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HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDIES ON SELECTED ORGANS OF MALE RATS AFTER ETHANOL TREATMENT

(With 21 Figures)

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دراسات هستوباثولوجية وهستوكيميائية على بعض أعضاء الجرذان بعد معاملتها بالكحول الإثيلى

سعاد حنفي محمود ، شعبان عبدالوهاب بيومي

كان الهدف من دراسة هذا البحث هو تحديد التغيرات الهستوباثولوجية والهستوكيميائية في أمعاء وخصية الجرذان بعد معالجتها بالكحول الإثيلي. إستخدمت مجموعتان من ذكور الجرذان. عوملت المجموعة الأولى بواسطة الكحول الإثيلى بجرعة ٠,١٠ مليجرام جرام يوماً بعد يوم ولمدة شهرين. بينما إستخدمت المجموعة الثانية كمجموعة كنترول وعوملت بالمحلول الملحى فقط. أخذت عينات من كل من خصية وأمعاء الفئران المعالجة والكنترول بعد ١٠ أيام ، ٢٠ يوم ، ٣٠ يوم وشهرين ثم اجريت نتائج الفحص المجهري للدراسة الهستولوجية والسهتوكيميائية. أظهرت النتائج الأولية نقص أقطار القنيات المنتجة للحيوانات المنوية وحدوث تدمير لكل الخلايا المولدة للحيوانات المنوية وزيادة في سمك طبقة الألياف والنسيج البينى حول الأنابيب المنوية كما لوحظ تأثير ضار وإتلاف في الخلايا المعوية وإنفصال خلاياها عن الغشاء القاعدى وإنكماش وتآكل في طلائية الأمعاء.كذلك حدث نقص شديد في كمية ودرجة إصطباغ جهاز جولجى كما حدث نقص شديد في المحتوى البروتيني وحامض الفوسفاتيز القاعدي وهذا النقص يتناسب تناسبا طردياً مع زيادة مدة المعالجة.

SUMMARY

The objective of this study was to determine the histological and histochemical changes in the intestine and testis of the rats treated with ethyl alcohol. Two groups, 25 animals, of male rats were used. The first group was treated with ethanol (oral dose of o.1 ml /kg day after day) for two months. While the second group served as control and treated with saline. The results indicated that male reproductive function was sensitive to ethanol administration which varied according to the dose and duration. Also, the present study revealed mild intestinal lesions

which progressed into severe changes and have got good correlation to the administered drug.

Key words: *Histology, histochemistry, intestine, testis, ethanol, rat*

INTRODUCTION

The metabolic effects of chemicals causing disease have been studied in many organs and are usually attributable to the interaction with a specific metabolic pathway. Ethyle alcohol (ethanol) is a good example. It causes a wide range of central nerve system disorders, drowsiness, impaired mentation, liver damage, pancreatitis and testis dysfunction (under wood, 1992).

Dreisbach and Robertson (1987) reported that the pathologic findings in acute fatalities from ethanol include edema of the brain and hyperemia and edema of the gastrointestinal tract.

Many treatments employed for the chronic administration of ethanol resulted in body weight loss of the ethanol treated animals, usually (although not always) due to decrease intake of diet (Van thiel et al., 1977). Also, the authors reported that, the adverse effect of alcoholism upon male reproductive function are well documented.

Several investigators have shown changes in the reproductive tracts of male patients and laboratory animals. The most widely noted changes included diminished spermatogenesis (Anderson et al, 1980 and Willis et al., 1983).

Abou-Egla et al. (1985) founded that treatment with alcohol induce damage in hepatic cells and decrease or impairment of testis function.

The present study aimed to investigate the pathologic effect of ethanol on histologic and histochemical parameters of male rat tests in order to give answer for the relation between infertility and alcoholic metabolism and to examine the accumulated dose response relationships between ethanol and the proliferative processes of the small intestinal mucosa.

