

## Histopathological changes of rodent liver infected with a larval cestodes recorded at Sohag, Egypt

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Rec. 10 Feb, 2016 Accept. 12 Mar, 2016

### Abstract

Rodents are important in many ecosystems because they reproduce rapidly, and can function as food source for predators, mechanisms for seed dispersal and as diseases vectors. Rodents may act as reservoir hosts for important human parasitic diseases. The present study was focused on histopathological changes of rodent liver infected with a larval cestodes recorded at two locations situated at Sohag, Egypt. This study reported infection rate of 56% and 32% for rodents at the two locations, respectively. The study also showed that the incidence in females is higher than in males in the second location compared with that of the first location. Microscope examinations of the liver tissue sections revealed a wall cyst of larval cestode, *Cysticercus fasciolaris* in parenchyma consists of two compressed layers of highly proliferative fibrous connective tissue and inflammatory cells mainly lymphocytes. Inflammatory reaction was seen in the hepatic parenchyma around the cyst. Also, there are dilatation and congestion in the central and portal veins and infiltration of the liver parenchyma with inflammatory cells. The hepatocytes in the central and portal areas showed fatty degenerative changes. While, in the portal area the histological tissue of the liver showed microvesicular steatosis and edematous infiltration. Signs of inflammation including sinusoid widening and prominent kupffer cells were noted.

**Key words:** Rodents, liver, cestode larva, histopathology.

### Introduction:

Rodents are a key mammalian group and found in many environments throughout the world. Rodents are a major agricultural, urban and social pest across many of developing world (Singleton *et al.*, 2003). Rodents are important in many ecosystems because they reproduce rapidly, and can function as food source for predators, mechanisms for seed dispersal and as diseases vectors (Aplin *et al.*, 2003; Wilson and reeder, 2005; Okoye and Obiezue, 2008). Rodents may act as reservoir hosts for important human parasitic diseases (Walsh *et al.*, 1993; Mayer *et al.*, 1995; Singleton *et al.*, 2003; Kimpel *et al.*, 2006).

Rodents are hosts to number of ectoparasites such as lice, mites and ticks that can transmit viral, bacterial and protozoan parasites to man and animals (Soliman *et al.*, 2001). In addition, they can harbor many different protozoan and endoparasites (Mahida, 2003).

Numerous studies on the parasite fauna of rodents have been carried out in recent years (Stojecevic *et al.*, 2004; Bajer *et al.*, 2005; Fuentes *et al.*, 2007; Gomez *et al.*, 2008; Tung *et al.*, 2009).

*Cysticercus fasciolaris* is a larval and cystic stage of *Taenia taeniaeformis* and common parasites in the liver of rats and mice (Hanes, 1995,

Malsawmtluangi and Tandon 2009). The intermediate hosts of *T. taeniaeformis* are mouse, rat, cat, muskrat, squirrel, rabbit, other rodent, bat, and human. The *C. fasciolaris* commonly found in a liver of intermediate hosts were infected through contaminated water or feed materials with infected cat faeces (Miyazaki, 1991). In an intermediate host, the *C. fasciolaris* cysts were found in different structured forms and they can be recognized by multiple hepatic cysts (Hanes, 1995).

*Taenia taeniaeformis* is a cestode of family Taenidae and it occurs as adult tapeworms in the small intestine of carnivores as definite hosts and are transmitted to rodents as intermediate hosts where they develop as fluid filled larvae in different organs. *Cysticercus fasciolaris*, the larval form of *T. taeniaeformis* is also known in the literature as *Taenia crassicollis*, *Hydatigena fasciolaris*, *Strobilocercus fasciolaris* and bladder worms (Hsu, 1979).

Larval cestodes (cysts) produce some pathological changes in rat's liver such as adhesions, granulation of tissue, acute and chronic inflammations and hepatic sarcoma (Jithendra and Somvanshi, 1999; Mahesh *et al.*, 2006).

Fibrosarcoma of liver associated with *Cysticercus fasciolaris* was suggested as an

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appropriate model for investigating parasitic carcinogenesis and pathogenesis in wild rats and rodents (Hart-Elcock *et al.*, 1989).

