end of the experiment. Rabbits fed on 10g/kg pelleted ration of *C. Procera* had an increase in *Eimeria* oocysts count, so it hasn't effect on *Eimeria* Sp. as well as it hasn't toxic effect. While rabbits fed on 20g/kg pelleted ration of *C. Procera* showed reduction in the number of oocysts in faeces as well as it hasn't toxic effect on hematological, biochemical parameters and biological organs so it can be used as a therapeutic agent against rabbit coccidiosis. With regarding to rabbits fed on 50 and 100g/kg pelleted ration of *C. Procera* there were an increase in the number of *Eimeria* oocysts in faeces as a result of off food of rabbits due to the bitter taste of the plant and the toxicity of these doses on rabbits so that high doses cannot be used as anticoccidial agent.

Finally, the normal values of hematological, biochemical parameters and disappearance of tissue alterations in the intestine and liver of rabbits infested with coccidian in histopathological examination after feeding of 20g/kg pelleted ration of *Calotropis Procera* suggest that this plant is safe and have a potential anticoccidial effect at this dose.

Key words: *Calotropis Procera*, rabbits, Coccidiosis.

Introduction

In recent years, there has been increasing commercial production of rabbits as a source of protein. The consumers prefer rabbits for their low cholesterol and fat contents (Al-Husseini, 1992) therefore; rabbit production became one of the most important animal resources in Egypt. In addition to this commercial value, these animals are used as very important models for medical research and as pets (Yousif, and Abdul-Aziz, 1995). Coccidiosis are a common and worldwide protozoal disease of rabbits, it causes significant mortality in domestic rabbit. Rabbits that recover frequently become carriers. There are 2 anatomic forms: hepatic, caused by *Eimeria stiedae*, and intestinal, caused by *E magna*, *E irresidua*, *E media*, *E perforans*, *E flavescens*, *E intestinalis*, or other *Eimeria spp.* Transmission of both the hepatic and intestinal forms is by ingestion of the sporulated oocysts, usually in contaminated feed or water. (El-Akabawy, et al., 2004).

Anticoccidial activity of the studied *Calotropis Procera* on broiler chickens suggests its use as an alternative anticoccidial agent to chemotherapeutic drugs for *Eimeria* species control (Zaman et al, 2012).

Calotropis Procera belongs to the family of Asclepiadaceae locally known as ushar. Commonly grows in various parts of Egypt and other countries. A soft wooded, evergreen perennial shrub having few stems, few branches and relatively few leaves concentrated near the growing tip. (Adams, 1995; Ahmed et al., 2005; Liogier, 1995; Sharma et al., 1997 and Howard, 1989). Cardenolides, triterpenoids, anthocyanins, alkaloids, resins and proteolytic enzymes are the main constituents of *C. Procera* latex. (Khan and Abdul Malik, 1989 and Al-Yahya et al., 1990).

C. Procera is often found growing in open habitat with little competition and it is also grows favorably in dry habitat. The giant milkweed has been found to be effective in the treatment of leprosy, fever, menorrhagia, malaria and snake bites. (Parrotta, 2001). The plant possesses potential anthelmintic, analgesic, anticancer, anticoagulant, anti-

inflammatory, antimicrobial, purgative and antipyretic properties (**Jain et al., 1996**). *C. Procera* flowers causes temporary paralysis of red stomach worm in sheep and notably reduces egg count percent of gastrointestinal nematodes in naturally infected sheep (**Iqbala et al., 2005**).

So that the study was planned to determine the effect of *C. Procera* on rabbit coccidiosis.

Materials and methods

1-Plants:

Collection and processing of plants sample, *Calotropis Procera*, was taxonomies in Department of Botany, Faculty of Science, South Valley University, Egypt. Fresh leaves were collected from Qena Province, Leaves were properly cleaned and shade dried for 5-7 days at 32-35°C and relative humidity 50 - 60%. The dried leaves were powdered mechanically using commercial electrical stainless steel blender.

2- Experimental ration:

Powdered leaves of *Calotropis Procera* were mixed with rabbit ration of 10% protein at ratio of 10 - 20- 50 and 100g / kg rabbit ration.

