

قسم : الباثولوجيا .
كلية : الطب البيطرى - جامعة أسيوط .
رئيس القسم : أ. د. / محمد ابراهيم الشرى .

دراسة عن التغيرات الباثولوجية الناتجة عن تقسم الأرناب بعد تناول نبات اللانتانا كامارا

محمد يوسف ، محمود عبدالظاهر ، أحمد حافظ *

عند تناول الارانب اللانتانا كامارا الأخضر الطازج وكذلك الاوراق الجافة و خلاصة الأوراق الجافة تسبب ذلك في حدوث تغيرات باثولوجية في الكبد كانتفي المرحلة المبكرة بعد تناول النبات عبارة عن يرقان واستحالات كبدية وكذلك سقوط وموت الخلايا المبطنه للقنوات المرارية وظهور التهابات بحدارها . أما في المرحلة المتأخرة فلقد وجد تكاثر في الخلايا المبطنه للقنوات المرارية وتكونت قنوات مرارية جديدة صغيرة وفي الحجم وفي النهاية حدث تليف في بعض اجزاء الكبد .

أيضا وجدت تغيرات في الكلية عبارة عن استحالات كلوية في المرحلة المبكرة بعد تناول النبات وتليف في بعض الاجزاء في المرحلة المتأخرة .

أما في الرئة فلقد وجد تكاثر في الخلايا الموجودة بين الحويصلات الهوائية .

* قسم الأمراض الباطنة .
رئيس القسم : أ. د. / ابراهيم سكر .

Depts of Pathology and Medicine,
Faculty of Vet. Medicine, Assiut University,
Heads of Depts. Prof. Dr. M. El-Sherry and Prof. Dr. I. Sokkar.

**PATHOLOGICAL STUDIES ON THE EXPERIMENTAL INTOXICATION
OF RABBITS WITH LANTANA CAMARA**
(With 11 Figs.)

By
M.S. YOUSSEF; A.Z. MAHMOUD and A.M. HAFEZ
(Received at 16/12/1983)

SUMMARY

Lantana camara fresh and dried leaves, and lantana processed extract when administered to rabbits it produced early liver damage manifested by icterus and intensive hydropic degeneration of the hepatic cells. This was accompanied by desquamative and necrotic changes in the bile duct epithelium and inflammatory changes in their wall. A proliferative reaction of the epithelial cells lining the bile ducts was also observed. In late stages, biliary cirrhosis developed with intensive proliferation of bile duct epithelium and formation of newly formed ductules.

In the kidneys, the early lesions were mainly tubular nephrosis together with an inflammatory interstitial reaction. In late stages, the condition developed nephrosclerosis.

In the lung, interstitial proliferation of the mesenchymal elements was the main change observed in the late stages.

INTRODUCTION

Lantana camara is a poisonous plant present in tropical and subtropical areas including north and central Africa and America. Sheep and cattle are highly susceptible to the toxicity of Lantana camara. KNOTT (1955) noticed that the symptoms of lantana leaves poisoning were general illness and photosensitisation. In addition, WATT and BREYER-BRANDWIJK (1962) reported gastrointestinal disturbances. SASTRY and MAHADEVAN (1963) described extensive liver damage and death of sheep after administration of a single dose of 1.5 gm. of the total lantadenes isolated from the plant/30 kg. body weight. SEAWRIGHT (1965) obtained an extract from Lantana camara leaves which was toxic for sheep and guinea pigs at a relatively low dose rate and when given orally it causes paralysis of the musculature of the alimentary tract as well as pathological changes in the liver, kidneys, gall bladder and lung of the sheep.

Analysis of the literature showed that although a lot of work has been frequently raised concerning the early changes of lantana poisoning, the effects of prolonged administration of the plant have not yet been cleared.

The aim of this work to describe the pathological changes produced in the parenchymatous organs of the rabbit through prolonged administration of Lantana camara fresh, dried leaves and processed extract of the plant.