MATERIALS and METHODS

A total number of 25 adult male rats (Rattus rattus) weighing from 100 – 120 gm was obtained from the animal house of the faculty of medicine, Assiut university. The animals were arranged into two groups, twenty animals in the first group received oral dose of 0.1ml ethyl alcohol, day after day for 2 months. Five untreated control rats were used in the second group and received 0.1ml saline solution by i.p

administration. Both the control and experimental groups were caged separately and fed a standard laboratory rat diet and water adlibitium for the duration of experiment. All animals from both groups were sacrifice after 10, 20, 30 and 60 days post-treatment. Specimens were taken from the testis and small intestine and were processed as follow:

Fixation: The fixative used in this study included:

- 10% neutral formaline for general histological and histochemical methods.
- Aoyama silver nitrate for Golgi apparatus.
- Cold acetone for alkaline phosphates enzyme.

Dehydration and clearing:

Dehydration was done using ascending grades of ethyl alcohol. Then clearing was performed in xylene. In case of acetone fixation, benzene and chloroform were used for clearing.

Impregnation:

The specimens were impregenated in 3 changes of paraffin wax and then embedded in paraplast. Paraffin section were cut at 5-7U thickness and the following stains were used.

- Harris's haematoxylin and eosin for general histological examination.
- The calcium phosphate method for alkaline phosphatase enzyme. (Gomori's technique, 1952).
- The bromophenol blue method for the total protein. (Mazia et al. 1953).
- Aoyama silver nitrate method for Golgi apparatus. (Gatenby and Beams, 1950).

RESULTS

I- Clinical observations:

The average body weight of ethanol treated animals showed a marked reduction over 2 months, the period of experiment. This was in contrast to the control group which showed slight increase in average body weight.

Table 1: Comparative effect of ethyl alcohol treatment on the precent of body weight change.

Period of experiment	Control animal	Treated animal
10 days	+ 2%	- 10%
20 days	+ 20%	- 16%
30 days	+ 22%	- 20%
60 days	+ 24%	- 24%

+ represent increased precentage change. - represent decreased precentage change.

II- Histopathologic observations:

The small intestine.

The lesions which had developed during ethyl alcohol administration were restricted to the innermost mucosal epithelium layer of the intestine and no change could be detected in any of the peripheral layers at any stage.

After 10 days of alcoholic treatment, the lesions in all remained mild and were represented merely as separation of mucosal epithelium from underlying basal membrane (Fig. 1). After 20 days, the ethanol–induced lesions had become severe and were manifested as extensive fragmentation of mucosal epithelium as well as atrophy of glandular epithelium (Fig. 2). After a period of 30 days treatment, separation of the mucosal layer of the intestine along with shrinkage and focal erosion of the mucosal epithelium were detected. In addition, the detached epithelium in the intestinal lumen showed the sign of necrobiosis in the majority of animals (Fig. 3). After 60 days, the degeneration of the absorptive cells is increased and the secretory granules of goblet cells are decreased. Also a slight inflammation in the lamina propria of the mucosa was observed (Fig. 4).

The testis:

In this study, two remarkable histopathological changes were observed in the treated animal with ethyl alcohol; lack of specific spermatogenic cells and severe atrophy of the seminiferous tubules. After 10 days of treatment, the seminiferous tubules appeared atrophied compared to the control. A major abnormality was an apparent decrease in the size of the tubules followed by destruction of the spermatogenic cells. (Fig. 5). After 20 days of treatment, the tubules were devoid of spermatozoa, the cells were hypertrophied and widly separated. The surrounding tissues of the tubules show an increase in thickness and stainability (Fig. 6). After 30 days of treatment, the tubules were severely shrunken, with irregular contours. Sertoli cells and Spermatogonia are more dense and closely packed. Some tubular lumina were completely obliterated. The surrounding tissues of the tubules showed an increase in thickness and stainability (Fig. 7). After 60 days of treatment, the degenerated tubules with irregular contours were detached from the basement membrane and severely shrunken. The tubules contained no spermatogenic cells except for some spermatogonia (Figs. 19, 21).

III- Cytological observations:

The small intestine.

The Golgi bodies are demonstrated by the standard silver nitrate impregnation ($AgNO_3$) method. The Golgi zone was easily recognized as a supranuclear mass of accumulated argentophile bodies, occuping a possition between the argentophobe nucleus and the brush border.

