

Effect of lysate of *Sarcocystis gigantea* in rats

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(Received June 22, 2009; Accepted June 30, 2010)

Abstract

This study was conducted to examine the effect of lysate isolated from macrosarcocysts of *Sarcocystis gigantea* after inoculation into albino rats to follow up the pathological effect on heart, lung and skeletal muscle fibers. The results showed the presence of pathological changes, represented by hyperatrophy, congestion, associated with hyaline degeneration in myocardium of heart. In lung tissues severe congestion in each right and left lobes concomitant with emphysema, thickening was observed in wall of bronchioles and blood vessels at 3-7 days have been seen. In skeletal muscles the result revealed the presence of multinuclear areas which infiltrated with inflammatory cells accompanied by hyaline degeneration in muscle fibers at 3-7 days after treatment.

Keywords: Sarcocyst; Lysate; Pathological lesions; Rat; Macrosarcocysts.

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تأثير اللايست لطفيلي *Sarcocystis gigantea* في الجرذان

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الخلاصة

تضمنت الدراسة الحالية معرفة تأثير اللايست المعزول من الاكياس العضلية كبيرة الحجم لطفيلي المكيسات العضلية *Sarcocystis gigantea* بعد حقنه في الجرذان البيضاء لملاحظة التأثيرات المرضية على كل من القلب والرئة والعضلات الهيكلية. اظهرت النتائج وجود تغيرات مرضية عيانية ونسجية تمثلت بوجود تضخم Hyperatrophy واحتقان Congestion يصاحبهما تنكس زجاجي Hyaline degeneration في القلب، اما في الرئتين فقد لوحظ وجود الاحتقان الشديد في كل من الفص الايمن والايسر يرافقها نفاخ Emphysema وتتخذ جدار القصيبات وجدار الاوعية الدموية عند اليوم الثالث واستمر الى اليوم السابع. اما في العضلات الهيكلية فقد لوحظ وجود العديد من اليور النخرية المترسحة بالخلايا الالتهابية مع التنكس الزجاجي في الالياف العضلية في اليوم الثالث واستمر الى اليوم السابع من المعاملة.

Introduction

The sexual reproduction of the *S. gigantea* occurs in the intermediate hosts (sheep, goats, cattle or horses), eventually forming cysts in the skeletal muscle (Sarcocysts) that are filled with bradyzoites. Lysates is a bradyzoites extract of a sexual stages of *Sarcocystis spp.* are toxic when injected into various mammalian hosts such as rabbits and rats (1). The substances imparting toxicity have not been chemically defined and their mechanism of action, a lysate

of *S. cruzi* bradyzoites was shown to possess lectin activity separable from toxin activity (2). The lysate produced a reaction in rabbit similar to that produced by bacterial endotoxin, which have the ability to stimulate macrophages to produce the monokine called cachectin or tumor necrosis factor TNF, the TNF has been implicated as a cause of a profound wasting, loss of body weight, emaciation, this effect results from the action of TNF- α or other cytokines, either directly suppressing insulin-like growth factor 1 (IGF-1) in various tissue or acting indirectly via reduced

feed intake or by action on the pituitary gland (3,4). Lysates which has been regarded as the cause of eosinophilic myositis (EM) syndrome, which has been described as a subacute to chronic inflammatory process of skeletal and cardiac muscle particulate and soluble antigen from schizont and merozoites may attract the large number of cells observed perivascularly and interstitially (5).

The present study was to explore the effect of lysate which was isolated from *S. gigantea* on weight and pathology in albino rats.

Materials and methods

Twenty male 4-5 weeks old albino rat were used, they were divided into two equal groups. Group 1 (G1) is the control group (not inoculated with lysate), Group 2 (G2) was inoculated, intraperitoneally (IP) with lysate of *S. gigantea* which was prepared according to (2), each rat received about 1 ml the lysate contained 1 mg total protein per ml, these animals were revealed in laboratory house of Vet. Medicine. Rats weight obtained before and after inoculation during 3-7 days, rats were sacrificed 3-7 days post inoculation to determine the gross pathological changes and specimens were taken from lung, heart and skeletal muscle and fixed in 10% neutral formaline, processed routinely by ethyl alcohol and xylol and 4-6µm thick sections were prepared and stained with hematoxylin – eosin stain (H&E) (6).

Results

Animal weight

Table (1) showed reduction in weight of rats treated with lysate after 3 days in G2 as compared with control group. This reduction continuously at seven days the mean of weight gains reached 181.2 gm as compared with control rats which reached to 204.2 gm.

Gross pathological finding

Macroscopic findings

After 3 days of inoculation of lysate into rats, gross pathological change which occur characterized by: In lung, lesions represented by severe congestion with some areas of gray hepatization in both right and left lobes. These lesions continuously at 7 days after inoculation. Heart, showed cardiomegaly at 3-7 days of inoculation. Skeletal muscles revealed multifocal discrete 2 mm-5 cm in diameter, greenish to yellowish nodules within the affected muscles.

