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Experimental Infection with Foodborne Parasite (*Trichinella spiralis*) Induced Damage and Apoptosis in Rat Skeletal Muscle Fibers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Apoptosis, a type of host-cell death, frequently causes tissue damage in the deaths caused by parasite infections. Current study aimed to study the histopathological and Immunohistochemical changes in rats skeletal muscles after experimental trichinellosis infection.
Methodology: A total of 20 male rats (Sprague Dawley) were divided into 2 groups (Negative control and positive control or Infected). At the end of the experiment; skeletal muscles fron control and infected rats were fixed in 10% formalin and examined for histopathological changes using haematoxylin and eosin stains and Immunohistochemical changes against a poptotic P53 proteins and anti-apoptotic Bcl2 immunoreactivities.
Results: Current results revealed that; diffuse degenerative changes all over the muscle fibers with massive numbers of *T. spiralis* encysted larvae that surrounded by a collagen capsule and mild

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inflammatory cellular infiltration in addition to elevation in apoptotic P53 proteins and reduction in anti-apoptotic Bcl2 in addition to muscle muscles injury.
Conclusion: It can conclude that; experimental infection with *Trichinella spiralis* can induce damage and apoptosis in rat skeletal muscle fibers.

Keywords: Trichinella spiralis; rats; apoptosis; skeletal muscles.

1. INTRODUCTION

Trichinellosis is a risk to public health as well as economic issues with the production of porcine animals and food safety [1]. One of the most common and prevalent zoonotic diseases in the world is the genus Trichinella [2]. Food is the primary source of transmission for this parasite disease [3]. Trichinellosis is caused incidentally when humans eat undercooked meat which contains Trichinella larvae [4]. Any type of Trichinella cannot infect humans if meat is properly cooked and frozen. The best method is to cook meat for one minute at 71°C Celsius. Furthermore, proper cooling to - 60°C for 2 minutes or - 55°C for 6 minutes typically kills Trichinella species. It has a tiny, light, thread-like appearance. Worms are tighter anteriorly than posteriorly in both male and female worms. Trichinella species start their life cycle by eating meat that has live larvae inside a cyst wall that is either raw or undercooked. The acidic environment (pepsin and hydrochloric acid) in the host's stomach causes the larvae to be freed from the cyst wall [5]. The quantity and frequency of exposure to contaminated meat affects the severity of Trichinellosis. There are three categories for the severity of the infection: light (0–10 larvae consumed), moderate (50–500 larvae consumed), and severe (more than 1000 larvae consumed). Humans and a number of other animal hosts that were infected with *Trichinella spiralis* developed an energetically costly liver organomegaly [6]. Other frequent side effects include CNS symptoms, cardiac inflammation, and/or pneumonia [7]. The most frequent symptoms are gastrointestinal (adult-caused) symptoms such nausea, vomiting, diarrhoea, fever, periorbital oedema, and myalgia, but In severe circumstances, further effects of larvae migration can include encephalitis [8]. When cells are subjected to external stimuli that harm them, the expression of genes is regulated [9,10]. According to Karmańska et al. [11] *Trichinella spiralis* induces apoptosis in the lamina propria of the small intestine as well as in striated muscle. Also; Boonmars et al. [12] who studies the role of apoptotic P53 during the formation of *Trichinella spiralis* cyst. This study has evaluated how experimental trichinellosis affected the rats' skeletal muscles and its relationship to apoptosis.

2. MATERIALS AND METHODS

2.1 Animals Groups

This study included 10 male albino rats (Rattus norvigicus), apparently healthy and free of parasitic infection and divided into 2 groups.

Group 1: Control group (-ve), in which normal healthy non-infected animal.

Group 2: Control Infected group (+ve), in which rats were challenged with 1000 larvae of *Trichinella spiralis*.

2.2 Sample Collection

At the end of the experiment, overnight fasted rats were anaesthetized with diethyl ether, dissection took place for 5 weeks and *T. spiralis* muscle larvae was obtained from laboratory bred infected rats. Larval preparation and extraction of inoculums were made as per Dunn and Wright [13].

2.3 Histopathological Assessment

Skeletal muscles were fixed in 10% formalin, and the fixed samples were paraffin sectioned then examined using haematoxylin and eosin stains, as described by Tousson [14].