In the treated cells, the Golgi apparatus was radomly perinuclearly distributed and often closely packed. The amount of argentophil Golgi bodies was variable in adjacent cells (Figs. 8,9).

The testis:

The Golgi apparatus is seen as a distinct argentophil cytoplasmic organelle which perinuclearly distributed and often closely packed together. In normal interphase, the nondividing nuclei are argenotophobe and appear colourless with no nuclear details (Fig. 10), while the normal dividing nuclei as well as in pathologic conditions argentophilic nucle can be distinguish (Fig. 11). In the treated animals, there was a decrease in the amount and stainability of Golgi bodies compared with those of the control.

IV- Histochemical observations

The small intestine:

Staining with mercury bromophenol blue (Hg-BpB), the total proteins are demonstrated which involves the nuclear structures and cytoplasm in the form of granules or diffuse manner.

In control rats, a highly positive reaction for Hg-BpB appeared in the basement membrane of the lining epithelium and in the connective tissue of the lamina propria. The epithelial cells showed a variable reaction in the form of fine granules in their apical parts. The secretion inside the lumen exhibited a strong positive reaction.

In all groups, treatment with ethyl alcohol caused an obvious change in the amount and distribution of the total proteins. In affected cells, there was an increase in the intensity of the positive reaction for total proteins in the basement membrane of lining epithelium and in the muscle layer of the intestine (Figs. 12, 14), while others showed a decrease in the intensity of the reaction in the apical parts of the lining cells and more in the lamina propria compared to the control (Figs. 13, 14).

Alkaline phosphatase enzyme was detected as deep brown granules of different sizes in the cytoplasm, while the nuclei gave no indication of enzyme activity. In the control, a highly positive reaction for alkaline phosphatase was observed in most connective tissue cells

present among mucosal folds. The epithelial cells showed strong diffused reaction in their apical parts with some variation. Also, the secretions inside the lumina exhibited strong positive reaction. In the treated animals, there was a general decrease in the intensity of the alkaline phosphatase reaction. This decrease was proportional to the duration of experiment and was obviously marked in the connective tissue present among the mucosal fold. The surface columnar cells covering the villi showed moderate intensity of diffused and granular form mainly in the striated border (Figs. 15, 16).

The testis:

In the control group, the peritubular tissues stained positive with mercury bromophenol blue indicating that it is rich in proteins. The stain was highly intense in Sertoli cells, spermatogonia, primary and secondary spermatocytes, taking the form of granules both in cytoplasm and nuclei. The interstitial cells appeared deeply stained due to the presence of large amounts of proteins.

In the treated animals, a general decrease in total proteins was evident after ethyl alcohol treatment, the peritubular tissues of the seminiferous tubules exhibited a moderate bromophenol staining (Fig. 17). The cytoplasm of spermatogonia and spermatocytes took a slight reaction while their nuclei were more stained. This is due to the reduction of total proteins in these cells. Spermatids as well as the Leydig cells were slightly stained. In other words, a decrease in total protein in various structures of the testis is observed in comparison to the control (Figs. 17-19).

Concerning alkaline phosphatase reaction in the control group, a positive reaction was observed in the peritubular tissues of seminiferous tubules as well as in the spermatids and interstitial cells. The nuclei of spermatogonia gave moderate reaction, where the spermatocytes showed a slight reaction for alkaline phosphatase.

In the treated animals a highly diffused reaction was observed in the peritubular tissue of seminiferous tubules as well as in the interstitial cells, while the spermatocytes exhibited a slight reaction for the enzyme.

Although there was a decrease in alkaline phosphatase activity, the concentration and hence the stainabitily of the reactive sites showed some variation. This decrease in the concentration of the enzyme was variable in the neigbouring tubules, or even in adjacent cells in the same group (Figs. 20, 21).

DISCUSSION

Presently, a greater emphasis is being undertaken to examine the chronically sublethal consequences of alcohol poisoning in animals. Quite often, the concentration of alcohol which are not quickly fatal, have been found to induce serious organal pathologies that directly interfere with vital life processes (Underwood, 1992).