Histopathological findings

Histological sections of lungs rat treated with lysate at 3 days showed multifocal areas of necrosis infiltrated with neutrophils and macrophags and some eosinophils in addition to hemorrhage, thickening in wall of alveoli and

emphysema (Fig. 1 and 2). Also at seven days the lesions that mentioned above are seen, in addition to thickening in wall of bronchiol, hyperplasia in lymph node around bronchi as well as accumulation of inflammatory exudate in lumen of bronchi (Fig. 3) pulmonary arteries, revealed hypertrophy of endothelium and thickening in wall due to proliferation in vascular smooth muscle cells in the media (Fig. 4), congestion of blood vessels and atelectasis also noticed (Fig. 5). Histopathological section of heart revealed congestion of blood vessels, vacuolar degeneration of myocyte associated with infiltration of inflammatory cells after 3 days (Fig. 6), while after 7 days edema and hyaline degeneration have been seen (Fig. 7).

Table 1 : Effect of lysate on rat weight via three and seven days.

Period of inoculation (days)	Animal weight (gm)	
	Group 1 (control)	Group 2 (treated with lysate)
3	201	203
	250	207
	216	225
	213	210
	210	213
	Mean = 218	Mean = 211.6
7	205	140
	220	180
	201	201
	190	190
	205	195
	Mean = 204.2	Mean = 181.2

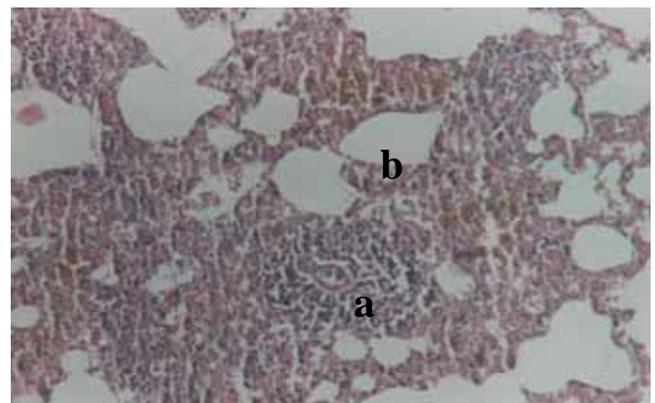


Fig. 1: Histomicrograph of rats lung inoculated with lysate isolated from *S. gigantea* after 3 days showed inflammatory foci (a) emphysema (b). H&E. 400X.

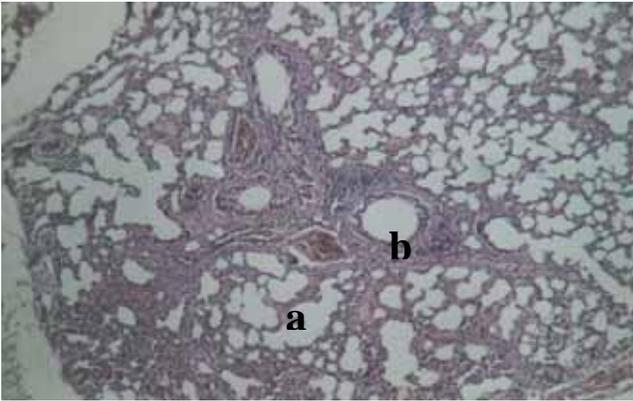


Fig. 2: Histomicrograph of rats lung inoculated with lysate isolated from *S. gigantea* after 3 days showed thickening in wall of alveoli (a) and bronchiol (b). H&E. 400X.

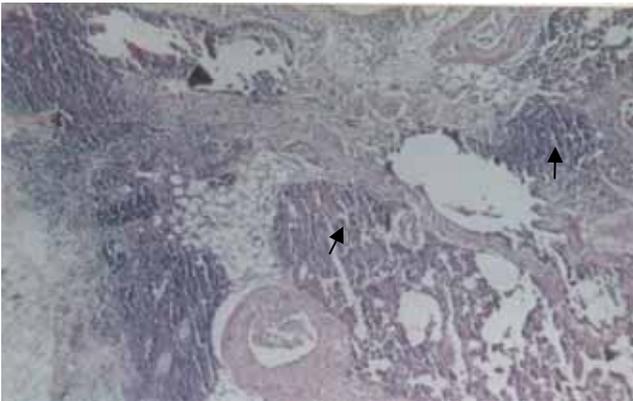


Fig. 3: Histomicrograph of rats lung inoculated with lysate isolated from *S. gigantea* after 3 days revealed hyperplasia in lymphoid follicle of bronchiol (→). H&E. 400X.

