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CLINICAL AND HISTOPATHOLOGICAL STUDIES OF ASPERGILLUS FLAVOUS IN NILE TILAPIA *(OREOCHROMIS NILOTICUS)*

(With One Table & 14 Figures)

By

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دراسات اكلينيكيه وهستوباثولوجيه على فطر الأسبرجيلس فلافس فى أسماك البلطى النيليه

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تم هذا البحث على أسماك البلطى النيلى لدراسة التأثير الاكلينيكى والهستوباثولوجى باستخدام فطر الأسبرجيلس فلافس. تم حقن ٢٠ سمكه ب ٢ر ملل من معلق الجراثيم للفطر وذلك عن طريق الغشاء البريتونى بعد عد الجراثيم فى المحلول المعلق باستخدام الهيموسيتومتر ليحتوى كل ١ ملل على مليون جرثومه. أما ضوابط التجربه فقد تم حقن ١٠ سمكات ب ٢ر ملل من الماء المقطر عن طريق الغشاء البريتونى. تم دراسة التغيرات الاكلينيكيه وتحديد نسبة لوفيات خلال فترة التجربه. وكانت نسبة الوفيات هى ٣٠٪ فى الأسبوع الأول ، ٦٥٪ فى الأسبوع الثانى ، ٩٠٪ فى الأسبوع الثالث وكذلك تم وصف الأعراض الاكلينيكيه وعمل الصفات التشريحيه المتمثله فى فقدان القشور وتحلل الزعنفه الخلفيه وغمقان الجلد وانتفاخ منطقة البطن. أما التغيرات الهستوباثولوجيه فقد تم وصفها خلال الثلاث أسابيع فى الكبد والبنكرياس والطحال والكليه الخلفيه والأمعاء. كانت من أهم التغيرات هى ظهور تورمات حبيبيه متعدده فى هذه الأعضاء فى الأسبوع الثالث من التجربه. كان هناك نشاط واضح لمركز الميلانو – مكروفاج فى التورمات الحبيبيه عند ثلاثة أسابيع وأعزى هذا النشاط الى دور هذا المركز فى التهام الجراثيم وتنشيط الجهاز المناعى.

SUMMARY

Clinical and histopathological studies were made using the spore suspension of *Asperiglus flavous* in Nile tilapia (Oreochromus niloticus). Intraperitoneal inoculation of 0.2 ml spore suspension resulted in obvious clinical signs, which manifested by darkening of the skin, loss of scales, and sloughing of fin rays. The mortality rate

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during the course of experiment was 90%. The livers, spleen, posterior-kidney, and intestine were the main organs affected, especially at three weeks post-inoculation. Multiple discrete granulomatous reaction were observed in the internal organs at three weeks post-inoculation. Activation of Melano-Macrophage centers (MMC) inside the granulomas was observed suggesting the role of these centers as a defensive mechanism in Asperigellomycosis.

Keywords: Clinical, histopatholoical, Studies, Asperigillus Flavous, Nile Tilapia

INTRODUCTION

Mycosis in fish can lead to serious problems in aquaculture with marked decrease in production. There is a major concern for many fungi, if they are primary or secondary invadors. Moreover, there is a limited knowledge for many fungal species about their pathogenicity. For example, Aspergillus Spp. Known to be pathogenic in fish only since, *1983 by OLUFEMI and ROBERT's*

Rainbow trout *(Salmo gairdneri)*, Channel catfish *(Ictalurus punctatus)*, Nile tilapia *(Oreochromis niloticus)*, and Common carp *(Cyprinus carpio)* were found to be affected with aflatoxin and developed toxic and hepatocarcinogenic syndromes *(ASHLEY et al., 1965, HALVER, 1965, JANTRAROTAI et al., 1990)*. Moreover, the immune mechanisms incolved in the pathogenesis of these fungal agents are still a fertile area of research.

The purpose of this study was to describe the clinical signs and histopathological changes in Nile tilapia *(Oreochromis niloticus)* after intraperitoneal inoculation with the spore suspension of *Aspergillus flavous* previously isolated and characterized from Nile fishes at Assiut, Egypt.

MATERIALS and METHODS

Fish:

Thirty Nile tilapia *(Oreochromis niloticus)* fish of an average size of 20-30g were caught from Ibrahamia tributary at Assiut and kept in glass aquaria containing 40L decholrinated water for two weeks.