3- Experimental animals:

Thirty six white New-Zealand male rabbits at age of 8 weeks and weighting between 1 and 1.5 kg were obtained from the farm of the Faculty of Agriculture, South Valley University; Rabbits were examined prior to the experiment to determine the infested rabbits with coccida. Six of them were healthy and free from coccidiosis and thirty of them were naturally infested with coccidiosis. Rabbits were housed in clean separate metallic cages .They was supplied with clean water.

3-I-Experimental design:

Thirty six rabbits were divided equally into six groups each group containing six rabbits for 15 days as following:

Group (1):

It was used as negative control (coccidian free) and fed on ration free of *C. Procera*.

Group (2):

It was used as positive control, naturally infested rabbits with intestinal and hepatic coccidiosis and fed on ration free of *C. Procera*.

Group (3):

Naturally infested rabbits with intestinal and hepatic coccidiosis and fed on pelleted ration contained 10g of *C. Procera* dried leaves powder / kg ration.

Group (4):

Naturally infested rabbits with intestinal and hepatic coccidiosis and fed on pelleted ration contained 20g of *C. Procera* dried leaves powder / kg ration.

Group (5):

Naturally infested rabbits with intestinal coccidiosis and fed on pelleted ration contained 50g of *C. Procera* dried leaves powder / kg ration.

Group (6):

Naturally infested rabbits with intestinal coccidiosis and fed on pelleted ration contained 100g of *C. Procera* dried leaves powder / kg ration.

4-Sampling:

(A) Fecal sample:

Faecal samples were collected immediately after defecation of rabbits from each group at zero (before feeding), 5th, 10th and 15th days post *C. Procera* dried leaves powder feeding in a separate plastic sac, each labeled with the needed data such date of collection and any apparent lesions. These samples were homogenized through mortar and examined microscopically and the oocysts were counted, oocyst per gram of feces (OPG), using McMaster chamber according to methods described by (Charles, 1998).

A-I-Parasitological examination:

Fecal samples were examined for the presence of *Eimeria* oocysts by concentration floatation technique according to Kruse and Pritchard, (1982).

A-II- Total oocyst count:

It was performed according to Charles, (1998) to achieve the intensity of coccidian infestation, as well as to evaluate the therapeutic effect of the used *Calotropis Procera* dried leaves powdered in rabbits ration.

(B)Blood sample:

Two blood samples were collected from each group on 15th day post *C. Procera* dried leaves powder feeding to the rabbits. The first sample was collected in a clean dry test tube containing ethylene diamine tetra-acetate (EDTA) as anticoagulant for hematological examination. The second sample was collected in a clean dry test tube without anticoagulant for biochemical analysis.

B-I-Hematological analysis:

The blood samples will be analyzed for Erythrocytic count (RBCs) and hemoglobin (Hb) concentration and as described by Baker et al., (1998).

B-II-Biochemical analysis:

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated by using the test kits supplied from Randox Laboratories (Cat. No. SC2643) spectrophotometrically. Both ALT and AST are expressed in U/l (Reitman and Frankel, 1957).

(C) Samples for histopathological examinations:

Rabbits were scarified on 15th day post *C. Procera* powder feeding. Post mortem examination was carried out on all scarified and dead rabbits. All the detected lesions in the parenchymatous organs were recorded. Specimens from liver and intestine were taken immediately and fixed in 10% neutral buffer formalin. After 24 hours all specimens were transferred to new neutral buffer formalin then all specimens were washed, dehydrated and embedded in paraffin blocks, sectioned at 4-5 mn and stained by H&E, examined by light microscopy (Robinson, 1977).

5- Statistical Analysis:

Statistical analysis was done using one-way analysis of variance (ANOVA). It was done to compare between control and other treated groups, followed by post-hoc analysis (Dunnett's test) using SPSS (Statistical Package for Social Sciences) version 17 according

to **Borenstein et al., (1997)**. The data were presented in form of mean \pm Standard Error (SE). The difference was considered statistically significant when $P < 0.05$.