The present study revealed that treatment with ethyl alcohol resulted in body weight loss of the treated animals. This observation is in agreement with Van Thiel et al. (1977). In contrast Abou-Egla et al. (1985) stated that uses of alocholic beverages caused an increase of body weight.

The present investigation have shown that ethyle alcohol was greatly deleterious to the intestine of rats even in sublethal concentration and induced significant histopathological alterations in it. However, it was interesting to note that the lesions which had developed during treatments, were restricted to the innermost mucosal epithelial layer of the intestine and no change could be detected in any of the peripheral layers at any stage. Separation of the mucosal layer of the intestine along with shrinkage and necrosis of its cells and atrophy of glandular cipthelium have been observed during lethal and sublethal ethyl poisoning.

Dreisbach and Robertson (1987) reported that the pathological findings in acute fatalities from ethanol include edema of the brain, hyperemia and edema of the gastrointestinal tract. Postmorten findings in patient dying after chronic ingestion of large amounts of ethanol include degenerative changes in the liver, kidney and brain, atrophic gastritis and cirrhosis of the liver.

Smith and Aronson (1988) reported that in moderate doses alcohol stimulates gastric acid production and enhances back-diffusion of hydrogen ions. At higher doses the gastric acid output decreases, and the gastric mucosa becomes congested and hypertaemic. There may be vomiting and in severe cases acute gastritis.

In the present study, the animals suffered from weakness, loss of appetite and decrease in body weight, temporary irritability in all body parts causing uneasiness and scratching. Also, treatment with ethyl alochol lead to the development of atrophic gastritis.

Price and Wilson (1992) reported that when alcohol is ingested in combination with asprine, the effect is more deleterious than the effect of either taken alone. Diffuse hemorrhagic erosive gastritis is known to occur with heavy alcohol and asprine use and may lead to the necessity

of resection. Also they reported that heavy alcohol, hot tea and smoking may predipose to the development of atrophic gastritis.

In this study, two remarkable histopathological changes were observed in testis of the treated animal; a lack of spermatogenic cells and severe atrophy of the seminiferous tubules. Rossi and Bestelti (1981) founded that the testis with the lowest weight had tubules with a markedly reduced diameter when spermiogenesis was blocked at spermiocyte I/II. Also, Abou-Egla et al. (1985) found that a decrease in the activity of the testis was detected after treatment with alcohol and impairement of its function after nicotine treatment.

Our results also showed that treatment with ethyl alcohol produced an alteration in the distribution and stainability of Golgi bodies in both intestine and testis of the treated rats. The cytochemical studies of Piqueras et al. (1987) suggested that prenatal exposure to alcohol alters some Golgi apparatus functions. Thus ethanol could directly affect enzyme activities of the Golgi apparatus and/or ethanol may have a direct or indirect effect on protein synthesis or on factors, such as hormones that could modulate this process.

The present study, revealed that treatment with ethyl alcohol led to a significant decrease in the amount of total proteins. This finding may give rise to retardation in spermatogenesis. Abou-Egla et al. (1985) reported that serum luteinizing hormone, follicle stimulating hormone, prolactin and testoterone were lowered in male rats fed low protein diet. Also, they reported that treatment with either nicotine or alcoholic beverage, led to a significant decrease in albumin and globulin.

Alkaline phosphatase enzyme was detected in the connective tissue present among the mucosal folds and the surface columnar cells covering the villi of the small intestine. Also it was only detected in the basement membrane of the seminiferous tubules and with moderate reaction in the nuclei of spermatogonia and spermatocytes. According to Ahmed (1988), alkaline phosphatase plays a role in spematogenesis, intermediate carbohydrate metabolism and synthesis of testicular hormone. The importance of the enzyme for sex hormone synthesis was supported by Chowdhury and Mukherjea (1976). The localization of lakaline phosphatase in the wall of the seminiferous tubules play a role in the active transport of phosphate group (Tice and Barnette, 1963).

A moderate activity of the enzyme in the nuclei of spermatocytes and spermatogonia have been described by Manicini et al. (1952). They suggested that the enzyme activity might be necessary for the formation of nucleoprotein during the process of growth and reproduction.