The present study aims to:

- Describe the collected larval cestode from liver rodents.
- Study histopathological changes in the infected liver.

**Materials and methods:**

The present work was carried out in two trapping area, (Shandwell farm and surrounding houses). The study area was chosen for the present work because it is highly infested with rodents also the selected area occupied with different fruit trees (palm and mango), summer and winter field crops and vegetables.

All rodents (Genus: *Rattus*) were trapped alive using specially made wire traps, each of which measures (24 x 11 x 9 cm) in both sites. Traps were baited with meat and carrot and sampling was carried out randomly

50 rodents (25 individuals from each site) were caught alive from the study areas during the period of collection from May 2010 till April 2011. The collected rodents were dissected in the laboratory, where liver was removed from the digestive tract, put in separate labeled Petri-dishes containing saline solution, and then examined separately under stereo-binocular microscope searching for larval cestode parasites.

The larval cestodes were picked up by delicate forceps and put in another clean Petri-dish containing the saline solution. These larvae were relaxed in cold water during 24 hours, fixed in hot alcohol-formalin- acetic acid, stained with carmine stain, dehydrated in graded series of ethyl alcohol, cleared in toluene and mounted on slide with Canada balsam.

After preparation larval cestodes were examined and measured with an ocular micrometer of Olympus microscope (mm) and photographed.

For histopathological studies, small pieces of rodent liver were fixed in Carnoy's fluid dehydrated in graded series of alcohols, embedded in paraffin wax, sectioned at 5-7µm and stained with haematoxylin and eosin. The stained sections were examined under a research microscope and then photographed.

Identification of the present parasites was carried out according to the keys of (Leong *et al.*, 1979; Miyazaki, 1991; Ambu *et al.*, 1996 and Moudgil *et al.*, 2014) for helminthes (larval cestodes). The present host was identified by (Harrison & Quah, 1962; Medway, 1983 and Payne *et al.*, 1985).

**Results:**

Rodents known to carry a variety of organisms that many cause diseases in humans and domestic animals. So, it is important to survey their parasites in the present study.

50 rodents (25 individuals from each site) were collected and dissected in the laboratory. 56% and 32% from the collected rodents were infected with larval cestodes inhabiting, liver in the first and second sites, respectively. The pooled infection percentage of rodents by parasites are 44% in the two sites (Table 1). Table (2) shows the number of infected rodent females and males in the two sites of collection. In the first sites, 8 individuals of rodent females (53%) and 6 males (60%) were infected with larval cestodes. While, in the second sites, 6 individuals of rodent females (37.5%) and 2 males (22%) were infected with larval cestodes. It is noted that the infection percentages of rodent females are higher than that of the males in the second site and vice versa in the first one. Table (3) shows the number of infected organs of rodent females and males with larval cestodes in the two sites.

| sites             | Total numbers |              |       |
|-------------------|---------------|--------------|-------|
|                   | Infected      | Non-infected | Total |
| First             | 14            | 11           | 25    |
| Second            | 8             | 17           | 25    |
| Total             | 22            | 28           | 50    |
| Total infection % | 44%           | 56%          | 100%  |

**Table 1:** Total number of infected rodents in the two sites of collections. 25 individuals of rodents in each site.

| sites           | No. of female |            |       | No. of male |             |       |
|-----------------|---------------|------------|-------|-------------|-------------|-------|
|                 | Infect        | Non-infect | Total | Infect      | Non- infect | Total |
| 1 <sup>st</sup> | 8             | 7          | 15    | 6           | 4           | 10    |
| 2 <sup>nd</sup> | 6             | 10         | 16    | 2           | 7           | 9     |

**Table 2:** Number of infected rodent females and males in the two sites of collections. 25 individuals of rodents in each site.

| Cestodes | Infected organs | First site |      | Second site |      |
|----------|-----------------|------------|------|-------------|------|
|          |                 | Female     | Male | Female      | Male |
| Larva    | Liver           | 5          | 2    | 3           | 0    |

**Table 3:** Number of infected organs of rodents with larval cestodes in the two sites of collections.

During gross examination, the liver of rodents showed 1-15 chick peas-like cysts of larval cestode, *Cysticercus fasciolaris* (5-10 mm) in diameter with creamy white – color. These cysts were observed mostly in the caudal and lateral lobes of the liver and embedded in the liver parenchyma (Pl. 1A, B).