MATERIAL and METHODS

In the present study, the experiment was carried out on twenty male and female adult healthy Baladi rabbits ranging from 1.2 to 1.5 kg. in weight. In addition, three rabbits were

served as untreated controls. The animals were kept in a controlled climate and examined at frequent intervals before and during the experiment for parasitic infestation specially coccidia species. All the rabbits seemed to be free from infectious diseases during the experiment. In the control period (two weeks), the animals fed a dry ration. At the beginning of the experiment, the rabbits fed the control dry ration together with 10 gm. lantana camara fresh leaves daily for 7 successive days. After that, 30 gm. of lantana fresh leaves were added to the control ration for another 7 successive days, however, the animals only fed 15 gm. of the plant daily. At this time, ten animals were sacrificed, examined and samples were taken for histological examination. The rest of animals continuously fed the control ration mixed with 2 gm. of lantana processed extract for only one day. Lastly, the rabbits fed 120 gm. of lantana fresh and dried leaves, without the control ration, daily for 10 successive days. At the end of the experiment, the animals were sacrificed and exposed for post-mortem examination. Specimens from the liver, kidneys, lungs, stomach and intestine were taken for histological examination. Tissues were fixed in 10% neutral buffered formalin and Carnoy's fluid and paraffin sections were stained with Harris haematoxylin an eosin, Mallory trichrome and periodic acid Schiff reaction.

RESULT

Gross findings:

In animals which were sacrificed after 14 days, the liver was congested and increased in size. On cutting, greenish-yellow foci were found allover the parenchyma. The wall of the main bile ducts was congested and the mucosa revealed ulceration. The liver of animals which were killed at the end of the experiment also showed greenish-yellow foci, which were distributed allover the hepatic parenchyma, as well as greyish-white areas. The bile ducts became more prominent and had a thickened wall.

Kidney lesions were also observed. In the early stages, the kidneys were enlarged and congested. On cut section, greenish-yellow foci were found together with pale yellowish streaks which were extending from the cortex to the medulla. In the late stages, the kidneys were slightly increased in size and congested. On cutting, irregular greyish-white spots were found both in the cortex, specially in the subcapsular area, and medulla. Sometimes the capsule was thickened and closely adhesive to the underlying kidney tissue.

In addition to the liver and kidney lesions, the lungs in the early stages appeared oedematous and haemorrhagic, while in the late stages focal greyish-white consolidated areas were also present.

No pathological changes were recognised in the stomach. The small intestine only showed congestion in the early stages, while in the late stages the mucosa appeared thickened and folded.

Histopathological findings:

In rabbits which were sacrificed after 14 days of feeding Lantana camara leaves, examination of the liver revealed diffusely distributed hydropic degeneration (Fig. 1). In some instances, focal areas of coagulative necrosis together with pronounced hyperaemic sinuoids were also found. In few livers, an evidence of the beginning of chronic inflammatory reaction was detected. The process began with congestion and mononuclear cellular infiltration, mainly lymphocytes and macrophages, in some portal tracts (Fig. 2). In addition, few fibroblastic proliferations with collagenous fibers formation were also present specially around the bile ducts which showed early proliferation of their epithelium and infiltration of their wall with mononuclear cells (Fig.

LANTANA CAMARA INTOXICATION IN RABBIT

3). The central veins were dilated, showed adventitial proliferation and mononuclear cellular infiltration around it. The portal blood vessels were also dilated and filled with blood. Mononuclear cells were also found infiltrating among the liver cells in some hepatic lobules. Bile thrombi were observed in some bile canaliculi. In the main bile ducts, the wall was oedematous with dilatation of the lymphatics and hyperaemia of the adjacent blood vessels. Their epithelium also showed necrosis, desquamation and ulceration.