Finally, treated rat with ethyl alcohol was found to alter Golgi apparatus and enzymatic activity resulting in disturbance of protein synthesis. Moreover intestinal lesions and decrease or impairment of testis function was also present.

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FIGURES

Fig. 1: Section of 10 days treated rat intestine, howing separation of mucosal layers from underlying basal membrane. H&E (x 400).

Fig. 2: Section of 20 days treated rat intestine, showing atrophy of the glandular epithelium. H&E (x 400).

Fig. 3: Section of 30 days treated rat intestine, showing the sign of necrosis. H&E (x 400).

Fig. 4: Section of 60 days treated rat intestine, showing degenerative change of absorptive cells and in lamina propria. H&E (x 400).

Fig. 5: Section of 10 days treated rat testis, showing reduction in testicular cells & seminiferous tubular diameter. H&E (x 400).

Fig. 6: Section of 20 days treated rat testis, a significant increase of interstilial tissues between the tubules. Spermatogonia & spermatocytes showing pyknosis of their nuclei. H&E (x 400).

Fig. 7: Section of 30 days treated rat testis, showing fusion of the basement memberane of the tubules which became wrinkled, A various degenerative changes of the tubules were also present. H&E (x 400).

Fig. 8: Section of 10 days treated rat intestine, showing separation of mucosal layer from basement membrane, atrophy of glandular cells and decreased in the amount & stainability of Golgi Zone. $AgNO_3$ (x 400).

Fig. 9: Section of 30 days treated rat intestine, showing errosins in mucosal epithelium, reduction in the amount & stainability of Golgi bodies. $AgNO_3$ (x 400).

Fig. 10: Section of 20 days treated rat testis, showing occasional decrease in the position and distribution of the Golgi bodies, $AgNO_3$ (x 400).

Fig. 11: Section of 30 days treated rat testis, showing argentophil pyknotic nuclei $AgNO_3$ (x 400).

Fig. 12: Section of 10 days treated rat intestine, a strong positive reaction for total proteins in the basement membrane, in mucosal cells & the secretion in the lumen. Hg-BpB (x400).

Fig. 13: Section of 20 days treated rat intestine, showing destruction of mucosal barrier & decreased in the amount of total proteins in comparison to the control. Hg-BpB (x400).

Fig. 14: Section of 30 days treated rat intestine, small erosions are common. A moderate staining for total proteins was observed in the lamina propria, muscle layer, serosa and the apical parts of the lining cells. Hg-BpB (x400).

Fig. 15: Section of 20 days treated rat intestine, the surface columnar cells covering the villi showing moderate enzymatic reaction mainly in the striated boder in diffuse & granular form. Gomori. for Alk. Ph. (x400).

Fig. 16: Section of 30 days treated rat intestine, showing decreased for Alk. Ph. reaction in the connective tissue cells among the mucosal folds. Gomori for Alk. Ph. (x400).

