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Sarcotoxin Effect on Leukocytic Finding and Phagocytic Activity in Mice

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Abstract: The total leukocytes counts in three groups of inoculation (3, 5 and 7 mg) with Sarcotoxin of *Sarcocystis gigantea* showed severe depression in their numbers comparing with control group (2200, 2375, 1950 and 5500) respectively, but there was no changes in effect between two post inoculation period. Appearance of lowering in numbers of lymphocytes, neutrophils, monocytes, eosinophils in inoculation groups comparing with control one was significantly. Blood stream clearance of Nitroblue Tetrazolium stain (NBT) has been evaluated to establish the phagocytic activity of neutrophils from mice experimentally inoculated with Sarcotoxin, the phagocytic cells calculated and there was decreasing in numbers of these cells in the treatment groups (inoculation) comparing with control one significantly (316, 284, 225 and 778), respectively. Hours of post inoculation time had no influences on these parameters (total, differential leukocytes count and phagocytic activity).

Key words: Sarcotoxin, leukocytic, phagocytic, mice, inoculation, influences

INTRODUCTION

S. gigantea species is common, ubiquitous, sporozoan parasites of sheep, it is macrocyst species which produce grossly visible cysts or Macrosarcocysts (ME) in muscle which is often found in oesophageal muscle and are an occasional cause of carcase condemnation in some countries, it have worldwide distributions, including Iraq, specially in Mosul (Kia, 2003; Al-Hyali and Daoud, 2002; Al-Taee and Al-Hyali, 2007).

The cyst is large enough to be seen with the naked eye. Although, apparently non-pathogenic, the cyst contains a very powerful toxin called, Sarcotoxin or Sarcocystin, which if injected Intraperitoneally (IP) to rats, is lethal in doses as low as 1 mg kg^{-1} B.W (Al-Taee and Al-Hyali, 2007). Functionally, macrophages are a diverse group of cells, acting at many levels in their response to foreign materials. They are important in the development and regulation of the immune response since, they can act both as accessory cells and suppresser cells, in addition, macrophages appear to play a role in the resistance of the host to the attack by the parasites (Herscowitz *et al*., 1981).

The aim of the current study is to identify the effect of Sarcotoxin on the leukocytic finding and evaluate the phagocytic activity of neutrophils that could be displayed by Sarcotoxin.

MATERIALS AND METHODS

Sarcotoxin

Preparations of ME: The protocol of ME of *S. gigantea* from naturally infected sheep as described by Saleque *et al*. (1991).

Preparations of ME crude proteins: Ammonium sulfate has been employed for precipitating proteinous materials from cold homogenate extracts (Robyt and White, 1987), the proteinous materials were lyophilized and kept at 4°C for next investigations.

Fractionation of proteins: Were prepared and set up as described by Al-Taee and Al-Hyali (2007) done with some modifications; using 25 g of sephadex G-50 gel. A column of dimensions (126×1.3 cm) was used in packing of the gel slurry to 120 cm or a volume of 159 and 2 mL concentrated sample of proteinous materials of ME was slowly poured on the wall of the column, using a pipette into the top of the gel, 2 mL distilled water as a washer was added after observing that the sample has completely entered the gel bed. Elution of the proteinous materials of ME was eventually collected at flow rates of 2 mL/1 min the proteinous fractions obtained by gel filtration chromatography were dried in a powder by lyophilizer.

Experimental design: Twenty healthy laboratories breed white mouse of 2 month of age, were randomly allocated

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into four groups, five mouse to each one, group 1, group 2 and also, groups 3 was inoculated with 3, 5 and 7 mg mL^{-1} of Sarcotoxin IP, respectively, while control group 4 received IP with BPS only.

Leukocytic pictures: Blood samples were collected from retrobalber plexuses of each mouse after 2 and 4 h post inoculation to monitor Total Leukocytic Count (TLC) and Differential Leukocytes Count (DLC) were determined as described by Coles (1986), then absolute No. of leukocytes were calculated.

Evaluation of phagocytic activity: By using 2% NBT stain was prepared as Metcalf *et al*. (1986), 0.05 mL of NBT stain was mixed with the same amount of heparinized blood of each mouse in all groups in siliconized test tube. The mixture was incubated at 37°C for 30 min. Then, smears were made into clean slides, stained with 10% Giemsa stain and examined under oil immersion lens. Cells taken blue colour as a result of formazan reduction and those, which remained intact were counted up to 100 cells (Gaspar *et al*., 1992). Calculation was based on the following equation:

$$\text{Phagocytic index} = \frac{\text{Number of phagocytosed cell}}{\text{Total number of phagocytic cell}}$$

After that we calculated the absolute number of phagocytic cells.

Statistical analyses: Differences among control and treated groups were tested using student's test (t-test) for differences between 2 groups and Analysis of Variance (ANOVA) in differences between means. Level of significance at $p \leq 0.05$ (Bruning and Kintz, 1997).

RESULTS AND DISCUSSION

Leukocytic findings: The mean total and the absolute number of leukocytes counts after 2 and 4 h depicted in (Table 1 and 2) revealed a significant decline in the mean of TLC and absolute number of lymphocyte, neutrophil, monocyte and eosinophil in groups 1, 2 and 3 as compared with control group.

