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A study of the Cisplatin effect on testis of Infected mice with *Echinococcus granulosus*

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Abstract:

In the present work, 25 male Balb/c mice have been used (5 mice are not infected with *Echinococcus granulosus* and untreated with cisplatin used as a control group),(10 infected with *Echinococcus granulosus* untreated mice) and (10 infected with *Echinococcus granulosus* treated with Cisplatin 100 mg/m^2 weekly for three weeks),these 20 mice considered as test group. the histopathological study of testis specimen show that the infected untreated groups have no histopathological changes in comparism to the control group, while the infected and treated group shows significant histopathological changes such as that the seminiferrous tubules destroyed completely, there is loss of many seminiferrous tubules, extensive necrosis in rete testis and the cells of the efferent duct are flat and attenuated arrangement singly or in clusters with round nuclei.

Key words: cisplatin; testis; mice & histopathology.

الخلاصة:

الدراسة الحالية شملت (25) من الفئران نوع (Balb/C) عرضت للإصابة بطفيلي *Echinococcus Granulosus* وزعت الى (5) فئران غير مصابه وغير معالجه بعقار cisplatin اعتبرت كمجموعة سيطرة (10) فئران مصابه وغير معالجة و (10) فئران مصابة عولجت ب Cisplatin بجرعة 100 ملغم / م^2 أسبوعيا ولمدة 3 أسابيع واعتبرت هذه العشرين فأرا لتمثل مجموعة الاختبار. تبين من الدراسة النسيجية المرضية لمقاطع الخصى المأخوذة من الفئران المصابه وغير معالجةعدم وجود أية تغيرات نسيجية بالمقارنه مع مجموعة السيطرة في حين أظهرت مجموعة الفئران المصابه والمعالجه تغيرات في نسيج الخصى شملت تلفا كامل للنبيبات الناقلة للمني (Seminiferrous tubules) وفقدان العديد منها, كما تم ملاحظة تنخرات واسعة في الشبكة الخصوية (Rete testis) في حين أظهرت الخلايا المكونة القنيات ألصادره (efferent ducts) بتسطحها الرفيع والمرتبة بشكل مفرد او بشكل مجاميع وذات انويه مدوره.

Introduction:

Hydatidosis is a zoonotic disease caused by dog tapeworm of the genus *Echinococcus* .it is one of the most important cestode infection of man, domestic and wild animals [1]. Several drugs have been reported to be effective in the treatment of hydatid disease [2, 3] but up to the present date surgery remained to be the most accepted method of treatment of this disease [4]. No effective chemotherapy is currently available for the medical treatment of cystic and alveolar hydiatid disease in human [5].

In the recent years in addition to several anthelmintic drugs which have shown promising results in the reduction to the larval cystic mass[6], there is a noticeable effect of the drug Tinidazole and Praziquantel on killing the protoscolices [7,8] .

Cisplatin is chemotherapeutic agent used in treatment of many cancer such as carcinoma of testis, ovary, bladder, stomach, intestine, esophagus , lymphoma, leukemia & breast [9] it has been found that cisplatin have bactericidal effect against *E.coli* [10,11] . It has inhibitory effect on DNA synthesis of *Trypanosoma rhodesisnse & T. brusei* [12,13] Aim of study : Is to discuss the effect of cisplatin on the reproductive system of male infected mice with hydatid cyst

Materials and methods:

Sample collection

The hydatid cyst was obtained from AL-Basrah abattoir. Surface of hydatid cyst were sterilized by alcohol, the protoscolices are isolated from the hydatid cyst fluid & germinal layer kept in sterile closed beakers [14].

Infection of mice:

Twenty mice of 2 months old & weight of 25-30 gm have been infected with protoscolices (1500 protoscolices per each mouse) injected through peritoneal cavity, infections occur after 2 months of injection [15], used as test group that is further divided into two groups, 10 mice were treated by cisplatin (100mg/m^2) intravenously weekly for 3 weeks (through vein of tail) the other 10 infected mice left untreated for comparism. The last 5 mice are untreated and uninfected as control group.