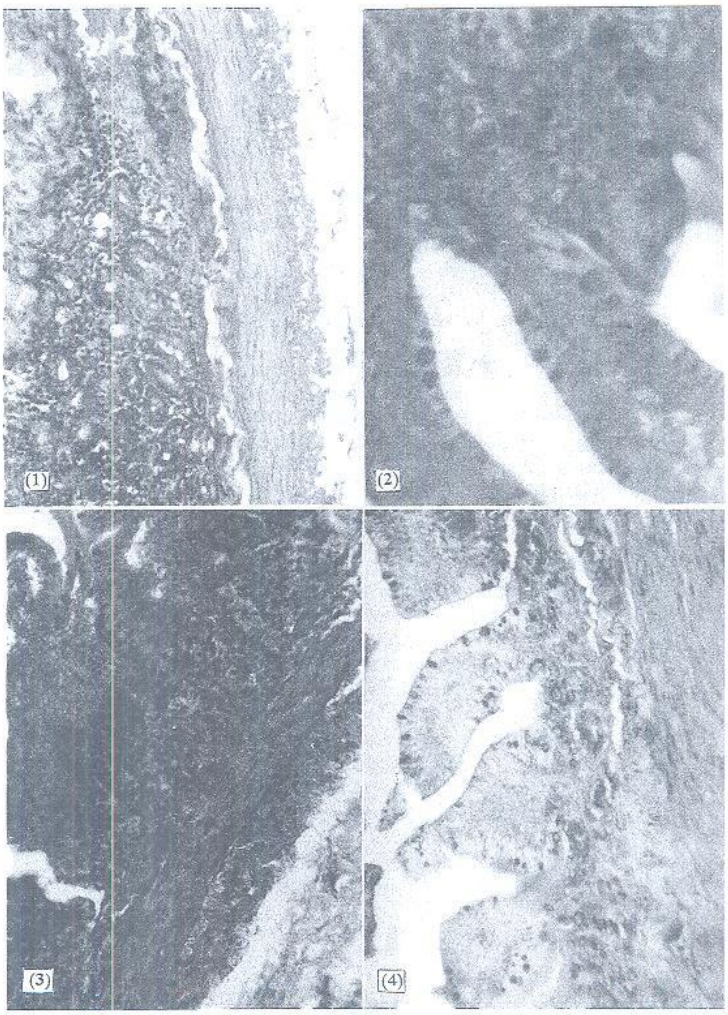
Fig. 17: Section of 10 days treated rat testis, showing an intense reaction for total proteins in the nuclear structure of the seminiferous tubules & a slight reaction in the peritubular tissue of the seminiferous tubules. Hg-BpB (x400).

Fig. 18: Section of 30 days treated rat testis, the nucleus of spermatogonia & spermatocytes took a dense stainability while the cytoplasm moderately stained. Hg-BpB (x400).

Fig. 19: Section of 60 days treated rat testis, the tubules contained no spermatogenic cells except for a few spermatogonia, others had vacuolated cytoplasm. The total proteins detected only in the nuclei of few spermatogonia. Hg-BpB (x400).

Fig. 20: Section of 10 days treated rat testis, showing an intense Alk. Ph. reaction in the basement membrane of the seminiferous tubules as well as in the interstitial cells. Gomori for alk. Ph. (x400).

Fig. 21: Section of 60 days treated rat testis, showing degenerated tubules with irregular contours detected from their basement membrance. A moderate enzymatic reaction in the wall of the seminiferous tubules and in some degenerated spermatogonia & spermatocytes. Gomori for Alk. Ph. (x400).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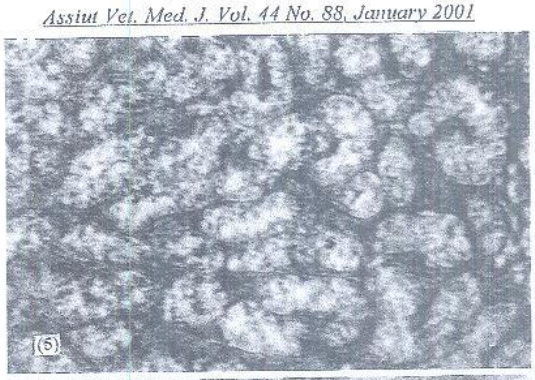

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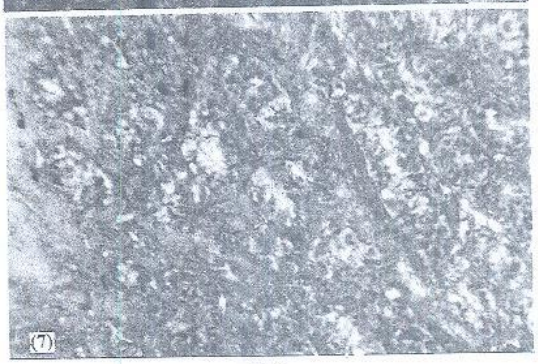