Results and Discussion

Medicinal plants being an effective source of both traditional and modern medicines are genuinely useful for primary health care. Herbal medicinal drugs are mostly considered to be harmless (**de Lima et al., 2011**). In the present study, a total number of 36 male rabbits were used to determine the effect of *Calotropis Procera* on rabbit coccidiosis.

1-Clinical signs:

In the 1st group which were coocidia free rabbits and fed on ration free of *C. Procera*, the rabbits appeared normal with no clinical signs, but in the 2nd group which were naturally infested with coccidian and fed on ration free of *C. Procera* there were common clinical signs of coccidiosis include a reduced appetite, depression, abdominal pain indicated by continuous body curvature (fig 1), and paleness of the mucous membranes due to bloody diarrhea and these symptoms were similar to the 3rd group which fed on 10g of *C. Procera* / kg ration. While the 4th groups which fed on 20g of *C. Procera* / kg ration, there were no clinical signs of coccidiosis and the rabbits appeared normal.

With regarding to the 5th and 6th groups that fed on high dose of *C. Procera*, 50 and 100g / kg ration, respectively had the following clinical signs, excitation, hurried respiration, lateral deviation of the head, moving in circle, the rabbits became emaciated, off food likely due to the bitter taste of the plant which attributed to palatability of the ration, and inability to stand ended by death (Fig2). These symptoms referred to the pulmonary and neurotoxic effect of *C. Procera* and these results agreed with **Mbako, et al., (2009)**

2- Gross pathological finding:

The gross pathological finding of non infested group (G1) showed normal internal organs during post- mortem examination, while the gross pathological finding of the infested group (G2) were similar to the gross pathological finding in rabbits fed on 10g of *C. Procera* dried leaves powder / kg ration which showed that the liver is grossly enlarged with linear, raised with gray-white to yellow lesions in the hepatic parenchyma (Fig. 3). The intestinal tract was congested, thickened and hemorrhagic with ulcerated wall. Rabbits fed on 20g of *C. Procera* dried leaves powder / kg ration showed normal organs without any pathological lesions. While there were congestion and hemorrhage all over the internal organs (lungs, heart, intestine, spleen, liver, kidneys and testis) and edematous swelling of the brain (Fig. 4) in rabbits fed on high dose (50g and 100g) of *C. Procera* dried leaves powder / kg ration.

From the previous results we noticed that 20g / kg ration of *C. Procera* dried leaves powder during 15 days of the study did not record any mortality and clinical signs of toxicity in rabbits, and it had a good effect on *Eimeria* Sp. infestation and these results are in agreement with data observed by **Mbako, et al., (2009)**. While there were mortality, one rabbit in both G2 and G3 due to coccidial infestation, while the mortality were three and four rabbits in the G5 and G6 respectively as a result of the toxicity of these doses of *C. Procera*.

3-Oocyst count:

The oocyst count of the infested group (G2) was greatly increased all over the experiment. Also there was an increase in the number of the oocyst in 5th, 10th and 15th day post feeding of 10g of *C. Procera* / kg ration; this indicates that the dose of 10g of *C. Procera* / kg ration had no effect on the coccidia. While the mean oocyst count of G4 was 26.47 that decreased to 16.00 on 5th day and exhibited a highly significance decrease on 10th and 15th day post feeding of 20g pelleted ration of *C. Procera*. These results showed that 20g of *C. Procera* pelleted ration / kg ration had anticoccidial effect. Our results confirmed by **Mahmoud et al., (2001)** who had found that oocysts production suppressed 4 days post- treatment and feces were completely free from coccidia oocysts between 7 and 17 days after treatment with single oral dose of 0.02 ml / kg body weight of *C. Procera* latex. On the other hand the mean oocyst count of G5 and G6 were 19.00 and 23.60 that decrease

to 14. 20 and 18.8 respectively on 5th day, while oocyst count exhibited a highly significant increase on 15th post feeding of 50g and 100g of *C. Procera* pelleted ration as shown in table (1).

4-Hematological results:

The hematopoietic system is one of the most sensitive targets for toxic chemicals and an important index of physiological and pathological status in human and animal.