2.4 Determination of Apoptotic Markers Expressions

Apoptotic P53 proteins and anti-apoptotic Bcl2 immunoreactivities were made in fixed tongue sections, which were evaluated using the avidin-biotin complex method [15 and 16].

3. RESULTS

3.1 Histopathological Examination

The histological changes in the rat skeletal muscle fibers in control and infected group have

been presented in Fig. 1. Normal myofibrillar structure with striations that consist of bundles of elongated were observed in the skeletal muscles in control group. Skeletal muscles in infected group showed diffuse degenerative changes all over the muscle fibers with massive numbers of *T. spiralis* encysted larvae that were surrounded by a collagen capsule and mild inflammatory cellular infiltration (Fig. 1B & 1C).

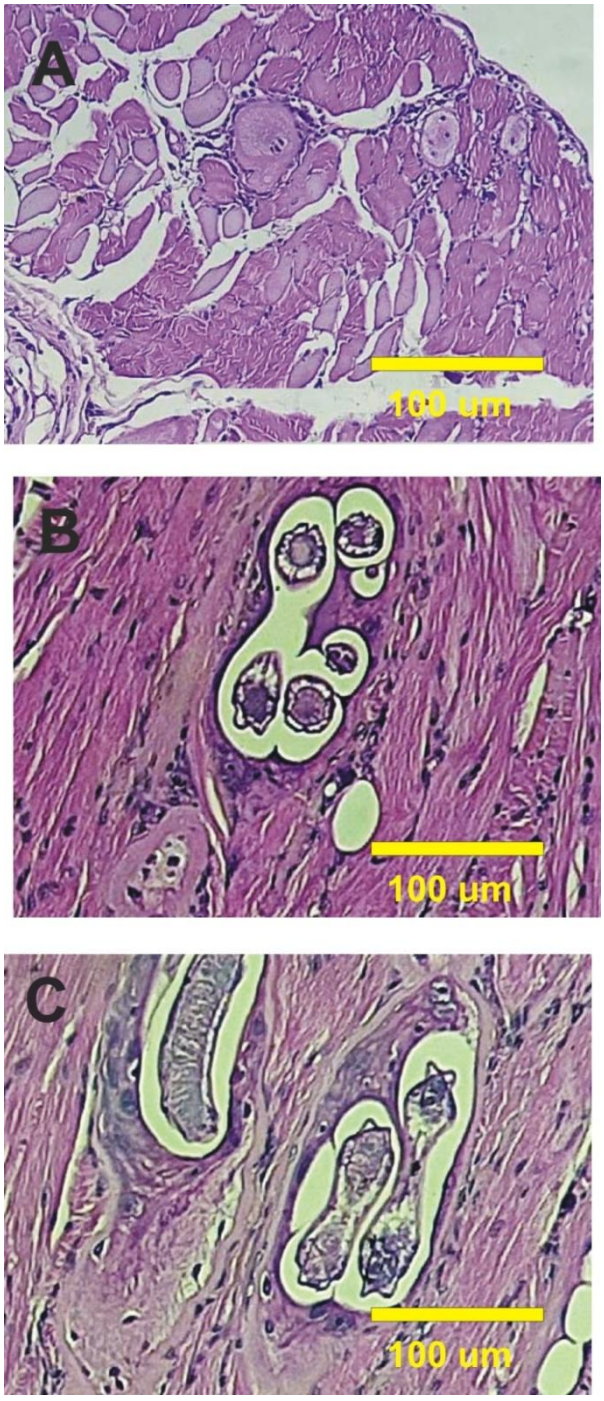

Fig. 1. A Photomicrograph of skeletal muscle fibers section in control and infected experimental groups. A: Normal myofibrillar structure with striations in skeletal muscles in control group. B&C: Muscle fibers with massive numbers of *T. spiralis* encysted larvae; encysted larvae were separated from thin cyst wall by spaces, muscle tissue has mild inflammatory cells

3.2 P53 Expression

The p53 expressions in the rat skeletal muscle fibers in control and infected groups are represented in Fig. 2. Negative or faint positive positive reactions for p53 expressions were detected in skeletal muscle fibers in control group, while moderate to strong positive reactions for p53 expressions were observed in skeletal muscle fibers in infected group (Fig. 2B & 2C).

