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HISTOPATHOLOGICAL ALTERATIONS OF *Rattus rattus frugivourus* INFECTED WITH *Echinostoma liei* (JEYARASASINGAM, HEYNEMAN, LIM AND MANSOUR,1972)

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ABSTRACT

Echinostoma liei (Jeyarasasingam, Heyneman, Lim and Mansour, 1972), was used for the first time as bio control agent for the wild rat *Rattus rattus frugivorus* Rafinesque .

Two levels of infection were used (250 and 500 metacercariae / rat) and histopathological alterations were studied in the affected organs (intestine ,liver and testis) . The investigation revealed that high infection of *E. liei* resulted in severe pathological alteration in rat intestine, and liver, such as edema, lymphocytic infiltration, necrosis and hemorrhage in the liver lead to death in addition to testis hypertrophy with seminiferous tubules devoid of sperms that may lead to sterility. Thus, *E. liei* could be considered a new effective biological agent for controlling wild rats causing either death or sterility and also safe to human since people in Egypt don't eat fresh water snails either raw or cocked).

Keywords : *Echinostoma liei, Rattus rattus frugivourus,* Histopathology **.**

INTRODUCTION

Parasites have detrimental effects on host fitness where both theoretical and experimental evidences suggest that parasite-induced reduction in host fecundity and/or host survival have a major influence on host population dynamics (Anderson and May, 1979).

Echinostoma infection has been shown to lead to identifiable damage to the small intestine, particularly erosion of the intestinal villi (Bindseil and Christensen, 1984).It has the capability to be a blood and tissue feeder (Balogun, 1991).High levels of crypt-cell proliferation and tissue hyperplasia were observed in the ileum of mice infected with *Echinostoma caproni* coinciding with the establishment of chronic infections (Cortés *et al.*, 2015).

Jeyarasasingam *et al*. (1972) found *E. liei* in the mid-small intestine in hamsters.

Huffman *et al*. (1986) studied the pathological effects of *E*. *revolutum* infection in golden hamsters and revealed that *E. revolutum* migrated from the small intestine to the liver and caecum in this host. This migration was only noted in acute heavy infections.

Light infections of echinostomes in animals don't cause significant pathology or symptoms, but heavy infections are associated with serious pathology, disease and even death (Carney 1991). In fowl, hemorrhagic diarrhea, progressive emaciation, weakness in flight, and death were

associated with infections of hundreds to thousands of flukes (Rim, *et al*. 1982) .

Histological examination of the small intestine of mice infected with *Echinostoma trivolvis* showed hyperplasia in the goblet and Paneth cell (Fujino*,et al*.1996).

Effects of various worm densities of *E. liei* on the establishment success, gut distribution, size, in utero egg counts and egg output of the parasites themselves as well as on the weight and levels of anemia and pathological features associated with infections in laboratory mice were studied by Balogun(1991).

Very little knowledge is available on the effect of *E. liei* on the wild rats .Therefore ,the objective of the present study was to assess the effects of *E. liei* on different organs of wild rat ,*R. rattus frugivorus* as a bio- control agent .

MATERIALS AND METHODS

Field collections:

Snails:

The first intermediate hosts of the *E. liei* ,*i.e*. *Biomphalaria alexandrina* (Ehrenberg), the intermediate host of *Schistosoma mansoni* Sambon and *Lanistes carinatus* Olivier and the second intermediate hosts *i.e. B. alexandrina , Helisoma duryi* (Wetherby) and *Physa acuta* Draparnaud, were collected from irrigation channels in, Giza ,Egypt , 2013, and reared in laboratory by the same technique which previously described by Azzam (1995) and Azzam and Belal(2006) .

Rodents:

Rats were alive-trapped from Giza Governorate and acclimatized in the laboratory for four week before use. Through this period, the rats were examined for parasites infection to exclude the natural infected individuals according to (Carney ,1991).

Experimental exposures:

For determination of the *E. liei* snail's infection, the first intermediate host collected snails *i.e*. *B. alexandrina* and *L*. *carinatu*s were placed in beakers contained dechlorinated water and kept under light for 2 h to induce cercarial shedding. Cercariae were identified as *E. liei* using standard keys of (Schell, 1985). Laboratory breeding snails which serve as second intermediate hosts *i.e. B. alexandrina ,H. duryi* and *P. acuta* (5-6mm shell diameter or highet according to the species) were placed in plates with cercariae in dechlorinated water for 24 h ,then transfer to plastic aquaria .After 7 -10 days of ad libitum feeding with fresh lettuce, snails were examined by high power light microscope,the number of echinostome metacercariae was counted then the snail was introduced to the rat *R. rattus frugivorus* without or after crushing. If the counting of metacercariae was unable in living snail, metacercarial cysts were obtained from the pericardial sac of the dissected snail and counted, then introduce to the rat**s.**

Initial infection and fecal analysis:

Two sets of five rats each administered either, 250 or 500 metacercarial cysts of *E. liei.* Rat from each cyst density set was necropsied

after death or killed on 21 days after infection .Additional uninfected control consisted of 5 rats, one of them was killed on the aforementioned days of the experiment .