In the rabbits which were sacrificed at the end of the experiment (after 25 days), the liver revealed extensive tissue proliferation in the portal tracts which extended to encircle the hepatic lobules with isolation of small islands of hepatic tissue (Fig. 4). Inflammatory cellular infiltration, mainly lymphocytes; macrophages; fibroblasts and few fibrocytes, were also present in the portal tracts. In addition, pronounced proliferation of the portal bile duct epithelium with newly formed ducts was observed (Fig. 5). Some bile ducts showed cystic dilatation (Fig. 6A), while others were compressed and with atrophied epithelium (Fig. 6B). In the hepatic lobules, the liver cells were dissociated from each other and smaller newly formed ductules were found infiltrating among the hepatic cell cords. The hepatic lobules were either atrophied, distorted and encircled with proliferating connective tissue or may be completely disappeared and replaced by a fibrous connective tissue with numerous newly formed bile ductules (Fig. 7). The main bile ducts showed pronounced hyperplastic proliferation with necrosis and desquamation of their epithelium.

Kidney lesions were also observed in the rabbits after feeding of *Lantana camara*. In animals which were killed after 14 days, examination of the kidneys revealed focal areas of acute interstitial nephritis. The most prominent changes were severe hyperaemia in the intertubular capillaries together with lymphoid cell infiltration and periglomerular oedema (Fig. 8). In the areas of acute interstitial nephritis, some tubules were destructed, others showed hyaline casts in their lumina (Fig. 9), while the majority of them revealed vacuolar and granular degenerative changes. In addition, few limited foci of coagulative necrosis were also found. Highly refractile eosinophilic granules were observed in the cytoplasm of the epithelial cells lining of some tubules (hyaline droplet reabsorption). Bile pigments were also present in the epithelium of the tubules. The glomerular lesions in this stage were characterised by swelling of the glomerular tuft, congestion and lymphocytic infiltration. In few glomeruli, the Bowman's space was dilated and filled with a pink-stained albuminous fluid. In the more advanced stages (rabbits which were killed at the end of experiment), close examination of the kidneys revealed focal areas of chronic interstitial nephritis which were mainly located subcapsular or in the corticomedullary junction. The more prominent feature seen in this instance was the intensive cellular reaction with some fibrosis. By using of a connective tissue stain, the fibrous tissue was mainly of the collagenous type. Fibroblasts, macrophages and lymphocytes were extensively infiltrating these areas (Fig. 10). The tubular changes were characterised by cystic dilatation of some tubules and hyaline cast formation in the lumina of others. Some tubules were atrophied, compressed and totally disappeared. Concerning the glomeruli, some of them were fibrosed, while others showed cellular infiltration, congestion of the tuft and dilatation of the Bowman's space with pericapsular fibrosis. In some other glomeruli, the capillary tuft was atrophied and the Bowman's capsule was dilated. In addition, the wall of the intertubular blood vessels showed adventitial proliferation and some capillaries were thrombosed.

The lung in the early stages showed congestion of the interalveolar capillaries along with a moderate pulmonary oedema. In the late stages, there was pronounced interstitial reaction characterised by proliferation of the mesenchymal elements and thickening of the interstitial septa together with the presence of collapse and areas of compensatory emphysema (Fig. 11).

The stomach showed no microscopical changes either in the early or late stages.

The small intestine showed severe hyperaemia and signs of catarhal enteritis in the early stages, while in the late stages, the mucosal epithelium showed proliferative changes with extensive lymphoid cell infiltration both in the villi and in the submucosa.

DISCUSSION

Losses of cattle and sheep through *Lantana camara* poisoning are prevalent in many tropical countries. STEYN and VAN DER WALT (1941) and LOUW (1943) have reported toxicity in cows and sheep respectively with the plant itself or with materials derived from it. The animals were affected with general icterus and severe photosensitisation. SANDERS (1946) studied the symptoms of lantana poisoning in cattle, before and after death, and stated that small amounts of the mature, frosted and dried leaves produced typical symptoms of chronic poisoning. KNOTT (1955) added that death may occur within few days and the post-mortem findings showed generalised jaundice and a swollen liver.