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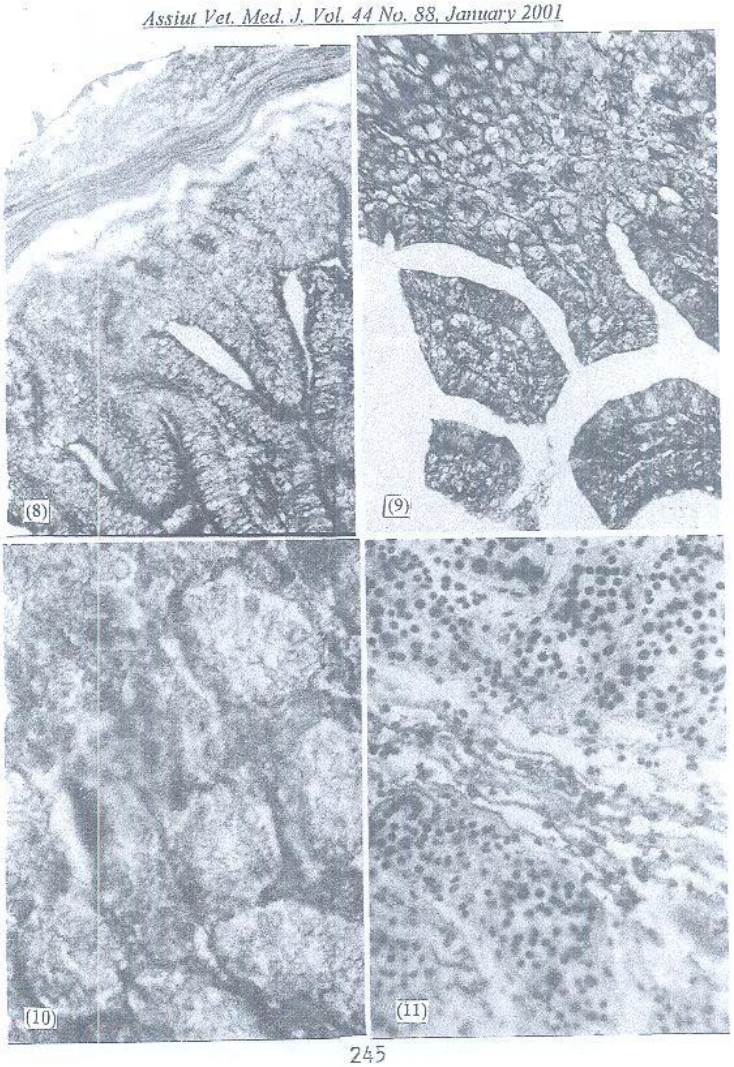
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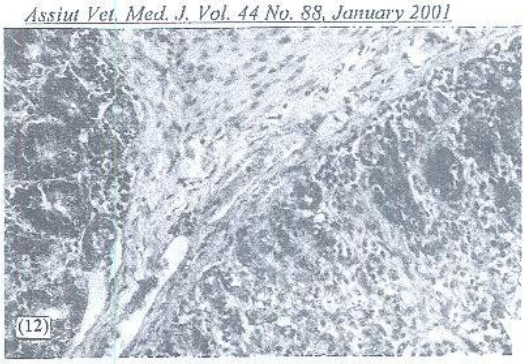

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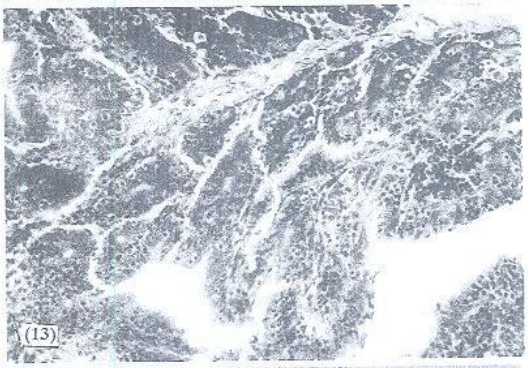

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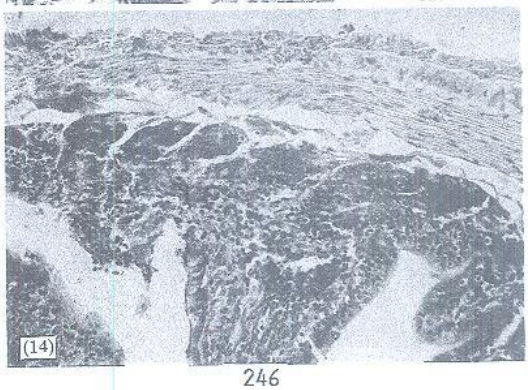