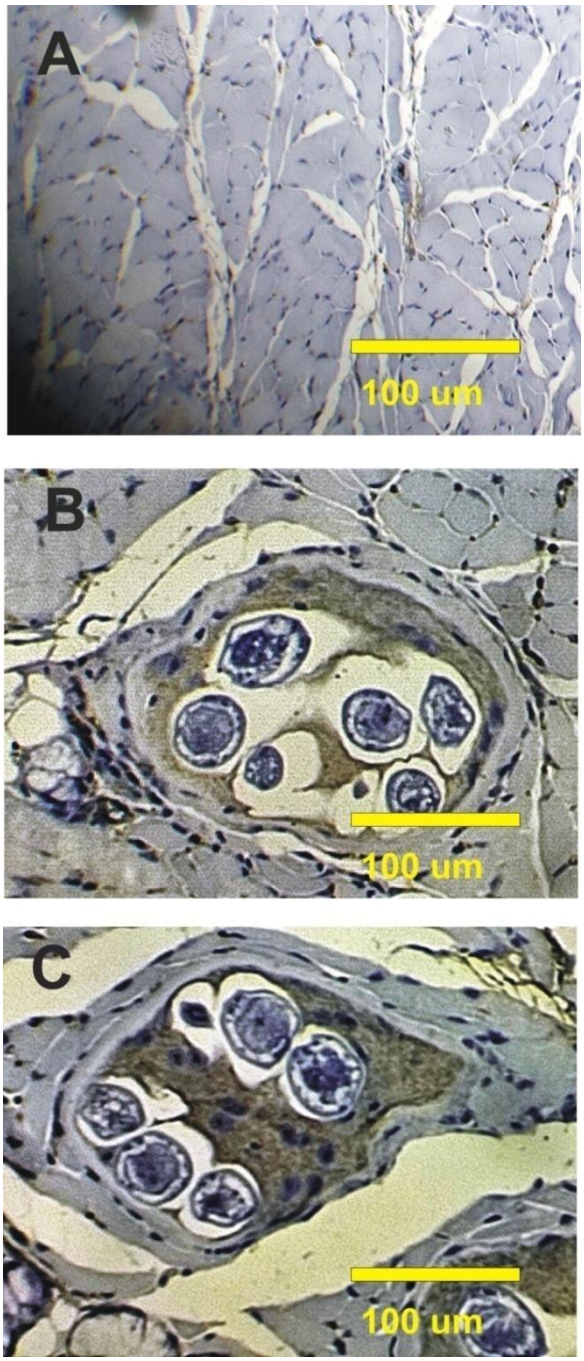

Fig. 2. A Photomicrograph of skeletal muscle section stained with P53 in section in control and infected experimental groups. A: Rat muscle fibers in control showed fine positive reactions for p53 expressions. B&C: Strong to moderatepositive reaction for P53 in muscle fibers of infected rats with *T. spiralis* in encysted larvae and intact muscles

3.3 Bcl2 Expression

The Bcl2 expressions in the rat skeletal muscle fibers in control and infected groups have been depicted in Fig. 3 Strong positive positive reactions for Bcl2 expressions were detected in skeletal muscle fibers in control group, while mild positive reactions for Bcl2 expressions were observed in skeletal muscle fibers in infected group (Fig. 3B & 3C).