In the present work, we are able to confirm that lantana camara leaves were toxic to rabbits.

Previously reported studies of experimental lantana poisoning (SEAWRIGHT, 1963) indicated that in early and mild intoxication, histopathological changes in the liver seemed insignificant compared with the degree of hepatic excretory dysfunction that was present. Studies of lantana affected sheep liver in the early stages of the condition indicated that in the peripheral parenchymal region, bile canaliculi were distended and hepatocytes appeared to be separated by a prominent wide yellowish line (SEAWRIGHT, 1965). Increased severity of intoxication in the guinea pigs (SEAWRIGHT, 1965) showed, however, that the peripheral parenchymal cells appeared to be specifically affected while the centrilobular parenchymal cells remained normal. The main microscopic changes in the liver of the guinea pigs were midzonal necrosis with degeneration of the periportal parenchymal cells and biliary ductular hyperplasia. The same author added that midzonal necrosis has not been observed in lantana poisoning of either cattle or sheep.

In the present experiment, our findings differed from those reported by SEAWRIGHT (1965). In the early stages of the condition, the affected rabbit livers showed severely diffuse vacular degeneration which was, in our opinion, the main cause of jaundice observed. This was indicated clinically by the early increasing amount of bilirubin in the blood of rabbits (EL-SAYYAD, HAFEZ, GAZIA and HASSAN, 1983). Moreover, the necrotic changes were not so pronounced and had no specific distribution. In addition, the proliferative changes in the bile duct epithelium appeared to be preceded by desquamative and necrotic changes. Together with these changes, inflammatory cellular infiltrations, mainly lymphocytes and macrophages, in the wall of portal bile ducts were observed along with oedema, dilatation of the lymphatics and vascular congestion. Extension of the inflammatory process from the portal tract into the hepatic parenchyma was also detected. Bile pigments were found both in the bile canaliculi and in the sinusoids. From these findings we concluded that the toxins of *Lantana camara* leaves may act by causing alterative changes in the bile duct epithelium (mainly desquamative and necrotic changes) and this was followed by proliferative changes and inflammatory reaction in the wall of the ducts and around it. However, the proliferative effect of the toxins must be considered as there were some bile ducts which showed proliferation of their epithelium from the beginning in the early stages.

In the late stages, extensive bile ductular hyperplasia together with a severe chronic inflammatory process (biliary cirrhosis) which also invaded the hepatic parenchyma were found.

LANTANA CAMARA INTOXICATION IN RABBIT

In our view, this was happened as a result of the direct effect of the toxins and as an indirect regenerative reaction due to the degenerative changes which occurred in the liver cells (the inflammatory process was observed in those hepatic lobules which showed extensive degenerative changes).

Concerning the kidney lesions, SEAWRIGHT (1965) stated that the least acutely affected guinea pigs and those which showed the least marked liver damage showed a greater degree of kidney damage than the most acutely affected animals. The former showed a moderate degree of vacular degeneration of the uriniferous tubules with marked hyaline cast formation and accumulations of proteinaceous fluid in the lumina. While in the kidneys of the most acutely poisoned guinea pigs, there was only a slight degree of vacular degeneration of the proximal convoluted tubules.

In the present experiment, kidney lesions in the early stages were mainly degenerative changes and hyaline cast formation together with acute interstitial nephritis. It seems probable that the primary changes were degenerative in nature and secondary followed by inflammatory changes, or the toxins may be directly initiated both degenerative and inflammatory changes at the same time. In the late stages, kidney lesions were mainly formed as a result of the toxic effect of the plant, however, the reparative and compensatory processes must not be neglected.