Preparation of spore suspension:

Well identified Asperigillus flavus strain previously isolated from different organs of Nile tilapia *(Oreochromis niloticus)* was used for experimental inoculation and prepared according to *OLUFEMI et al., (1983)* using Sabouard's dextrose broth supplemented with chloramphenicol and tween 80 as a diluent for spore counting using haemocytometer. The spore suspension was adjusted to contain 10^6/1ml spores.

Experimental design:

Twinty fish were inoculated intraperitoneally with 0.2 ml spore suspension of *Asperigllus flavous* and represented the treated group. Ten fish were received 0.2 ml distilled water intraperitoneally and represented the control group. Clinical signs and post-mortem changes were recorded during the course of experiment for three weeks.

Histopathology:

Fish were killed by pithing the brain tissue at 1, 2, 3 weeks post-inoculation. The liver, spleen, posterior-kidney, intestine of both treated and control group were taken immediately, fixed in 10% formalin, embedded in paraffin, sectioned at 4-6 u, and stained with H. & E., PAS stain *(McMANUS, 1946), and ZIEHL-NEELSEN STAIN (BANCROFT and STEVENS, 1982).*

RESULTS

Intraperitoneal injection of 0.2 ml spore suspension resulted in obvious clinical signs as well as post-mortem changes. The clinical manifestations of the inoculation were characterized by lethargic behavior, darkening of the skin, loss of scales, and sloughing of the fin rays at three weeks post-inoculation (Fig. 1). Abdominal distension was developed and observed during the first week of injection (Fig. 2). The mortality rate at the end of experiment was 90% (Table 1).

Gross lesions:

Clear-bloody stained fluid in the abdominal cavity was observed. The gills were pale in color. The livers, spleen, and intestine were congested during the course of experiment.

Histopathology:

The experimental study showed obvious changes in the liver, spleen, posterior-kindney, and intestine. Fig. 3 showed the normal architecture of the liver obtained from fish injected with 0.2 ml of distilled water and represented the control group. One week post-injection, the hepatocyte had sharp well-defined vacuoles in their cytoplasm, with peripherally located nuclei (Fig. 4). The pancreatic acini had high contents of zymogen granules. In some areas, fragmented cell membranes of the hepatocyte were observed.

On the second week post-injection, the degenerative changes become more advanced and progressed to hepatocellular necrosis. The pancreatic acini around the hepatic portal veins had necrosis with loss of the zymogen gmules.

The main striking features were observed in the liver by the third week post-inocultion. Multifocal granulomas were observed in the liver parenchym. These granulomas were of variable sizes and well demarcated from the surroundings by a connective tissue capsule (Fig. 5). The granulomas had a caseous necrotic eosinophilic centers, surrounded by a zone of large, foamy epithelioid cells and connective tissue capsule (Fig. 6). Pronounced chronic inflammatory cellular reactions expressed by lymphocytes and plasma cells were observed inbetween these granulomas (Fig. 7).

The spleen had multifocal granulomas by the third week post-inoculation in comparison with the control group (Fig. 8, 9). These granulomas of the spleen had the same appearance and arrangements of cells as those described in the liver at three weeks post-inoculation, beside abundant (MMC) as well as epithelioid cells (Fig.10).

Fig. 11 showed the appearance of granulomas in the posterior-kidney at three weeks post-inoculation. Oblitera-

tion of Bowman's capsule, necrosis of the tubular epithelium of both proximal and distal convoluted tubules were observed (Fig.12).

The intestine by three weeks post-inoculation had several multifocal granulomas attached to the serosa of the intestinal wall (Fig.13), which have the same appearance as those described in the livers, spleen, and posterior-kidney. PAS stain reaction showed PAS positive spores inside the granulomas, as well as hyphae in the intestinal lumen and kidney parenchyma (Fig.14). While, Zheil-Neelson stain was negative for mycobacterium.

DISCUSSION

The clinical manifestations of the spore suspension inoculation intraperitoneally in this study, resulted in abdominal distension, loss of scales, and darkening of the skin were reported under natural and experimental infection by *OLUFEMI et al. (1983) and SALEM et al.(1989)* respectively.

The histopathological findings of this study showed different degrees of lesions during the course of experiment for three weeks. For example, the livers had fatty change by the first week, which may suggest the role of insult (spore suspension) as an irritant. The damage occurred in the pancreas, which might followed by enzyme leakage such as lipase may support this hypothesis. By the second week post-inoculation the process progressed into necrosis. While, the third week post-inoculation showed the typical reaction against mycosis.