In the present study the hematological results revealed a significant decrease in the RBCs count and in Hemoglobin concentration (Hb) among G2 when compared with the –ve control group (G1). Similar finding were strongly supported by **Dakshinkar and Dharmadhikari, (1985)** who reported that rabbits infested with *Eimeria* spp. were anemic with lower values of RBCs count and Hb concentration. While low dose of 10g /kg pelleted ration of *C. Procera* dried leaved powder revealed a highly significant decrease ($P<0.05$) of

RBCs count and Hb concentration due to intensive bloody diarrhea caused by *Eimeria* infestation leading to anemia and this dose has no effect on coccidiosis treatment, but there were improvements in hematological parameter as an increase in RBCs count and Hb concentration in rabbits fed on 20g/kg pelleted ration of *C. Procera* dried leaved powder suggested that the plant has no effect on hematological parameters as they do not affect the circulating blood cells or their production. Our results were similar to **Dada et al., (2002); El-Shafey et al. (2011) and Guy et al., (2011)** who found that the daily oral administration of latex of *C. Procera* on rat during 7 and 14 days has no significant effects on blood parameters.

The hematological parameters of rabbits fed on high dose (50 and 100g/kg ration) of *C. Procera* revealed a highly significant increase ($P<0.05$) of RBCs count and Hb concentration attributed to polycythemia, a condition where RBCs count, Hb% and total red cell mass are above normal levels which confirmed by **Evans, (2008)**, who stated that polycythemia may be due to generalized tissue hypoxia as shown in table (2).

5-Biochemical results (Hepatic parameters):

AST and ALT are found in cytoplasm and get releases in serum; elevation in the concentration of AST and ALT is usually an indicator of hepatic injury (**Chavda et al., 2010 and Zhang et al., 2012**).

The value of AST and ALT showed highly significance increase ($P < 0.05$) in both infested group (G2) and in rabbits administrated 10g /kg ration of *C. Procera* (G3) these results were due to infestation of rabbits with hepatic coccidiosis and this dose was very low and had no effect on rabbit coccidiosis. While the value of AST and ALT showed significance decrease ($P < 0.05$) in rabbits administrated 20g /kg ration of *C. procera* in all the experimental period when compared with infested rabbits (G2) and these results were due to the effect of this dose on hepatic coccidiosis. Also the obtained results elucidated, highly significant increases ($P < 0.05$) of AST and ALT in rabbits administered high doses of 50g and 100g/ kg pelleted ration of *C. procera* dried leaves powdered. These results are confirmed by **Jimoh and Odutuga, (2001)** who reported that, these increases may be due to damage/or dead liver cells under the toxic effects of high active principles of plant extract that cause diffusion of these enzymes from the intracellular sites. In contradiction to these results, **(Ali, 2006)** recorded decreases in ALT and AST after 15 days of treatment with *Calotropis* extract (Ushar) and these results illustrated in table (3).

6-Histopathological finding:

The intestine of rabbits infested with coccidiosis (G2) had both macrogametes and schizonts invaded the lamina propria of the intestine as well as hypertrophy of goblet cells was observed (Fig.5). Moreover, these macrogametes were invaded the intestinal crypts and were surrounded by intense lymphocytic reaction. Several developmental stages and crescent-shaped merozoites were invaded the muscular layer of the intestine.

Rabbit is the only animal that suffers from hepatic form of coccidiosis leading to severe economic losses. The histopathological changes in the livers of rabbits infested with coccidiosis (G2) were manifested by features of cholangitis. These changes were expressed by hyperplastic bile duct epithelium, periductal lymphocytic infiltration, and hyperemia of blood vessels. Hyperplasia of bile duct epithelium forming more or less papillary projections into the lumen with invaded developmental stages of *Eimeria* spp. Moreover hepatocellular necrosis was observed and associated with developmental stages and several crescent-shaped merozoites, (Fig.6), these necrotic changes were expressed by cytoplasmolysis and karyorehexis.