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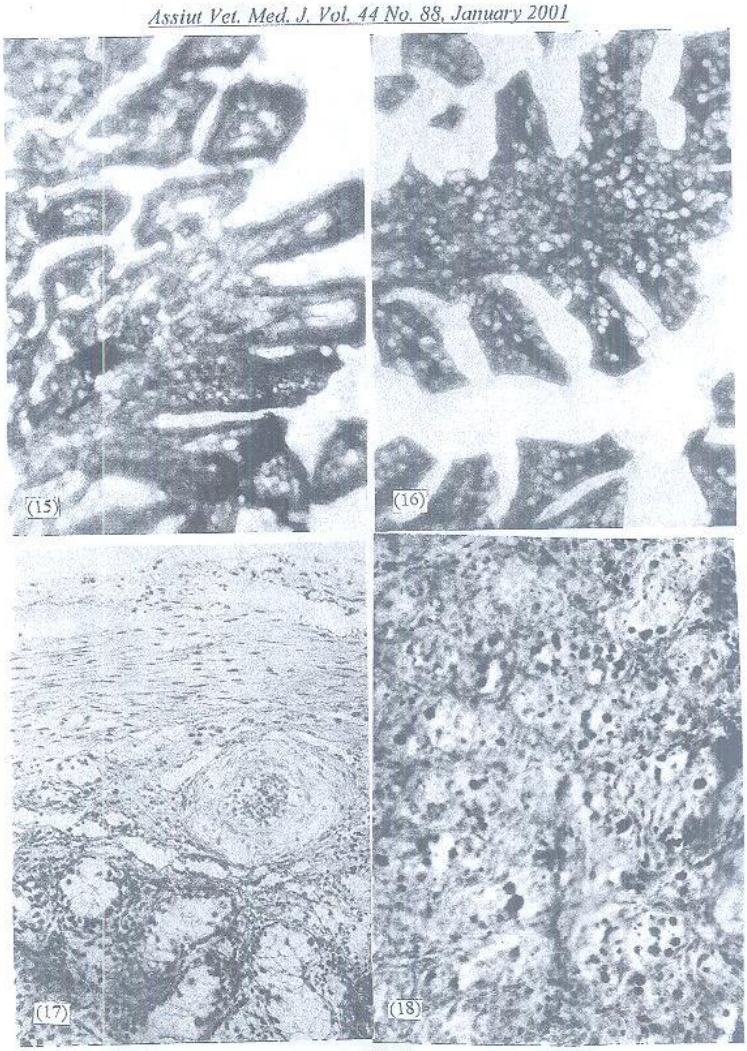
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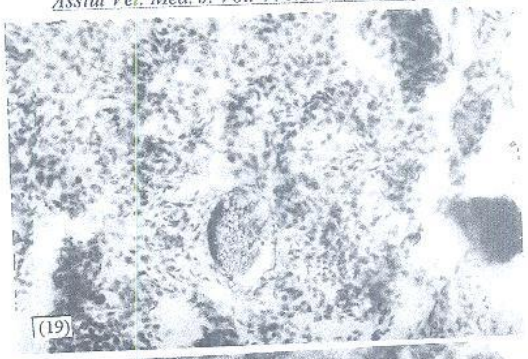

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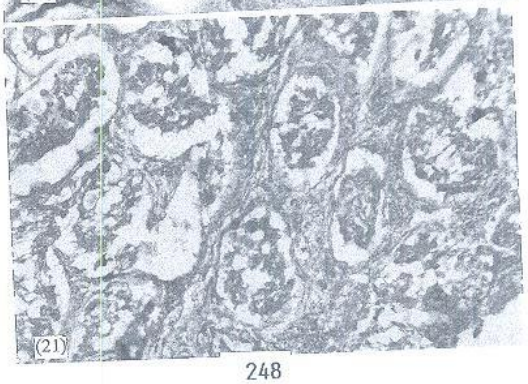

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