When the cysts were opened by an incision in the wall the fluid escaped and the small tape worm (15-21cm) long appeared using dissecting microscope. The tapeworm appeared to has a rostellum and scolex with vesicles filled with fluid at the end of the worm (Pl. 1C).

Morphological examinations of stained present larva revealed typical characteristics of taeniid cestodes with the presence of an armed rostellum having two rows of large and small hooks and four suckers on the scolex, a long neck and pseudo-segmentation of the entire body length with a terminal bladder, which is in consistence with the larvae of *Taenia taeniaeformis* (Pl. 1D, E, F).

Certain of the helminthic parasites of rodents produce pathological changes which would probably affect the general health of the host. Microscope examinations of the liver tissue sections revealed a wall cyst of larval cestode, *Cysticercus fasciolaris* in parenchyma consists of two compressed layers of highly proliferative fibrous connective tissue and inflammatory cells mainly lymphocytes. Inflammatory reaction was seen in the hepatic parenchyma around the cyst. It is noted that the segments of larva were seen inside the fibrous tissue cyst. The tegument of larva appeared as a thick and non-cellular layer. Also, there are dilatation and congestion in the central and portal veins and infiltration of the liver parenchyma with inflammatory cells. The hepatocytes in the central and portal areas showed fatty degenerative changes. While, in the portal area the histological tissue of the liver showed microvesicular steatosis and edematous infiltration. Signs of inflammation including sinusoid widening and prominent kupffer cells were noted (Pls 1G, H; 2A-J; 3A-H).

#### Discussion:

Rodents play a significant role in public health, Chiefly due to their role as carriers or reservoirs of microbes and parasites of zoonotic importance (Singla *et al.*, 2012).

In the present study, the parasites of rodents (Genus: *Rattus*) collected from Sohag Governorate was analyzed. 56% and 32% of the collected rodents

were infected with larval cestodes, *Cysticercus fasciolaris* inhabiting, liver in the first and second sites, respectively. The pooled infection percentage of rodents by parasites are 44% in the two sites. 53% of rodent females and 60% of males in the first site and 37.5% of females and 22% of males in the second site were infected with larval cestodes. The infection of both sexes of rodents by parasites was 46%. (El Shazly *et al.*, 2008) recorded 49.6 % and 56.3% infection percentages of rodents by parasites in Dakahlia and Menoufia, respectively. (Moudgil *et al.*, 2014) recorded 17.8% infection percentage of *Cysticercus fasciolaris* collecting from liver of rat.

The present study was performed on the rodents parasitized by larval cestodes. Hasegawa *et al.* (1994 and Tung *et al.* (2009) surveyed the endoparasitic infections in *Rattus rattus*, farm rodents and shrews in Taiwan and reported the occurrence of 4 cestode, 10 nematode and 1 protozoan, and recorded the occurrence of *Physaloptera* sp. and *Sarcocystis* sp.

Several authors worked on the helminthic parasites of the rodent *Rattus rattus* all over the world such as (Huq *et al.*, 1985; Faiyaz-ul-Haque *et al.*, 1990; Bhuiyan *et al.*, 1996; Bilgees *et al.*, 2001 and Sumangali *et al.*, 2007; Kia *et al.*, 2001). They investigated the endoparasites of rodents in Ahvaz, South west Iran and reported 12 different species of helminthic parasites. Mazeika *et al.* (2003). provided a new data on the helminthes fauna of rodents and reported the occurrence of larval form of 3 cestodes from must rat *Ondatra zibethicus*. Stojcevic *et al.* (2004) surveyed the rates in the rural regions of Croatia and reported the presence of 7 helminthic species. Waugh *et al.* (2006) reported the occurrence of 2 cestodes from the rodent *Rattus rattus* in Jamaica. Gomez *et al.* (2008) reported the presence of cestode in the Argentine brawn rat *Rattus norvegicus*. Rafique *et al.* (2009) reported the presence of 4 cestodes from the rodents captured from Pakistan.