In the present study rabbits infested with coccidia and fed on (10g/kg ration) of *C. procera* dried leaves powder and slaughtered at 15th day, their intestine and livers showed more or less similar changes of those infested with coccidiosis. These changes of the intestine were expressed by the presence of developmental stages of *Eimeria* spp. in the

enterocytes, lamina propria, and crypts as well as lymphocytic and plasma cell infiltration in the lamina propria and around the crypts (Fig. 7). While rabbit livers showed numerous bile ducts, periductal fibrosis, and periductal lymphocytic reaction (Fig. 8) and there were expressed hyperplastic bile duct epithelium into papillary folds, developmental stages, and eosinophilic infiltration. These results suggest that 10g/kg ration of *Calotropis Procera* haven't an obvious effect on the coccidial infestation in the rabbits.

In contrast, rabbit intestine infested with coccidia and fed on 20g/kg ration of *Calotropis Procera* and slaughtered at 15th day showing very few developmental stages within the enterocytes without any evidence for the protozoan in the lamina propria (Fig.9). The lymphoid follicles of Payer's patches were hypertrophic with active germinal centers. Moreover, developmental stages of coccidian entrapped within the lymphoid follicle of Payer's patches. These changes may suggested that 20g/kg ration of *Calotropis Procera* have an obvious effect on coccidian infestation via hyperactivity of Payer's patches and activation of lymphocytes and initiation of immune cascades resulted in overcome the parasitic infestation. Several studies have focused on the activation of Payer's patches and its involvement in immunity against rabbit coccidiosis (**Pakandl et al., 2008 and Reneux et al., 2003**).

Rabbit livers infested with coccidia and fed on 20g/kg ration of *Calotropis Procera* and slaughtered at 15th day showed the normal architecture liver with healthy nuclei. (Fig.10).

From the previous mentioned results we can concluded that low dose of 10g/ kg pelleted ration of *C. Procera* dried leaves powdered in rabbits ration proved that *C. Procera* is non toxic with no effect on *Eimeria* species due to an increase in the numbers of oosysts at this dose. While the dose of 20g/ kg pelleted ration of *C. Procera* dried leaves powdered in rabbits ration indicated that *C. Procera* is non toxic and a reduction of the number of oocyst in feces with disappearance of the tissue alterations in the intestines and livers suggested that this dose of plant could have a potential anticoccidial effect. On the other hand the high doses of 50 and 100g/ kg pelleted ration of *C. Procera* dried leaves powdered in rabbits ration indicated that *C. Procera* is toxic and can't be used as a therapeutic agent against coccidiosis.

Table (1): The effect of 10, 20, 50 and 100g of *C. Procera* pelleted ration /kg ration on oocyst count ($\times 10^3$) /g feces:

Oocyst count ($\times 10^3$) / g feces (OPG)					
Day	G2	G3	G4	G5	G6
0	5.80 \pm 2.61	29.00 \pm 7.1	26.47 \pm 2.78	19.00 \pm 4.81	23.60 \pm 6.64
5	10.60 \pm 3.75	35.16 \pm 9.9	16.00 \pm 3.92	14.20 \pm 5.55	18.80 \pm 5.33
10	18.00 \pm 5.89	42.74 \pm 10.2	6.800 \pm 1.03*	23.16 \pm 2.19	34.40 \pm 4.05
15	28.00 \pm 4.81	50.07 \pm 9.2	1.200 \pm 0.9*	39.60 \pm 9.01*	48.90 \pm 3.32*

Oocyst count (egg/gram)

* Significant at P<0.05 in comparison with the control

G2: Rabbit infested with coccidia (control+ve)

G3: Naturally infested rabbit with coccidia and fed on 10g/kg ration of *Calotropis Procera*.

G4: Naturally infested rabbit with coccidia and fed on 20g/kg ration of *Calotropis Procera*.

G5: Naturally infested rabbit with coccidia and fed on 50g/kg ration of *Calotropis Procera*.

G6: Naturally infested rabbit with coccidia and fed on 100g/kg ration of *Calotropis Procera*.