REFERENCES

- El-Sayyad; Samia, M.; Hafez, A.M.; Gazia, Nabila, A. and Hassan, N.K. (1983): Some liver function tests in rabbits fed Lantana camara leaves and extract. *Assiut Vet. Med. J.*, Vol. 10, 20: 133-137.
- Knott, S.G. (1955): Toxicity of Lantana camara in cattle. *Queen. Agric. J.*, 80, 200. Through *Biol. Abstr.*, 31, 4743, (1957).
- Louw, P.G.J. (1943): Toxicity of Lantana camara in sheep. *Onderstepoort J. Vet. Sci. Anim. Ind.*, 18, 197.
- Sanders, D.A. (1946): Symptoms of Lantana camara poisoning in cattle. *J. Am. Vet. Med. Ass.*, 109, 833, 139.
- Sastry, M.S. and Mahadevan, V. (1963): Clinical symptoms of Lantana camara poisoning in sheep. *Indian Vet. J.*, 40, 78.
- Seawright, A.A. (1963): Studies on the experimental intoxication of sheep with Lantana camara. *Austr. Vet. J.*, 39: 340-344.
- Seawright, A.A. (1965): An extract of lantana toxic for the guinea pig. *J. comp. path.*, 75: 215-221.
- Seawright, A.A. (1965): Electron microscopic observations of the hepatocytes of sheep in lantana poisoning. *Path. Vet.*, 2: 175-196.
- Steyn, D.G. and Van Der Walt, S.J. (1941): Intoxication of cows with Lantana camara. *Onderstepoort J. Vet. Sci. Anim. Ind.*, 16, 121.
- Watt, J.M. and Breyer-Brandwijk, M.G. (1962): The medicinal and poisonous plants of Southern and Eastern Africa. E. and S. Livingstone, Ltd., 1047.

DESCRIPTION OF THE FIGURES

- Fig. (1): Liver showing hydropic degeneration. H. & E. stain. 12.5 x 25.
- Fig. (2): Liver showing inflammatory cellular infiltration in the portal tract. H. & E. stain. 12.5 x 16.

- Fig. (3): Liver showing desquamative and regenerative changes in a portal bile duct with mononuclear cells infiltrating its wall. H. & E. stain. 12.5 x 25.
- Fig. (4): Liver showing extension of the inflammatory process from the portal tract to the hepatic parenchyma with isolation of a small island of the hepatic tissue. H. & E. stain. 12.5 x 16.
- Fig. (5): Liver showing pronounced proliferation of the portal bile duct epithelium with newly ducts formation. H. & E. stain. 12.5 x 25.
- Fig. (6): Liver showing cystically dilated portal bile duct (A) 12.5 x 16 and another duct with compressed and atrophied flattened epithelial cells (B). 12.5 x 25. H. & E. stain.
- Fig. (7): Liver showing extensive fibrosis with complete destruction of the hepatic parenchyma and numerous newly formed bile ductules. H. & E. stain. 12.5 x 16.
- Fig. (8): Kidney showing periglomerular oedema with hyaline cast formation in some tubules. H. & E. stain. 12.5 x 25.
- Fig. (9): Kidney showing acute interstitial nephritis. H. & E. stain. 12.5 x 25.
- Fig. (10): Kidney showing chronic interstitial nephritis. H. & E. stain. 12.5 x 25.
- Fig. (11): Lung showing interstitial reaction with compensatory emphysema. H. & E. stain. 12.5x16.



Fig. (1)



Fig. (2)