Necropsy :

The small intestine was examined for worms, removed and measured from the pylorus to the caecum, cut into five equal-length sections and each section was opened longitudinally in 0.85% saline. Associated organs of the peritoneal cavity were also examined for worms and then stored in 70% ethanol or 10% formalin.

Histological preparations:

Histological examination was made of the changes evident at the site of attachment of the main cluster of the worms to the mucosa in the small intestine of infected rats, and at sites adjacent to these clusters. Sections of the intestine were removed and fixed immediately in formal acetic-acid (FAA) for two days. Corresponding areas of the small intestine in control rat were removed and fixed in the same way. These infected and uninfected tissue samples were then processed using routine histological techniques, (taken through ascending series of ethanol to xylene , impregnated with wax, followed by wax embedding), sectioned at 6 micrometers using a rotary microtome,then mounted on slides and stained using standard haematoxylin and eosin staining techniques (Balogun 1991). The slides were examined by research microscope and then photographed.

RESULTS AND DISCUSSION

The first pathological alteration appears during necropsy was un-normal color of rat liver as shown in Figure (1) or partially darken (Figure, 2), these may indicate to an oxidation in liver tissues. Another clear pathological alteration was testis hypertrophy(TSHYP) (Figure, 14) compared with control normal testis (NTS) (Figure, 13) which indicated that *E. liei* affected the testis although it is intestinal parasite.

Histological examination revealed that in all infected rats pathological alteration was apparent in the intestinal sections. Areas of the small intestine from uninfected control rat are shown in Fig.(3)with low power which showed the relationship between the villi (V) and the crypts of Lieberkuhn (CL) in the small intestine (jejunum) of an uninfected rat. The villi, long finger like projections, are prominent and the columnar epithelial cells (E) covering the villi. The lamina propria (LP) is seen to be arranged within each villus and there is a low crypt to villus ratio (approximately 1: 3.5) . Balogun (1991) found this ratio in uninfected mice 1:4. The tunica muscularis (Tm) is a relatively thin layer of muscle. Figure (4) is a high power view of the tip of a villus in an uninfected rat showing the internal structure of the villus and the ordered columnar epithelium. The very prominent brush border (B) which outlines the luminal side of the simple columnar epithelium is also clearly apparent.

At two exposure levels it was clear that all infected rats had consistent pathological features. These features included crypt hyperplasia (HC) with cell and villous atrophy (AV), and erosion of the villi (EV) (Figures 5&6),

resulting in a high crypt to villus ratio (1:4). Balogun (1991) found similar ratio in infected mice (1:5).

Some of the pathological changes (crypt hyperplasia) were moderate (Figure ,5), in median infections (250 metacercariae), accompanied with necrosis (HCN) (Figure ,6) which showed an area of the small intestine in a rat exposed to 250 metacercarial cysts, but more severe in high infection accompanied with sever necrosis(SHCN) , congestion of blood vessels (CBV) and lymphocytic infiltration (LI) (Figure, 7) which showed an area of the small intestine (jejunum) in a rat exposed to 500 metacercarial cysts.

Balogun (1991) found the same alteration in the intestine of the laboratory mice infected with *E. liei*. He also detected cellular infiltrations into the lamina propria of the eroded villi amongst all the intestines observed at the infected mice. He observed hyperplastic crypts and muscular hypertrophy of the tunica muscularis and found that histological pathogenic changes are not confined to the attachment sites. Moreover ,Huffman *et al.* (1988) reported histopathological response of *E. liei* and *E. revolutum* in single and concurrent infections in hamsters as erosion of intestinal villi with lymphocytic infiltration as the primary response. High levels of crypt-cell proliferation and tissue hyperplasia were observed in the ileum of mice infected with *Echinostoma caproni* coinciding with the establishment of chronic infections (Cortés *et al*., 2015). On the other hand, Seanphet *et al*. (2006) , reported crypt hyperplasia and lymphocytic infiltration in the intestine of *Rattus norvegicus* infected with the intestinal trematode worm *Centrocestus caninus* . Rats and mice experimentally infected with intestinal trematode worm *Pygidiopsis summa* (Digenea: Heterophyidae) showed mucosal pathologies in the small intestine that include villous atrophy, crypt hyperplasia, and mucosal inflammation (Seo *et al*. ,1986 and Jong *et al*. ,2014).

Figure (8) showed the normal histological structure of the control rat liver with no alteration observed and normal histological structure of the center veins(CV), branch of hepatic artery (BHA),branch of bile duct (BBD) , sinusoids (SI) and normal hepatocytes (NH).

Histological examination revealed also liver pathological alteration *i.e*. lymphocytic infiltration (LI), edema(ED) dispersed the hepatocytes(HPC) (Figure, 9), accumulation of hemosiderin pigments(HP), necrotic hepatocytes cell(NHPC) which were moderate (Figure, 10) in median infection (250 metacercariae) and sever with congestion blood vessels(CBV) (Figure, 11), accompanied with activity of Vankupffer cells(KUP) or macrophage and hemorrhages (HM) (Figure, 12) which lead to death in high infection (500 metacercariae).