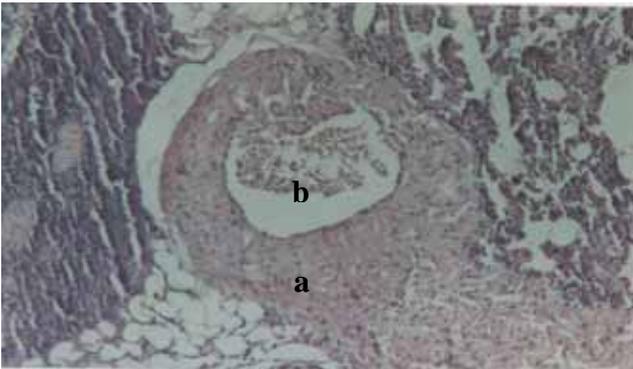


Fig. 4: Histomicrograph of rats lung inoculated with lysate isolated from *S. gigantea* after 7 days, revealed thickening in the wall of pulmonary blood vessels (a) with congestion (b). H&E. 400X.

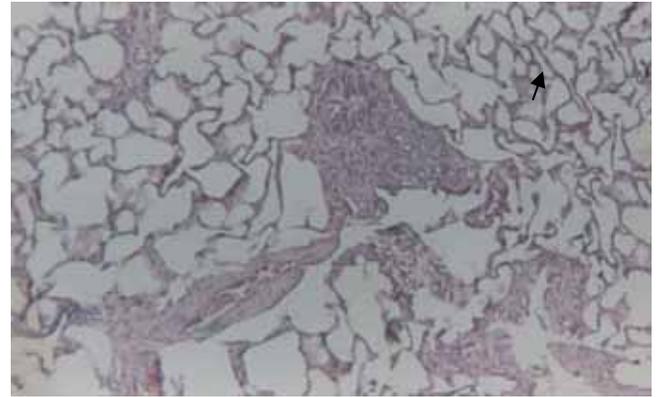


Fig. 5: Histomicrograph of rats lung inoculated with lysate isolated from *S. gigantea* after 7 days, revealed atelectasis in alveoli (→). H&E. 400X.

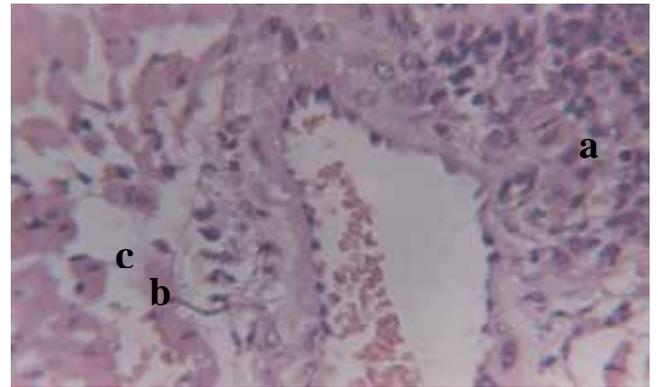


Fig. 6: Histomicrograph of rats heart muscle inoculated with lysate isolated from *S. gigantea* after 7 days, showed infiltration of inflammatory cells (a), hyaline degeneration (b) and edema (c). H&E. 400X.

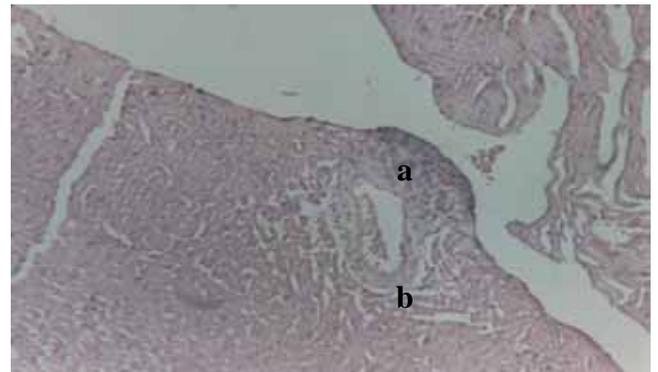


Fig. 7: Histomicrograph of rats heart inoculated with lysate isolated from *S. gigantea* after 7 days showed infiltration of inflammatory cells (a) and thickening in the wall of blood vessels (b). H&E. 400X.

Histopathological sections of skeletal muscles showed lesion, characterized by presence of multifocal necrosis each one infiltrated by inflammatory cells represent by eosinophils and neutrophils in addition to edema and hyaline degeneration of myofibriles at 3-7 days post inoculation (Fig. 8 and 9).