The present larval cestode, *Cysticercus fasciolaris* within the cysts (capsules) was observed in the liver rodents which has single or multiple (1-15) cysts from 5-10 mm in diameter. Each cyst is pea-like, creamy white and embedded on the surface of the liver parenchyma. There are some data on the parenchyma of mice and rat's liver. Singla *et al.* (2003; 2012) and Al-Najjar *et al.* (2009) recorded 1– 3 cysts from 4-15mm and 1-10 cysts from

4mm in diameters in rats, respectively. The second authors recorded 1-3 cysts in mice from 3-6 mm in diameter. Al-Jashamy *et al.* (2009) recorded in rats 1-5 cysts from 2-2.5 mm in diameters. In addition, Mahesh *et al.* (2006) recorded in rats 2-7 cysts from 8-16 mm in diameters. Moudgil *et al.* (2014) recorded in rat's multifocal hepatic cysts from 3-8mm in diameter and (1-6) cysts in the liver rodent's species.

Each present cysts contained live motile larva nearly coiled in the connective tissue cavity, measured 15-21mm in length and looked like a small tape worm. Singla *et al.* (2003, 2012) and Al-Najjar *et al.* (2009) reported 30-92mm,  $22.8 \pm 0.7$  mm and 4-15cm in length of the larvae, respectively.

Morphological examinations of stained present larva revealed typical characteristics of taeniid cestodes with the presence of an armed rostellum having two rows of large and small hooks and four suckers on the scolex, a long neck and pseudo-segmentation of the entire body length with a terminal bladder. This larva develops in hepatic parenchyma according to (Soulisby, 1982; Al-Jashamy and Islam, 2007 and Moudgil *et al.*, 2014). The morphological characters of the present larva is corresponding to the metacestoda, *Cysticercus fasciolaris* of the adult cestoda *Taenia taeniformis* which inhabits the small intestine of domestic cats.

*Cysticercus fasciolaris*, the larval stage of *T. taeniformis*, is a common parasite of rodents in India (Soulisby, 1982). Its occurrence in laboratory and wild rodent species has been reported by many workers (Nama and Parihar, 1976; Tyagi and Mishra, 1978; Jithendran and Somvanshi, 1999; Bhelonde and Ghosh, 2002; Sivakumar *et al.*, 2003; Singla *et al.*, 2003). *C. fasciolaris*, the metacestode of *Taenia taeniformis*, is found in the liver of rodents in the form of cysts (Soulisby, 1982). *C. fasciolaris* infection is clinically asymptomatic and is considered harmless (Singla *et al.*, 2003).

In the present study, microscopic examinations of the liver tissue sections revealed a wall cyst of *Cysticercus fasciolaris* in parenchyma consists of two compressed layers of highly proliferative fibrous connective tissue and inflammatory cells mainly lymphocytes. Inflammatory reaction was seen in the hepatic parenchyma around the cyst. It is noted that the segments of larva was seen inside the fibrous tissue cyst. The tegument of larva appeared