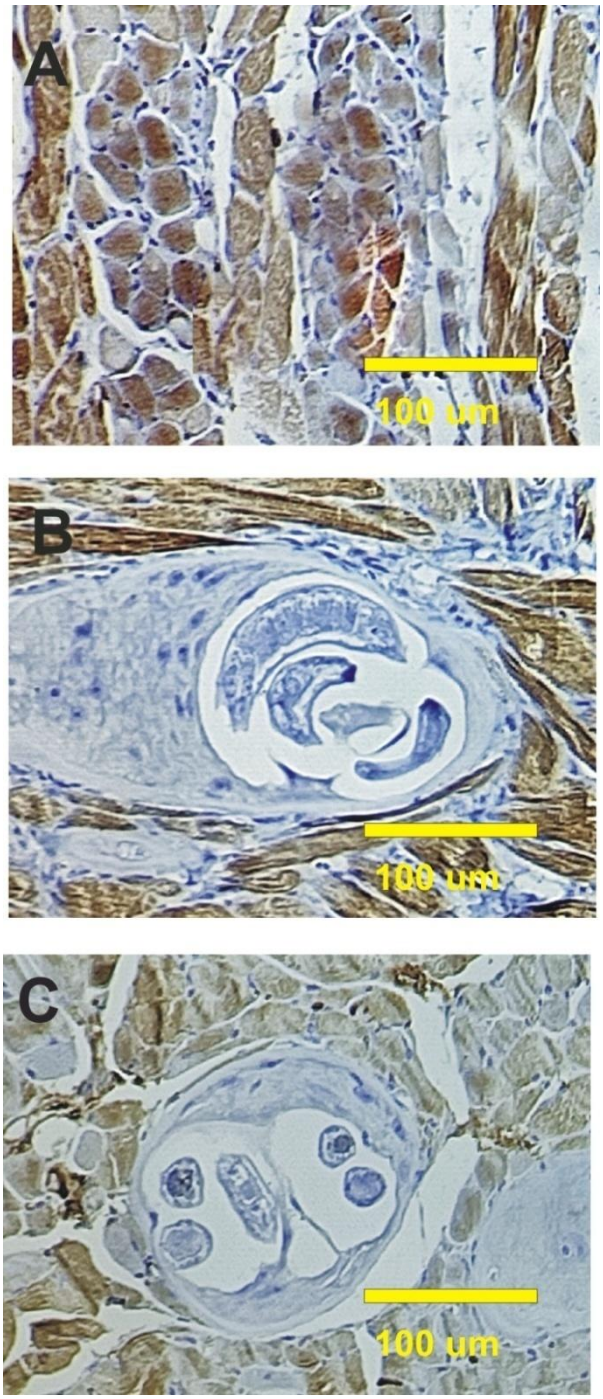

Fig. 3. A Photomicrograph of muscle section stained with Bcl2 in control and infected experimental groups. A: Rat muscle fibers in control group showed strong positive reactions for Bcl2 expressions. B&C: Mild positive reaction for Bcl2 in muscle fibers of infected rats with *T. spiralis* in encysted larvae and intact muscles

4. DISCUSSION

Apoptosis, a type of host-cell death, frequently causes tissue damage in the deaths caused by parasite infections. But rather than being harmful, parasite-induced apoptosis might promote host survival. different than the direct injury brought on by the parasite itself, there are a number of different mechanisms that contribute to the tissue destruction in trichinellosis. One of the main causes of this damage is the oxidative stress state that results from Trichinella infection, as demonstrated by the increased production of numerous stress indicators [17]. Therefore current study aimed to study the the effects of experimental trichinellosis on rat skeletal muscles damage. According to recent findings, *Trichinella spiralis* caused marked muscle fibrosis and widespread degenerative changes throughout the muscle fibres in experimentally infected rats' skeletal muscles. On the other hand, each encysted larva was surrounded by a collagen capsule and mild inflammatory cellular infiltration made up primarily of lymphocytes, eosinophils, and plasma cells, with areas of coagulative necrosis. Our results agree with Basyoni and El-Sabah [18] who found that; muscle cells in *Trichinella spiralis* infection revealed degenerative changes all over the muscle fibers and diffuse with massive numbers of *T. spiralis.*

P53 may indeed play some role in mitochondria-mediated apoptosis in the basophilic cytoplasm, but it is not indispensable for the induction of apoptosis in the basophilic cytoplasm. Parasites can provoke apoptosis by two means; either directly via active mediators or indirectly through the mediators of inflammation. Current results revealed that; skeletal muscles that infected with *Trichinella spiralis* induced a significant elevation of the apoptotic protein p53 and significant depletion in Bcl2 comparing with the control one. Our findings may be explained by Karmańska et al. [11] who reported that *Trichinella spiralis* induces apoptosis in the lamina propria of the small intestine and also in striated muscle. Also; Boonmars et al. [12] studies the role of apoptotic P53 during the cyst formation of *Trichinella spiralis*. These results also agreed with Etewa et al. [19] and Ibrahim et al. [20] who reported that infected rats with that *T. spiralis* showed degenerative changes with marked apoptosis.

5. CONCLUSION

In the present investigation, skeletal muscles that were infected with *Trichinella spiralis* induced diffuse degenerative changes all over the muscle fibers with massive numbers of *Trichinella spiralis* encysted larvae that were surrounded by a collagen capsule and mild inflammatory cellular infiltration; in addition to a significant elevation of the apoptotic protein p53 and significant depletion in bcl2 compared control.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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