Table (2): The effect of 10, 20, 50 and 100g of *C. Procera* pelleted ration /kg ration on RBCs count and Hb concentration

Groups	Hematological Parameter	
	RBCs (10^6 /uL)	Hb Conc. (g/dl)
G1	5.07 \pm 0.12	11.80 \pm 0.19
G2	3.70 \pm 0.06*	9.33 \pm 0.39*
G3	3.67 \pm 0.09*	10.40 \pm 0.25*
G4	4.33 \pm 0.03	12.27 \pm 1.33
G5	6.72 \pm 0.18 *	14.37 \pm 0.27 *
G6	9.06 \pm 0.25*	16.57 \pm 0.23*

G1: Non infested rabbits with coccidia (-ve control)

G2: Rabbit infested with coccidia (+ve control)

- G3: Naturally infested rabbit with coccidia and fed on 10g/kg ration of *Calotropis Procera*.
 G4: Naturally infested rabbit with coccidia and fed on 20g/kg ration of *Calotropis Procera*.
 G5: Naturally infested rabbit with coccidia and fed on 50g/kg ration of *Calotropis Procera*.
 G6: Naturally infested rabbit with coccidia and fed on 100g/kg ration of *Calotropis Procera*.

Table (3): The effect of 10, 20, 50 and 100g of *C. Procera* pelleted ration /kg ration on hepatic Parameter:

Groups	Hepatic Parameter	
	AST IU/L	ALT IU/L
G1	47.10±3.95	54.80±2.56
G2	97.00±4.62*	121.67±21.07*
G3	83.50±4.93*	101.0±19.69*
G4	78.00±3.51*	83.33±15.19*
G5	102.27±11.48*	145.67±8.02*
G6	121.33±9.73*	167.00±8.55*

- G1: Non infested rabbits with coccidia (-ve control)
 G2: Rabbit infested with coccidia (+ve control)
 G3: Naturally infested rabbit with coccidia and fed on 10g/kg ration of *Calotropis Procera*.
 G4: Naturally infested rabbit with coccidia and fed on 20g/kg ration of *Calotropis Procera*.
 G5: Naturally infested rabbit with coccidia and fed on 50g/kg ration of *Calotropis Procera*.
 G6: Naturally infested rabbit with coccidia and fed on 100g/kg ration of *Calotropis Procera*.



Fig.(1): Depression and abdominal pain indicated by continuous body curvature.

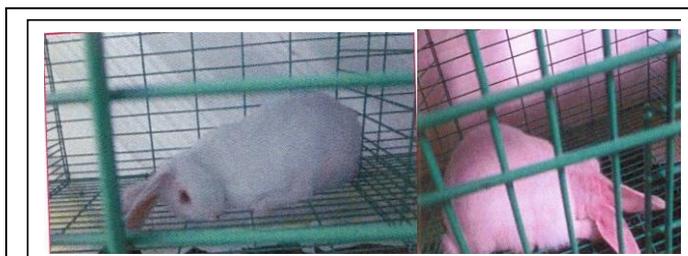


Fig. (2): Lateral deviation of the head and inability to stand ended by death.



Fig. (3): Raised, gray-white to yellow lesions in the hepatic parenchyma.



Fig. (4): Edematous swelling of the brain.

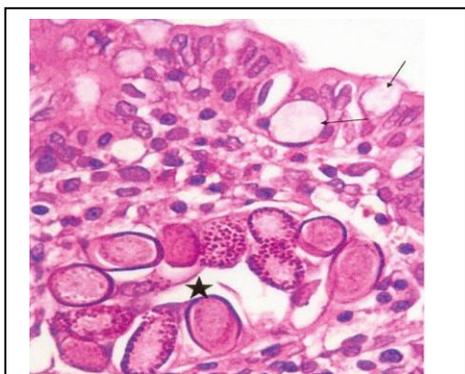


Fig. (5): Macrogametes and schizonts of *Eimeria* spp. (star) invaded the lamina propria of the intestine and hypertrophy of goblet cell (arrow). H&E. X 100 in rabbits intestine infested with coccidiosis.