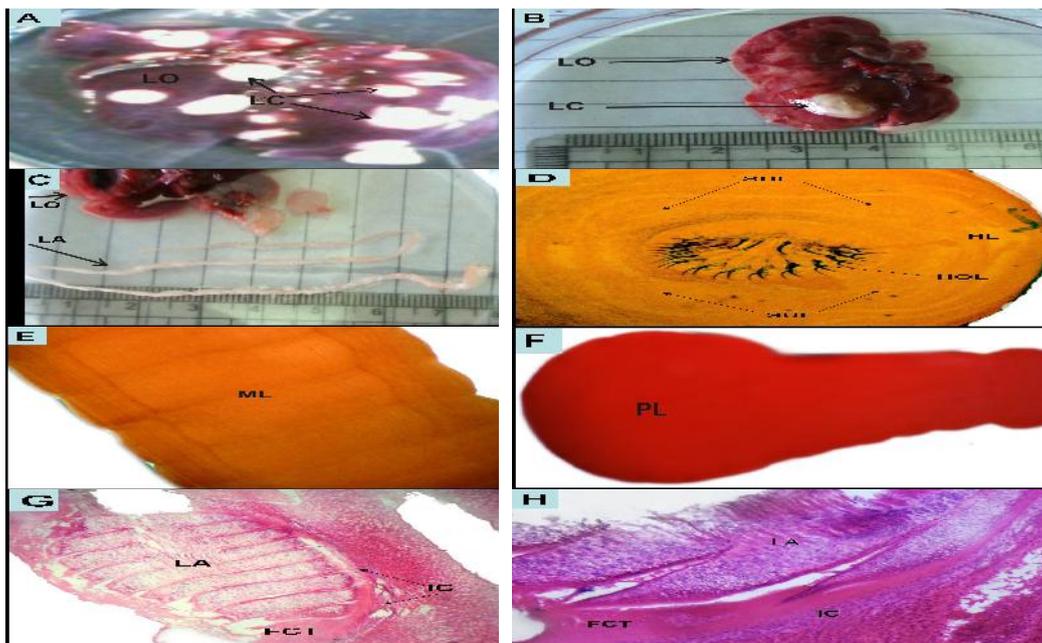
as a thick and non-cellular layer. Also, there are dilatation and congestion in the central and portal veins and infiltration of the liver parenchyma with inflammatory cells. The hepatocytes in the central and portal areas showed fatty degenerative changes. While, in the portal area the histological tissue of the liver showed microvesicular steatosis and edematous infiltration. Signs of inflammation including sinusoid widening and prominent kupffer cells were noted.

(Al-Najjar *et al.* 1999 and Singla *et al.*, 2003, 2012). reported a trophied hepatocytes around the cyst with various stages of degeneration The host connective tissue capsule cyst may give rise to sarcomas in older animals typically 12-15 months post-infection (Hanes, 1995; Al-Jashamy and Islam 2007; Al-Najjar *et al.*, 2009). The lesions around the cysts depicting infiltration of nuclear cells and eosinophils indicated acute inflammation whereas, granulation tissue with infiltration of fibroblasts and same lymphocytes indicated chronic inflammation (Moudgil *et al.*, 2014).

#### Abbreviations:

- AR = Artery
- BD = Bile Duct
- CV = Central Vein of liver
- DP = Dilatation of Portal vein branches
- EI = Edematous Infiltration
- FC = Fatty Changes of liver
- FCT= Fibrous Connective Tissue layer of cyst wall
- H = Hamorrhage
- HC = Hepatocytes
- HI = Hepatic Inflammatory reaction
- HL = Head of Larva
- HoL = Hooks of Larva
- IC = Inflammatory Cell layer cyst wall
- KC = Kupffer Cell of liver
- La = Larva
- LC = larval cysts
- LO = Liver Organ of rodents
- LT = Larval Tegument
- ML = Middle part of Larva
- MS = Micro-vesicular Steatses
- PL = Posterior end of Larval
- PV = Portal Vein of liver
- RA = Rostellum of Adult
- SUL = Sukers of Larval
- SW = Sinusoid Widening