Carney (1991),found that heavy infections of echinostomes are associated with serious pathology, disease and even death .Huffman *et al*. (1986), observed pathological features in the small intestine and liver in golden hamster infected with *E. revolutum*. They observed superlative lesions in the liver produced by bacterial infections which they believed to have been due to the transporting of intestinal flora by the parasite as it moved up the bile duct on its way to the liver. Huffman, *et al.*, (1988) noted the occurrence of *E. revolutum* in the golden hamster in the liver, gall bladder,

pancreas and the stomach , they attributed this spread to worm crowding in heavy infections after hamsters were exposed to 350 metacercarial cysts of the parasite. Extra intestinal infection resulted in marked necrosis and hemorrhage caused by the parasite in the liver parenchyma and the biliary system.

Figure(15) showed a normal well-defined interstitial cell layer(Leydig cells)(LC) separating the seminiferous tubules (ST) which are filled with spermatids(SPD), which occupied the entire lumen of these tubules , and spermatogonia (SPG) along the edge of seminiferous tubules and surrounded by well-defined smooth muscles (SM) .Although, *E. liei* is an intestinal parasite ,it cause un expected alteration in the rat testis . These alterations include, some disruption in the interstitial cell layer, edema (ED) that led to dispersed the seminiferous tubules which devoid of sperms (STDSP) (Figure, 16), sever proliferation of spermatocytes series(SPSS) and devoid of sperms of seminiferous tubules(STDSP) and edema (ED) (Figure , 17) in addition to thickening of testis wall(TW) (Figure, 18). These alterations may be due to host immune reactions to the parasite. Thus, the infection of rat with *E .liei* could affect the fecundity of the rat .

Infection with parasite can led to plasticity in the reproductive investment of hosts (Agnew *et al.* 2000; Hurd 2001). In rodent, *Peromyscus maniculatus* hosts, parasitic infection most often leads to reductions in current host reproduction (Schwanz, 2008), Infection has been recorded as reduced probability of reproducing, delayed reproduction, and reduced male mating effort Hurd 2001; Kolluru *et al*. 2002;Telfer *et al*. 2005).

It has been demonstrated that mice infected with *E. caproni* induced a serum antibodies response to the surface of the parasites (Simonsen and Andersen, 1986).

The immunocytochemical demonstration of vertebrate like peptides (VIP) have been confined to *E. liei* (Thorndyke and Whitfield, 1987and Riddell, *et al.*, 1991). Richard *et al.* (1989) detected substance P-like immunoreactivity in the central and peripheral nervous structures and in the glandular cells of the prostate. Riddell, *et al.*(1991),demonstrated the presence of FMRF amide-like immunoreactive cells in the central nervous system and reproductive system of *E. liei.* The nervous distribution of this peptide suggested a role as a peptidergic neurotransmitter while its localization within the reproductive system pointed to a direct involvement with copulation acting either locally or as a neurotransmitter. Anderson *et al* (1989), demonstrated the release of antigen from the surface of *E. caproni* cultured in vitro .

These literature make the authoresses suggest that the alteration occurred in rat testis infected with *E. liei* may due to release of some immunocytochemical which affect the testis, but this suggestion need more investigation to prove.

From the present results it could be concluded that high infection with *E. liei* cause severe pathological alteration in rat intestine, and liver hemorrhage lead to death in addition to testis damage which may led to sterility .

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Since eating raw snails and tadpoles was identified as an important mode of transmission of Echinostomatidae, (Carney1991).Thus *E. liei* could be considered a new biological method for controlling wild rats and safe to human (in Egypt people don't eat fresh water snails and tadpoles either raw or cocked) consequently this parasite represent a safe mean of rat control and could be taken in consideration in control programs of these harmful animals .

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التغيرات الباثولوجية فى الفأر المتسلق المعدى بطفيل الإيكينوستوما لياى

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فى هذه الدراسة إستخدم طفيل الإيكينوستوما لياى *Echinostoma liei* لأول مرة كعامل للمكافحة البيولوجية للفأر البرى المتسلق *Rattus rattus frugivorus* .

إستخدم معدلين لعدوى الفئران (250 و500 طور معدى متحوصل /الفأر) وتم دراسة التغيرات الهيستوباثولوجية (النسيجية المرضية) على الأعضاء التى تأثرت بالعدوى .

أوضحت النتائج أن العدوى الشديدة أدت إلى تغيرات مرضية فى الأمعاء وفى الكبد مثل الأوديما (إستسقاء فى الخلايا) وتسرب فى الخلايا اليمفاوية و نخر مع نزيف فى الكبد أدى إلى الوفاة .بالإضافة إلى تضخم فى الخصية وخلو الحويصلات من الحيوانات المنوية مما قد يؤدى إلى العقم .

وعلى ذلك فإن هذا الطفيل يعتبر طريقة جديدة من وسائل المكافحة البيولوجية الفعالة لهذه الفئران حيث أنه يسبب إما الوفاة أو العقم لتلك الفئران البرية وآمن للإنسان حيث أن الناس فى مصر لاتأكل قواقع المياه العذبة سواء مطهية أو بدون طهى