Histopathological study:

Specimens of testis were obtained from treated and untreated mice with cisplatin. they were fixed in buffered formalin (10%) , dehydration , clearing , embedded in paraffin wax (5-6 µl) thick section were cut and tissue sections were stained by hematoxylin & eosin[16] and examined under Compound light microscope .

Results:

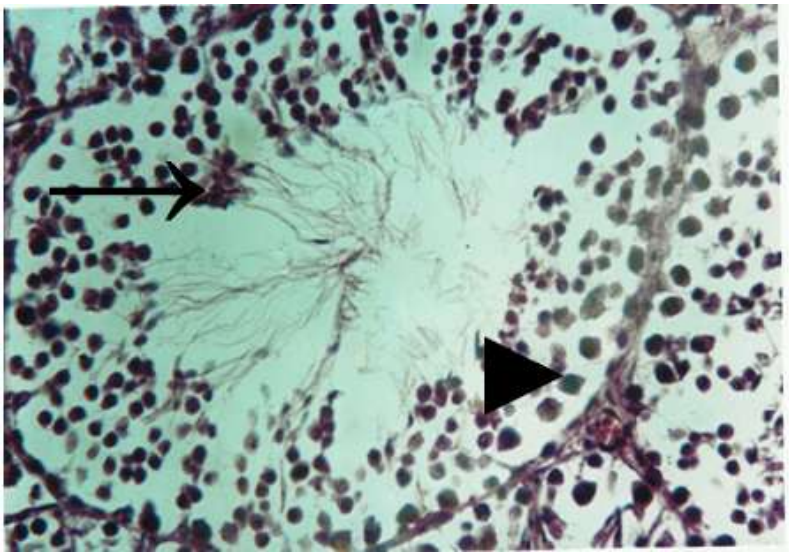
The mice of control group show the normal histological features of testis of mice as in **(Fig.1)**.the infected untreated mice did not show any pathological changes in their testis as **(Fig.2)** which shows interstitial connective tissue that supports the seminiferous tubules. , in the infected and treated group with cisplatin show a seminiferrous tubules in cross section .the cells within the lumen and the basement membrane have been completely destroyed and replaced by an ovoid collection of acidic pink- stained epithelioid cells (**Fig.3**).

In (**Fig.4**) Rete testis tubules of control group at higher magnification power appear that the duct lined with cuboidal epithelium .whereas in (**Fig.5**) Rete testis of untreated infected mice appears with same features as the control group having also cuboidal epithelium. While in **(Fig.6)** the Rete testis tubules of infected treated mice have extensive necrosis in tubules, loss of many seminiferrous tubules.

In **(Fig.7)** the efferent ducts of noninfected untreated mice shows lumen of the ducts have Wavy appearance which is normal characteristic feature with lining epithelium consisting of alternating columner and cuboidal epithelium, the ducts are embedded in dense connective tissue. in section of th e efferent ducts of untreated infected mice appear that the efferent duct are embedded in the connective tissue of the epididymis, the lumen of the ducts have a characteristic wavy

appearance, the lining epithelium is simple consisting of groups of cuboidal cells (**Fig. 8**) .

In (**Fig.9**) in the treated infected mice the cells lining the efferent ducts are seen to be very flat and attenuated, the spaces appear empty but in appropriately fixed tissue, mucinous secretions can be demonstrated within them. Clusters of cells with rounded nuclei and individual cells are present in some of the spaces, & completely deform the duct.