The absolute No. of phagocytic cell positively stained with NBT stain after 2 and 4 h showed a significant decline (316 cell in G1, 284 cell in G2 and 225 cell in G3) as compared with control group 4 (Table 3 and 4).

This research has clarified lowering which occurred in TLC of three groups which was inoculated with (3, 5 and 7 mg) of Sarcotoxin when it compared with control group after 2 h post inoculation to each dose, the results showed that the lowest numbers of TLC appeared in G3, which received 7 mg of Sarcotoxin, this point could be explained that the decrease in TLC may happened due to reduction numbers which had came out in lymphoytes, neutrophils, monocytes and eosinophils. So as Saha *et al*. (1985) they said that there is interaction between *S. gigantea* Lectin (SGL) and Toxin-containing Fractions (SGTF), that the later could suppressed the mitogenic capacity of SGL by 60-90% that the bradyzoic merozoite extract contains lectin toxin components (Dubey *et al*., 1989), this lectin is untoxic for rabbits and appears as an immunomodulator. However, it could be shown that the lectin activity counteracts the activity of Sarcotoxin (Tietz *et al*., 1986) and SGTF interacts with mononuclear cells differently than SGL, particularly by interfering with the mitogenic lectin, which playing role in increasing lymphocytes proliferation approximately, 71 fold over the background

Table 1: Effect of Sarcotoxin on TLC and DLC after 2 h in mice

		Absolute no. of leukocytes/mm^3			
Groups	TLC/mm^3	L	N	M	E
1	2200±0.125*	1020±0.033*	920±0.011*	65±0.006	98±0.002*
2	2375±0.100*	1324±0.031*	912±0.024*	65±0.006	0±0.000
3	1950±0.117*	1228±0.042*	603±0.029*	11±0.006	0±0.000
4	5500±0.025	2970±0.055	2079±0.015	149±0.006	220±0.007

Values are expressed as means±SE of 5 mouse per groups. L = Lymphocyte, N = Neutrophil, M = Monocyte, E = Eosinophil, *Means with the same column are significantly compared with control, group $p \leq 0.05$

Table 2: Effect of Sarcotoxin on TLC and DLC after 4 h in mice

		Absolute no. of leukocytes/mm^3			
Groups	TLC/mm^3	L	N	M	E
1	2100±0.111*	1005±0.083*	915±0.031*	56±0.005*	0±0.000
2	2300±0.134*	1316±0.077*	906±0.026*	54±0.008*	0±0.000
3	1910±0.120*	1209±0.064*	589±0.040*	11±0.001*	0±0.000
4	5584±0.117	2994±0.094	2087±0.031	235±0.009	235±0.002

Values are expressed as means±SE of 5 mouse per groups. *Means with the same column are significantly compared with control group, $p \leq 0.05$

Table 3: Effect of Sarcotoxin in phagocytic activity after 2 h in mice

Groups	Absolute no. of phagocytic cell/mm^3
1	316±0.17*
2	284±0.36*
3	225±0.28*
4	778±0.31

Table 4: Effect of Sarcotoxin in phagocytic activity after 4 h in mice

Groups	Absolute no. of phagocytic cell/mm^3
1	300±0.10*
2	281±0.14*
3	210±0.20*
4	797±0.25

Values are expressed as means±SE of 5 mouse per groups. *Means with the same column are significantly compared with control group, $p \leq 0.05$

(Tietz *et al*., 1989; Fayer and Elsaaaer, 1991). That reducing in concentration of TNF-α during the acute phase of infection coincided with the reduction in number of circulating leukocytes and massive infiltration of mononuclear cells (Tracey and Cerami, 1989) and this prove the reality of our results.

This study has showed decreasing in monocytes, neutrophils and eosinophils numbers due to direct depression effect of SGTF on bone marrow, production and proliferation of these cells right there (Lindsay *et al*., 2006). In the same manner, there is no any significant changes between both results in total leukocytic count according to time post inoculation. One of earliest functions of macrophages is their ability to take up particulate materials by phagocytosis, it has also been known that macrophages from animals infected with bacteria and parasites are morphologically and functionally modified, these changes include tumorcidal, increased microbicidal and phagocytic activates (Herscowitz *et al*., 1981). Parasitic agents such as *Toxoplasma* and *Besnoitia* sp. can induce the activated macrophages, the activated macrophages perform certain functions more efficiently than their normal resident counterparts. These functions may include intracellular microbicidal capacity and *in vitro* phagocytic capability. In phagocytic activity which revealed in this research we found that there is a progressive lowering in the absolute numbers of phagocytic cells using NBT related with hours post inoculation of Sarcotoxin IP in mice, that be very close with (Freire and Wilson, 1995) watches, due to decrease the number of neutrophil and its activity

CONCLUSION

The present study conclude that there is powerful depression effect of Sarcotoxin inoculate in TLC, also we had seen the depression effect of this toxin on the absolute number of phagocytic cells that engulphed NBT stain, which revealed the occupation forces that parasite shows to settle down in the host's organs.

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