The granulomas found in the liver, spleen, posterior-kidney, and intestine are a typical reaction to mycotic invasion and characterized by the formation of epithelioid granulation tissue *(JONES and HUNT, 1983)*. However, the granulomas observed in this study showed more or less unique arrangement, which was characterized by pronounced epithelioid cells and MMC.

MMC are widely distributed in the haemopotietic tissues of the spleen and kidney as spherical aggregates of pigment-containing cells. These centers, possess a delicate reticulin fibers and contain lymphocytes and pyroninophilic cells *(ROBERT'S 1989)*. It is also considered as a homologue for germinal centers of the lymph nodes in mammals. *LAMERS and DE HAAS, (1985)* proved the uptake and localization of *A.hydrophila* antigen in the MMC. Moreover, the fact that small lymphocytes home in the MMC suggests its active role in the immune response in fishes *(ROBERT'S, 1989)*. In this study, activation of he MMC in the periphery of the granulomas in the spleen and kidney may suggest antigen trapping had occur, which in our case is the spore suspension.

The obitleration of Bowman's capsule, the necrosis of the tubular epithelium of both proximal and distal convoluted tubules of the posterior-kidney may suggest *osmoregulatory* problem had occurred. The appearance of granulomas in conjunction with the intestinal serosa

may suggest a local reaction due to the sie of injecion. However, detection of the granulomas in the parenchymatous organs suggested systemic mycosis.

The mycologic and histopathologic observations in this study showed that *A. flavous* is a devastating pathogenic fungus which in turn, may lead to seri ous problems on fish health. Moreovei further attention should be made tc aquatic pathogenic fungi, especially i relation to the immune mechanisms i Nile fishes.

Figure Legends

Fig.1: Tilapia nilotica showed darkning of skin, loss of scales, and sloughing of fi rays after IP inoculation with A.flavus spore suspension at three weeks post inoculation.

Fig.2: Tilapia nilotica showed abdominal distension after IPinoculation with A.flavu. spore suspension at one week post-inoculation.

Fig.3: Control liver showed the hepatocytes arranged in cords with pancreatic acin around the hepatic portal vein. H & E, x 140.

Fig.4: Liver of Tilapia nilotica, one week post-inoculation had sharp well-developec vacuoles(F). H & E, x 140.

Fig.5: Liver of Tilapia nilotica, three-weeks post-inoculation showed mulifocal granulomas in the liver parenchyma. H & E, x 56.

Fig.6: Higher magnification of liver granuloma in tilapia showed the caseous eosinophilic necrotic center(c) and epithelioid cells(e). H & E, x 560.

Fig.7: Liver of Tilapia nilotica, three weeks post-inoculation showed pronouncec lymphocytic(L) and plasma cell (p) inbetween the granuolmas. H & E, x 560.

Fig.8: Control spleen of Tilapia nilotica. H & E, 56.

Fig.9: Spleen of Tilapia niloptica at three weeks post-inoculation had multifoca granulomas. H & E, x 56.

Fig.10: The splenic granulkomas showed aboundant melanomacrophage centers(c) and epithelioid cells(e). H & E, x 560.

Fig.11: Posterior kidney of Tilapia nilotica at three weeks post-inoculattion had the same appearance and arrangement of cells in granulomas of livers and spleen. H & E, x 560.

Fig.12: Posterior kidney of Tilapia nilotica, three weeks post-inoculation showed obilteration of Bowman's capsule(o) and necrosis of the tubular epithelium(n). H & E, x 560.

Fig.13: Intestine of Tilapia nilotica, three weeks post-inoculation had multifocal granulomas arising from the serosa(s). H & E, x 56.

Fig.14: PAS positive hyphae in the posterior kidney parenchyma. PAS reaction: x 560.