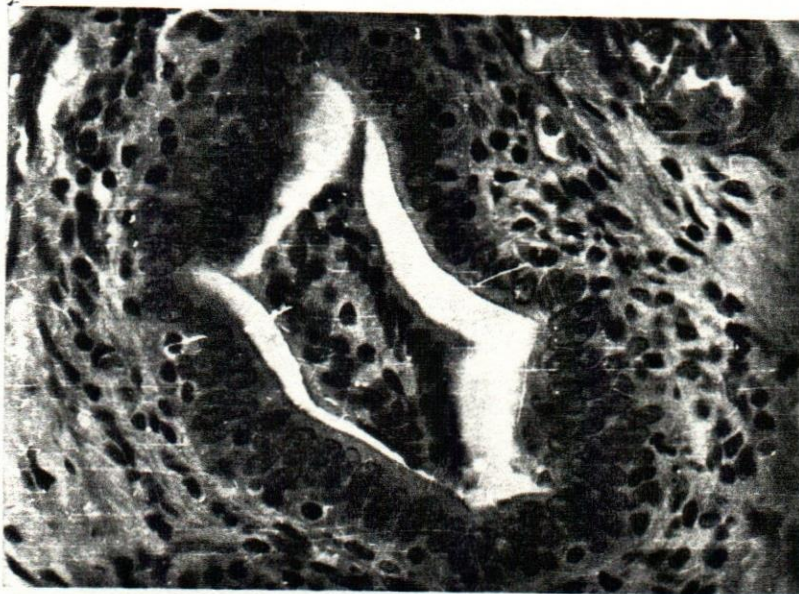


Fig. (3)

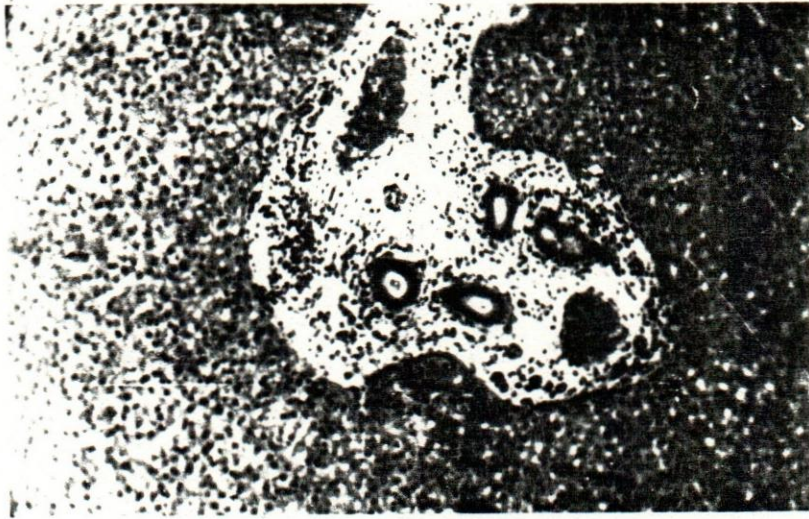


Fig. (4)

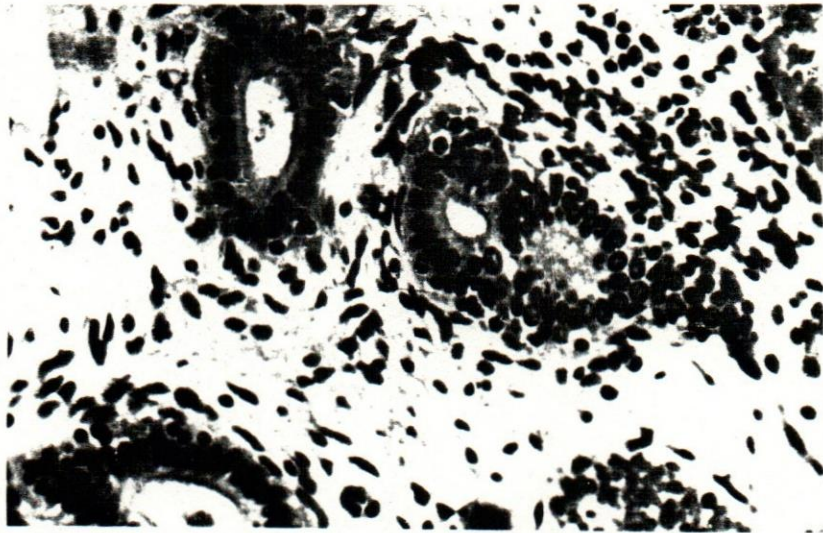


Fig. (5)