#### List of Photos



**Plate 1:** Photographs of rodent liver infected with cestode larva, *Cysticercus fasciolaris* showing.

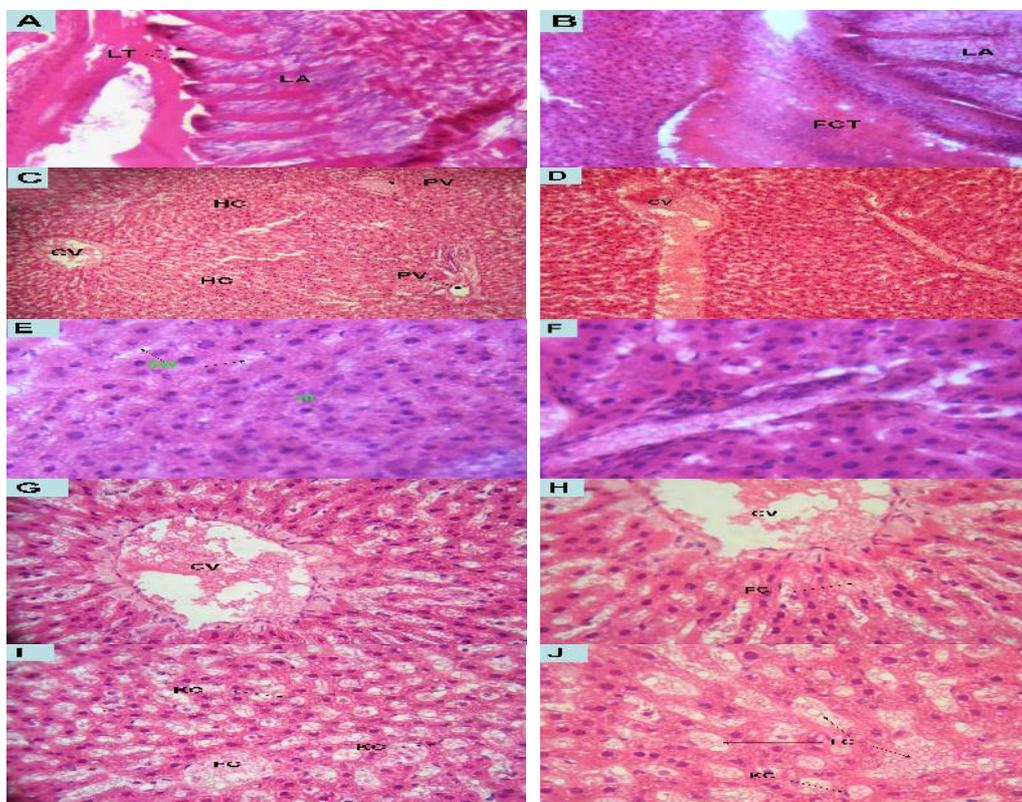
**A-** Multiple cysts within different lobes of liver.

**B-** Chick peas-like cyst of the larva

**C-** Larva after removing the cyst

**D, E, F-** Photomicrographs of larval cestode, *C. fasciolaris* showing four suckers and two rows of hooks (D), body segments (E) and Posterior end (F).

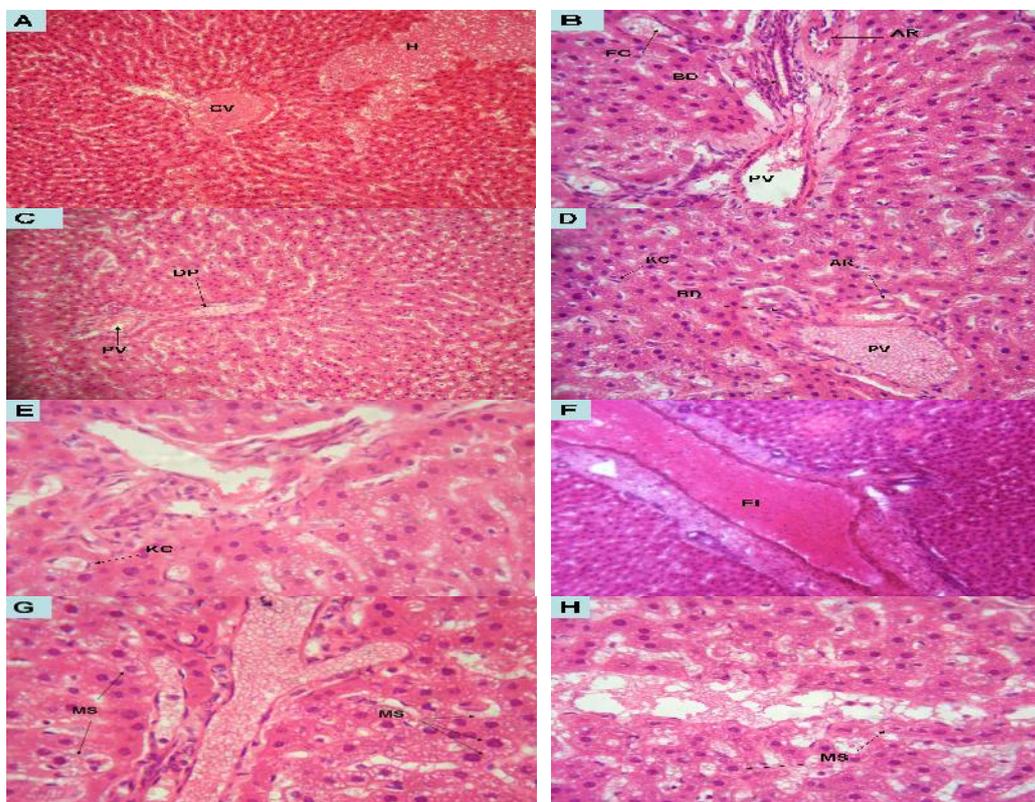
**G, H-** Photomicrographs of histological sections through rodent liver infected with larval cestode, *C. fasciolaris* showing wall of the cyst that consists of fibrous connective tissue and inflammatory cells layers (G) and attachment of larva with the liver (H).