Fig(1) Section of testis from mice not infected with *E.granulosus* and not treated with Cisplatin shows neumerous mature spermatozoa in contact with supporting sertoli cells(⟶) , spermatogonia (►){H&E, magnification 490X }

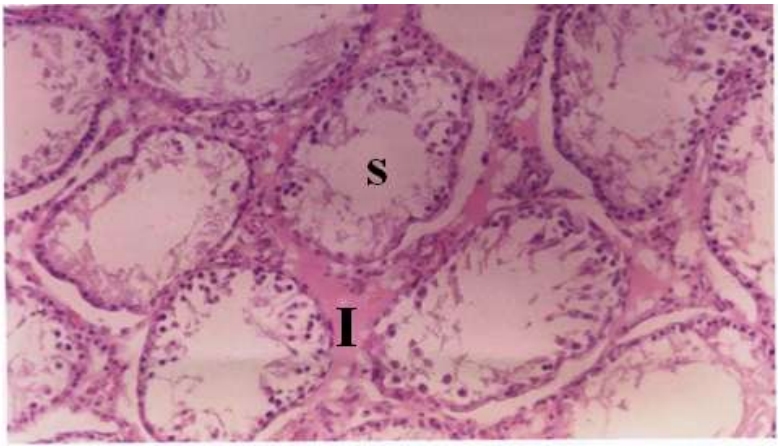


Fig (2) Section of testis of mice infected with *E. granulosus* untreated with Cisplatin After two month from infection shows interstitial connective tissue (I) and Seminiferous tubules(S) {H&E, magnification 390X }

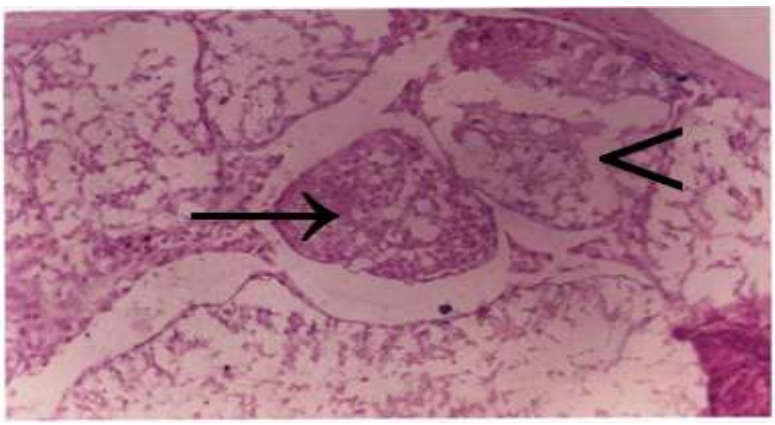
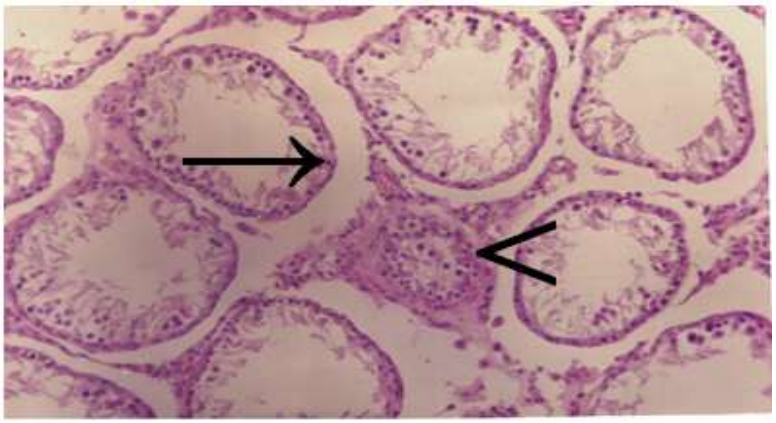


Fig (3) Cross section of testis of infected mice treated with Cisplatin for 3 weeks shows seminiferous tubules (<) is completely destroyed and replaced by an ovoid collection of pink-staining epitheloid cells(→).{ H&E, magnification 398 X}



Fig(4) Section of Rete testis tubules of non-infected untreated mice shows the lining Cuboidal epithelium of tubules(→) with surrounding connective tissue(<) { H&E, magnification 370 X}