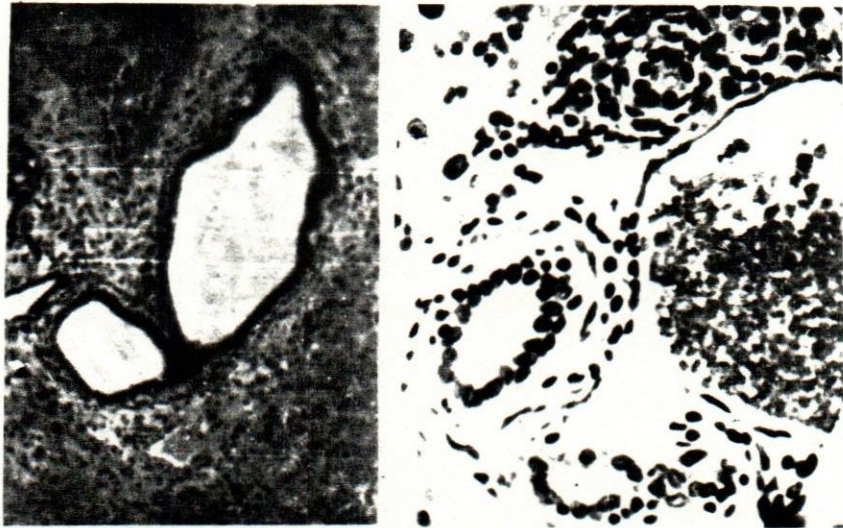


Fig. (6)

A

B

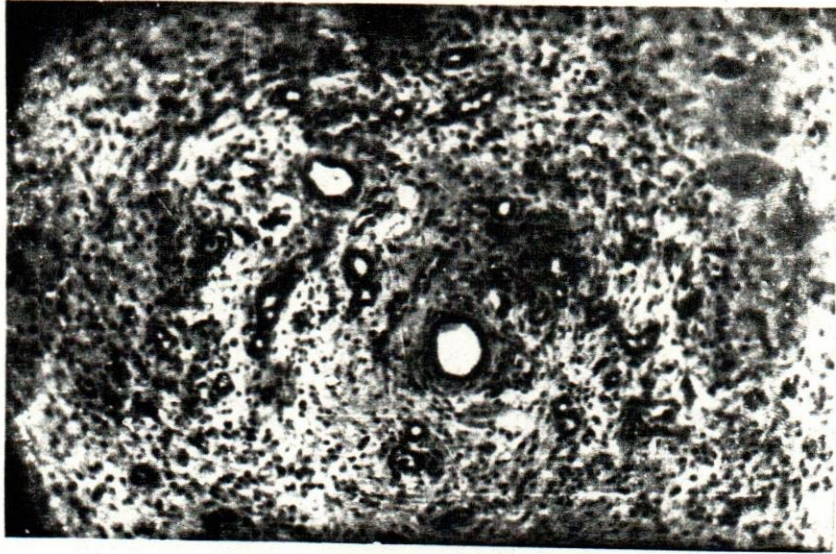


Fig. (7)

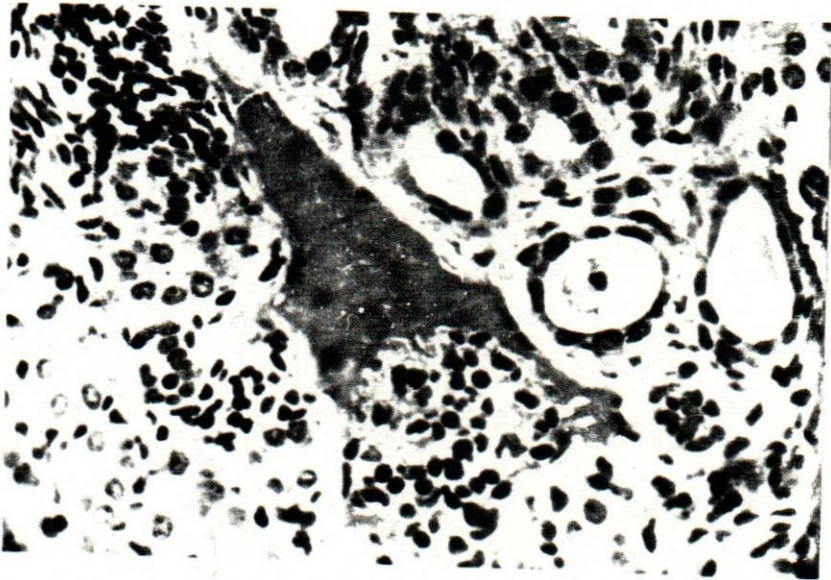


Fig. (8)