**Plate 2:** Photomicrographs of histological sections through rodent liver infected with larval cestode, *C. fasciolaris* showing.

- A-** fibrous connective tissue layer of the cyst wall
- B-** inflammatory cells layer of the cyst wall.
- C-** Congestion in central and portal veins
- D-** Congested and dilated veinules in central area
- E-** hepatic inflammatory reaction and sinusoid widening in central area

- F-** mild lymphocytes infiltration in portal area
- G-** Congestion in central vein
- H-** degenerative changes of hepatocytes
- I-** prominent kuppfer cells
- J-** fatty degenerative changes



**Plate 3:** Photomicrographs of histological sections through rodents liver infected with larval cestode, *C. fasciolaris* showing.

- A-**hemorrhage in central area
- B-**hepatic inflammatory reaction in portal area
- C-**sinusoid widening and dilated portal vein in portal area
- D-**congestion and dilatation in branches of portal vein
- E-**Degenerative changes in the hepatic cells and kuppfer cells in portal area.
- F-** Edematous infiltration
- G-**Microvesicular steatosis, and congestion and dilatation of blood vessel
- H-**Microvesicular steatosis

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## التغيرات المرضية النسيجية في كبد القوارض المصابة بيرقة الديدان الشريطية محافظة سوهاج، مصر

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### ملخص

التدبيبات الرئيسية في العديد من البيئات في جميع أنحاء العالم، وهي مهمة في العديد من النظم البيئية لأنها تتكاثر بسرعة و تعتبر من الآفات الزراعية والحضرية والاجتماعية الكبرى عبر العديد من بلدان العالم النامية. القوارض مصدر غذاء للحيوانات البذور، وكذلك ناقلات للأمراض ومخزن للأمراض الطفيلية البشرية الخارجية و الداخلية الهامة مثل القمل والقراد التي يمكن أن تنقل الفيروسات والبكتيريا والطفيليات وحيدة الخلية للإنسان والحيوان. وبالإضافة إلى ذلك، فإنها يمكن أن توي العديد من الأوليات والطفيليات الداخلية المختلفة. تم في هذا البحث دراسة التغيرات المرضية النسيجية في كبد القوارض المصابة بيرقة الديدان الشريطية في موقعين من مدينة شندويل (محافظة سوهاج) حيث سجلت هذه الدراسة نسبة . كما بينت الدراسة أن نسبة الإصابة في أعلى منها في الذكور في الموقع الثاني عن الموقع الأول. وبينت الفحوصات المجهرية النسيجية تواجد ورم والتهابات الخلايا الليفية الضامة في أنسجة كبد القوارض المصابة بيرقة الديدان الشريطية، كما تبين وجود توسع واحتقان في الأوردة الكبدية المركزية والبوابية، وكذلك حدث علامات التهاب متنوعة في خلايا الكبد في المناطق الوسطى والبوابية النزف والخلايا الدهنية والليمفاوية .....