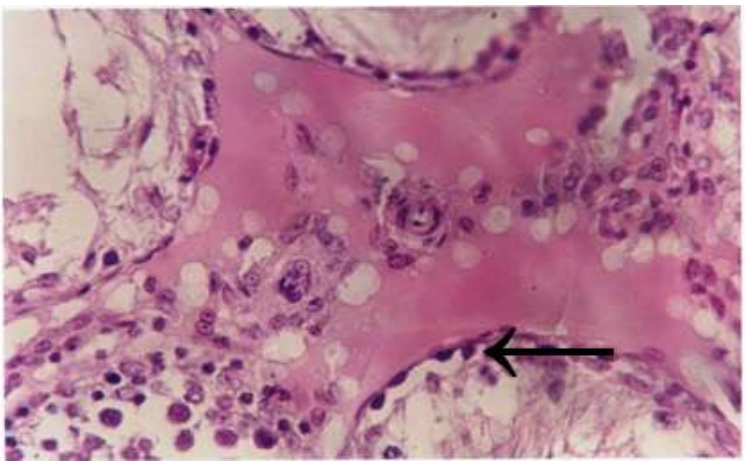


Fig (5) Section of Rete testis tubules of untreated infected mice show Cuboidal epithelium in the lumen of tubule (←). {H&E, agnification 480X }

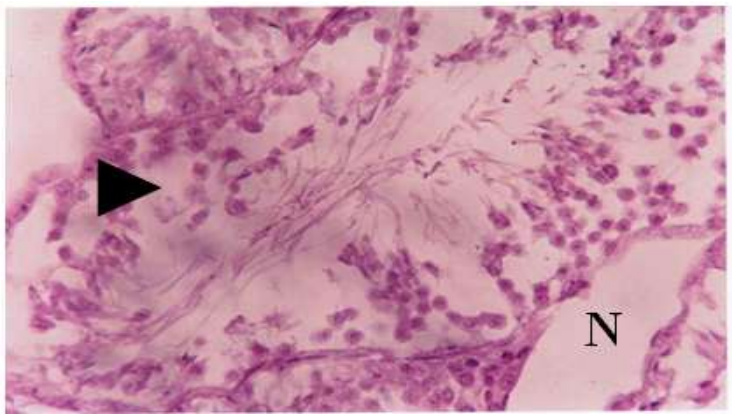
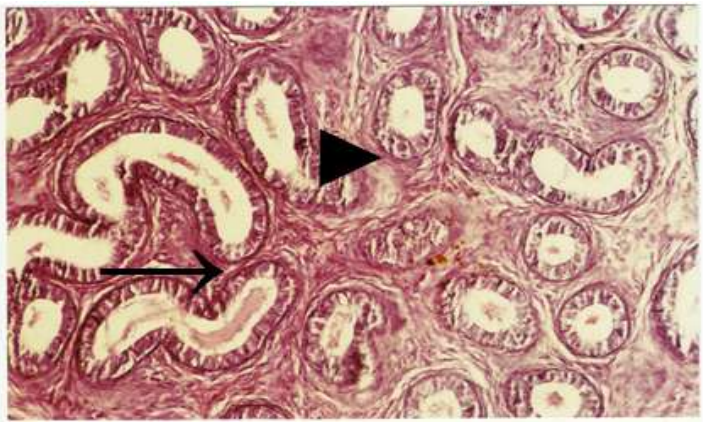


Fig (6) Section in Rete testis of treated infected mice show extensive necrosis in Rete testis tubules (N) and loss of many seminiferous tubules (►).{H&E, magnification 487X}



Fig(7) Section of the efferent ducts of untreated uninfected mice showing the lining Epithelium of cuboidal cells (→) , the ducts are embedded in connective tissue(►).{H&E, magnification 290X }

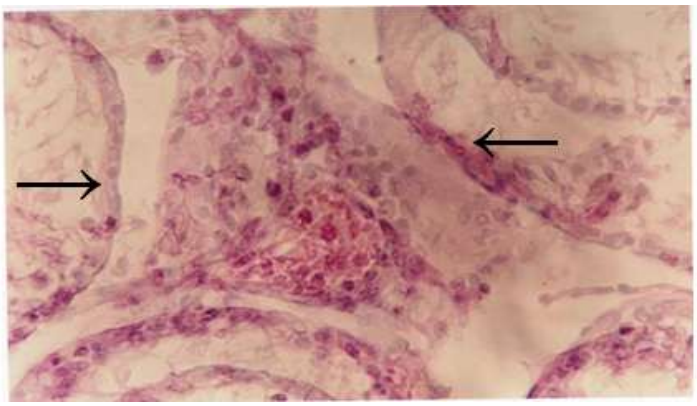


Fig (8) Section of the efferent ducts untreated infected mice showing, wavy appearance of lumen of duct which lined with Cuboidal epithelium(→){H&E, magnification 410X}