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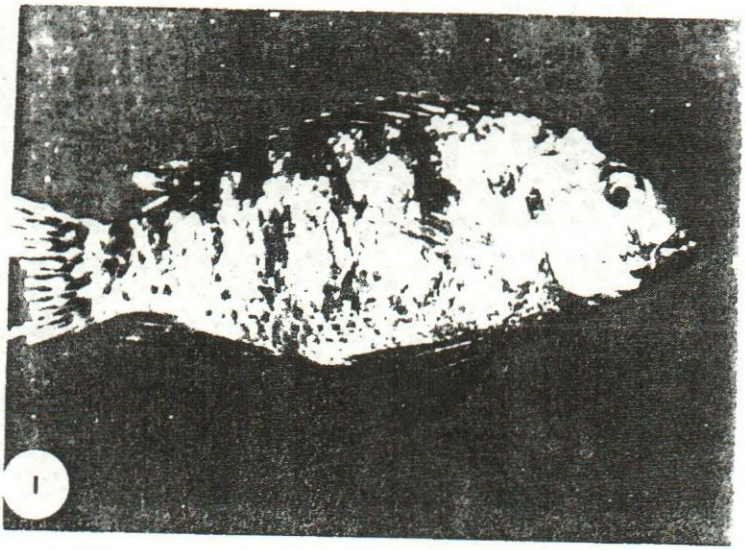
Table (1): Rate of mortalities among Tilapia fishes (*Oreochromis niloticus*) inoculated with 0.2 ml of spore suspensions of A. flavus

	1st week post inoculation		2nd week post inoculation		3rd week post inoculation		Control
	days	No. of dead fishes	days	No. of dead fishes	days	No. of dead fishes	-
	1st	-	1st	2	1st	1	-
	2nd	2	2nd	-	2nd	2	-
	3rd	-	3rd	2	3rd	-	-
	4th	1	4th	1	4th	1	-
	5th	1	5th	1	5th	1	-
	6th	2	6th	-	6th	-	-
	7th	-	7th	1	7th	-	-
Total dead fish		6		7		5	-
% of dead fish		30%		65%		90%	