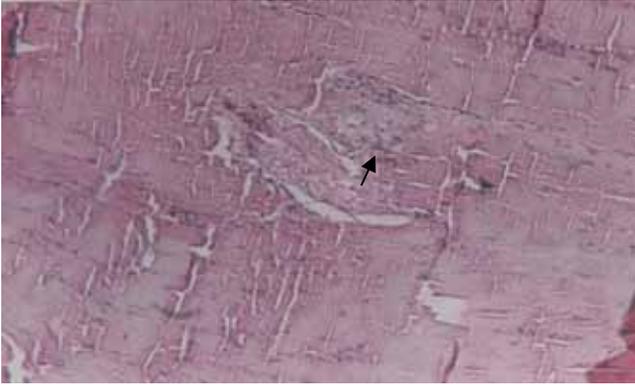


Fig. 8: Histomicrograph of rats skeletal muscle inoculated with lysate isolated from *S.gigantea* after 3 days revealed presence of necrotic foci infiltrated with inflammatory cells (→). H&E. 400X.

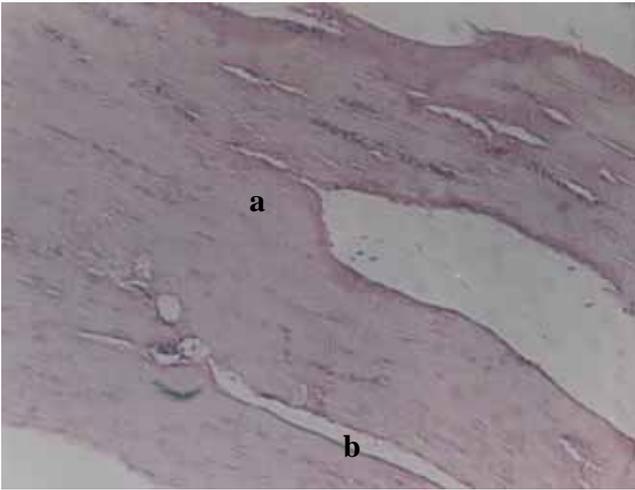


Fig. 9: Histomicrograph of rats skeletal muscles inoculated with lysate isolated from *S. gigantea* after 7 days revealed hyaline degeneration in myofibril (a) and edema (b). H&E. 400X.

Discussion

The significant reduction in weight of rat inoculated with lysate isolated from *S. gigantea*, was very close to (7,8) who reported that lysate have ability to release TNF- α which is responsible for the occurrence of anorexia and

cachexia, it has been observed that effect of lysate of the sarcocysts bradyzoite resembles to the acute phase of sarcocystosis in calves (9), they reported that those result from interleukin-1(IL-1) suppression of food intake and reduction of gastric motility (10) as well as reduced gut motility possibly due to elevated somatostatin (SSN) levels (11), elevated levels of plasma glycerol and free fatty acids may also contribute to decreased appetite, IL-1, through the stimulation of lymphocytes to produce IL-2, stimulates adipose tissue lipolysis ; TNF- α by suppressing synthesis of lipoprotein lipase, also contributes to adipocyte lipolysis, also it may be correlated with abnormal nitrogen metabolism that appear to result from the malabsorption and internal recycling of nitrogen lysate (acute sarcocystosis) associated with skeletal muscle wasting resulting from muscle protein degradation to produce amino acids needed during the acute phase response. TNF- α also blocks myogenesis, preventing skeletal muscle from competing for amino acids and ultimately inhibiting muscle growth (4).

Pathologically, the result demonstrated the occurrence of stages of pneumonia in lung in addition to congestion of blood vessels and thickening in wall, this indicated the effect of lysate to stimulate the release of chemical mediator which lead to proliferation of vascular smooth muscle cell this result agreed with (12,13).

The toxin may effect on host's immune response that lead to changes in the concentration of cytokine signal TNF- α , IL-6 and IL-1 that secreted from macrophages (14). In heart the results illustrated cardiomegaly, fatty change in addition to lymphocytic myocarditis, the result indicate that lysate of *S. gigantea* bradyzoites induced releasing of oxygen free radical which responsible of lipid peroxidation represented by fatty change appearance, and (15) refers to TNF- α during acute phase of sarcocystosis coincided with a reduction in the number of circulating leukocytes and massive infiltration of mononuclear cells, especially macrophages and lymphocytes, into various tissues, and this result agreed with (16,17).

Results of skeletal muscles indicated the presence of eosinophilic myositis associated with necrosis, this results correlated with previous study (18-21) and also agreed with the results of (1,22) who refered to the increases in the level of alanine amino transferase ALT to ensure the muscle destruction due to ability of lysate to stimulate the chemical mediators to induce this lesion. The pathogenesis of EM varies; a correlation between serum IL-5 levels and disease severity (23) suggests a role for this interleukin species in eosinophil activation.

The pathological consequences resulting from overproduction of cytokines TNF- α leading to a cascade of shock-like symptoms that could effect the survival of the hosts.

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