Fig. (9)

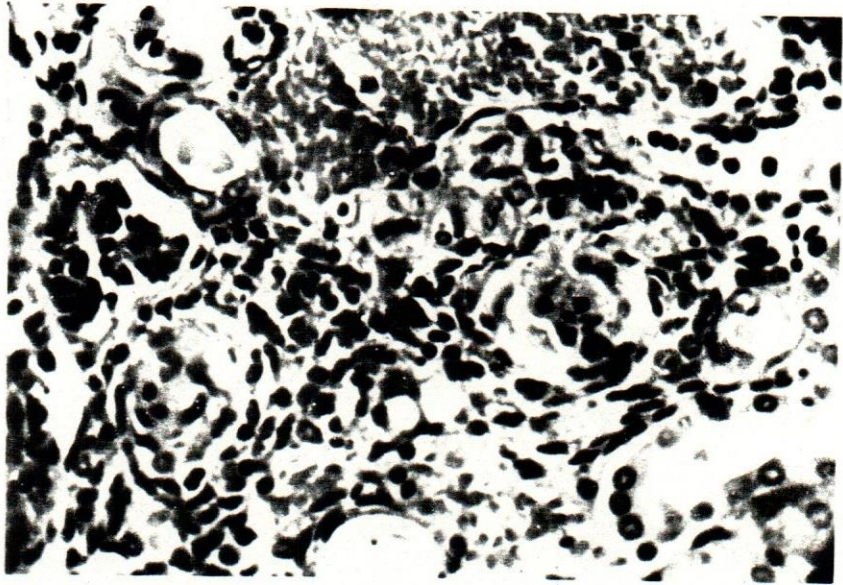


Fig. (10)

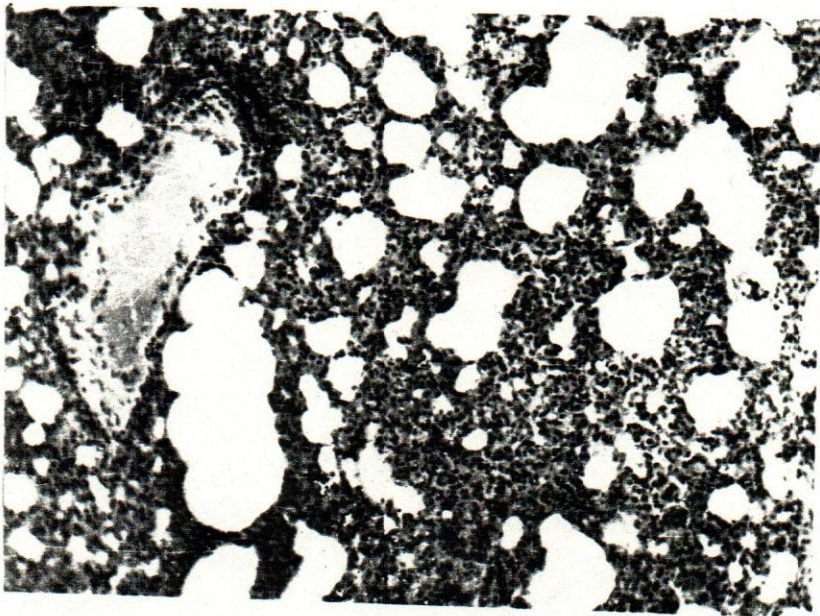


Fig. (11)