* Total number of inoculated fish were 20
* 10 fishes of control were inoculated with 0.2 ml distilled water.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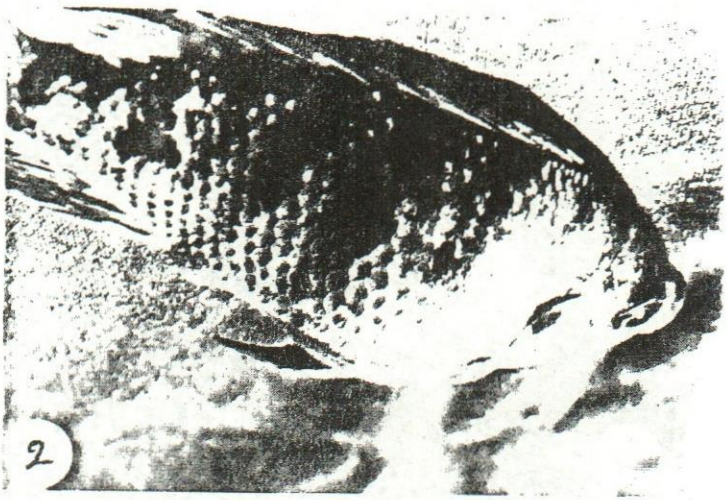
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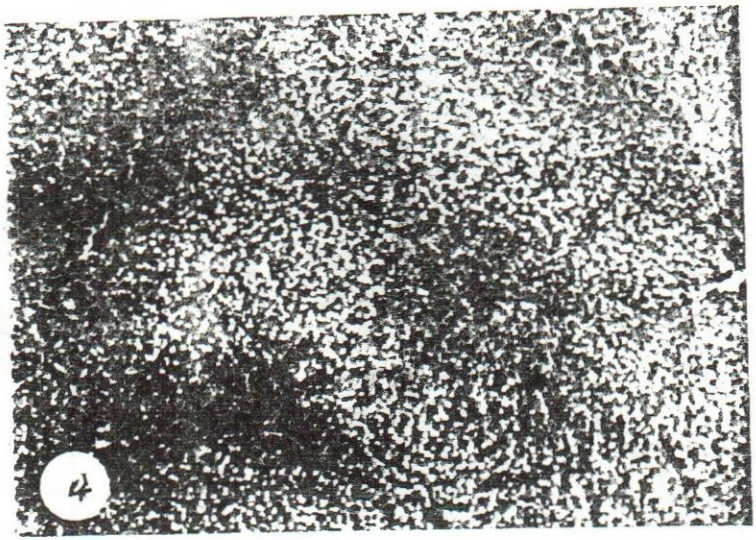
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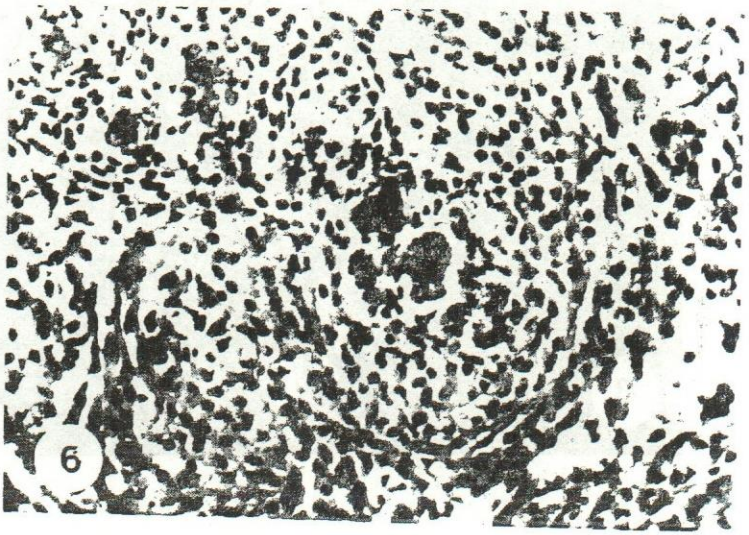
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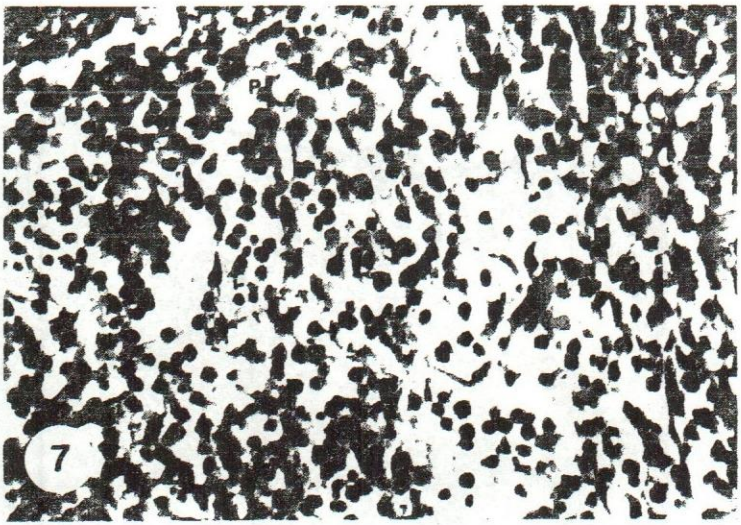