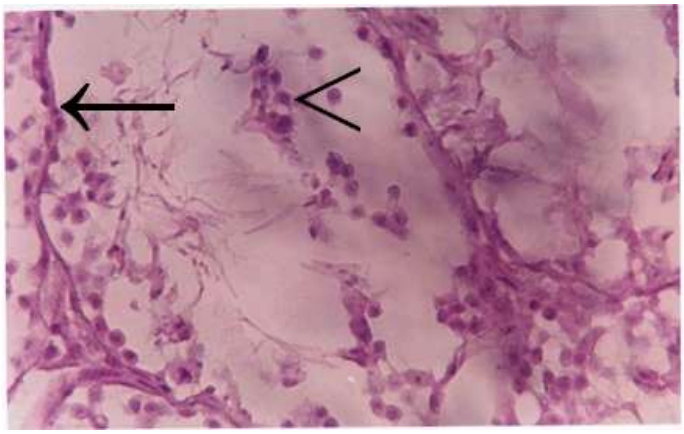


Fig (9) Section of the efferent duct of the treated infected mice show cells duct very flat and attenuated (←) with cells arranged single or in clusters with round nuclei(<) are present in some of the spaces . {H&E, magnification 489X}

Discussion:

Although Cisplatin which is anticancer agent have proved efficacy in treating *E. granulosus* infection in mice [17]. it has a significant side effect on the male Reproductive system of infected mice which is demonstrated by current study that find An extensive necrosis in Rete testis tubules, complete deformity in efferent ducts and Complete destruction of seminiferous tubules which may result in sterility because Spermatogenesis occurs in the seminiferous tubules [18] . On the other hand, in other study a group of Wistar rats was given 8 mg/kg Cisplatin after 5 days it cause inhibition of Testosterone production and testicular damage [19] . This in line with a similar study which finds that administration of Cisplatin to rats decreased sperm concentration ($p<0.05$) & sperm motility ($p<0.001$), increased total abnormal sperm rate ($p< 0.05$) as compared with control group [20]. In a recent study showed that Cisplatin – induced germ cell apoptosis in the mouse testes may result in decreased spermatogenesis and direct action of Cisplatin on dividing germ Cells through DNA binding [21].

In the present study histological changes in testis are suggested to be result from that Cisplatin act mainly by DNA binding, denature the double helix and cause intrastrand cross linking and as the testis is the main site of mitosis so is most commonly affected This also was suspected by Bertini[22]. This has been demonstrated by studies of Vawda & Davies [17] which found that there is decrease in sperm motility by an average of 36% & the rate of testicular DNA synthesis by 36%. They also prove that cisplatin did not affect the rate of protein synthesis; nor was there a change in testicular protein, RNA and DNA contents.

In the second half of test group the infected mice Show no changes in their reproductive system as the infection is limited to the gastrointestinal tract and no Cisplatin has been used on them, and this is consistent with previous studies which found there was low incidence of spontaneous apoptosis (which is, defined as normal programmed cell death) observed in control group which is also not treated with Cisplatin [21].

Conclusion:

Cisplatin has effect in killing hydatid cyst in vivo and in vitro, but because of its Significant damaging effect on male reproductive system it can not be used as Therapeutic modality for hydatid cyst in human. Since Cisplatin did not affect stem cell Spermatogonia, there appears to be

no danger of permanent sterility. The effect on Spermatogenesis is probably transient and reversible after withdrawal of the drug. The infection with E.granulosus has no direct or indirect effect on the male reproductive System.

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