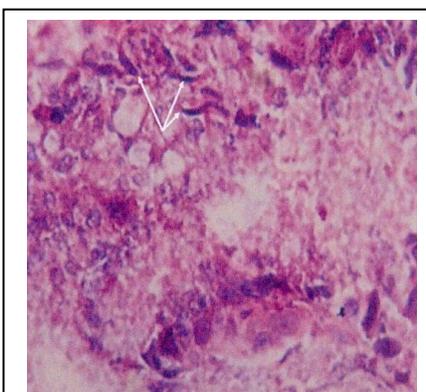


Fig. (6): Hepatocellular necrosis and invaded crescent-shaped merozoites (arrows) H&E. X400

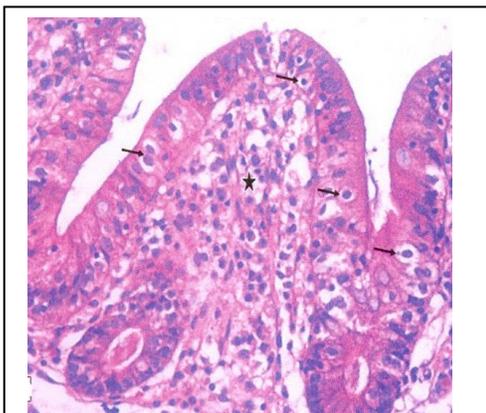


Fig. :(7) Developmental stages of *Eimeria* spp. Invading the enterocytes (arrow) lamina propria. Lymphocytic and plasma cell infiltration in the lamina propria (star) H&E. X 400 in rabbit intestine infested with coccidia and fed on 10g/kg ration of *C. Procera*.

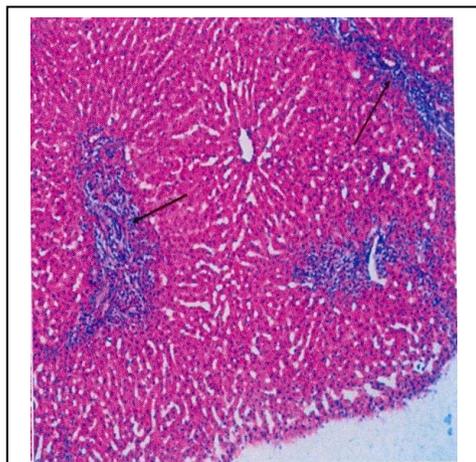


Fig. :(8) Periductal fibrosis and lymphocytic infiltration (arrow). H&E. X 100 in rabbit livers infested with coccidia and fed on 10g/kg ration of *C. Procera*.

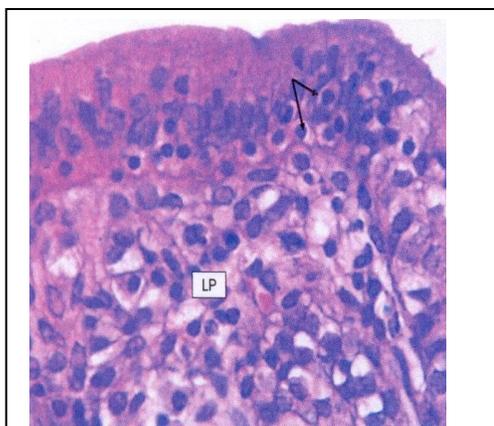


Fig. :(9) Very few developmental stages within the enterocytes (arrow). No evidence for the protozoan in the lamina propria (LP) H&E. X 400 in rabbit intestine infested with coccidia and fed on 20g/kg ration of *Calotropis Procera*.

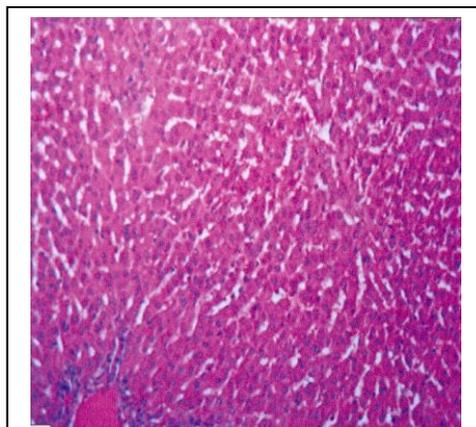


Fig. :(10) Normal architecture liver with healthy nuclei. H&E. X 100 in rabbit livers infested with coccidia and fed on 20g/kg ration of *Calotropis Procera*.

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