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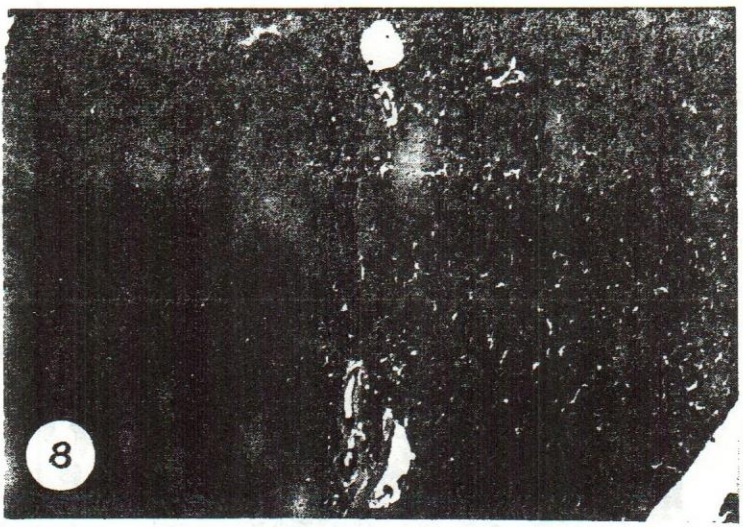
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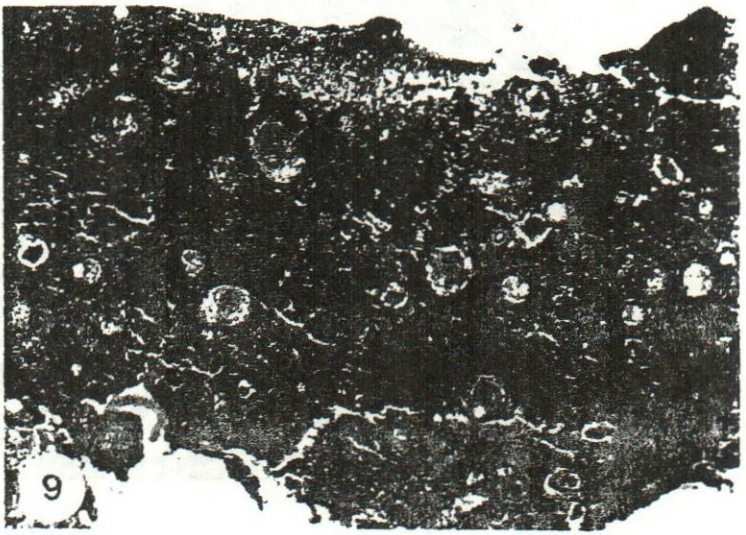
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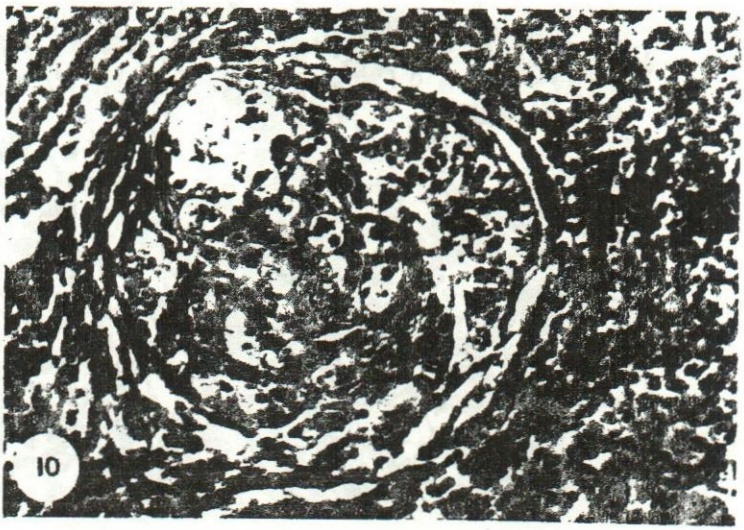
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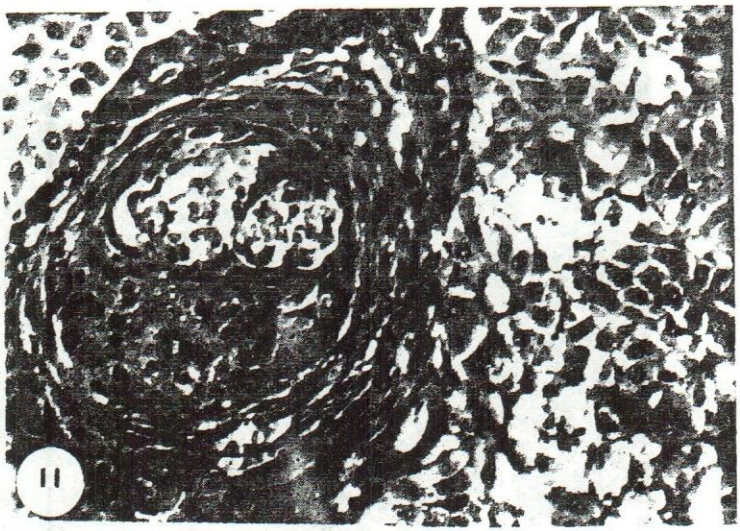
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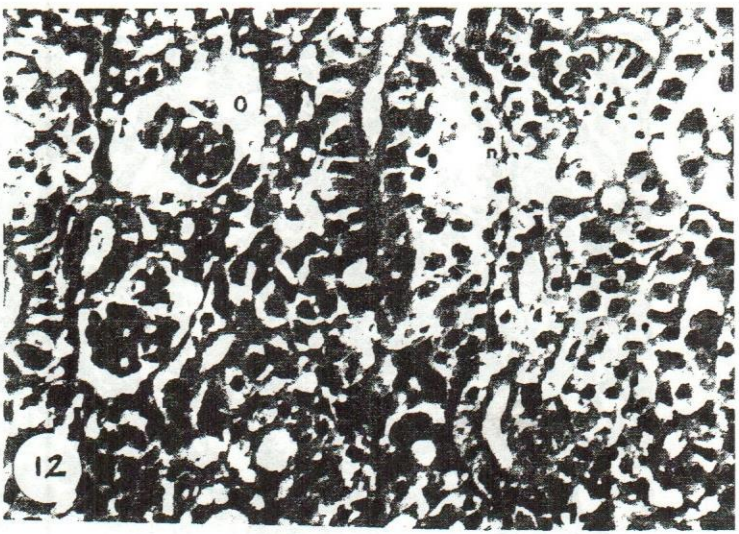
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