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The Influence of Some Physio-Chemical Properties of Sarcotoxin in Rats

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Abstract: The effect of physical (heat, pH level, precipitation and dialysis) and chemical (enzymes) evaluation of sarcotoxin toxicity were investigated. After the preparation of the macrosarcocysts extract, biochemical analysis using chromatography was carried out to characterize and define the sarcotoxin of *Sarcocystis gigantean*. The study involved 51 albino rats, which were divided into 6 groups. Result showed that inoculation of a 1 mg mL^{-1} concentration of protein (sarcotoxin) of Macrosarcocysts Extract (ME) Intraperitoneally (IP) into the rats was lethal. The findings also revealed that sarcotoxin was thermolabile at 70°C for 30 min. Protease and trypsin caused inactivation, while papain had no toxic effect. Sarcotoxin was found to be non-dialyzable, but perceptible. There was also no effect of a pH level 6-9, while acidity of 4-5 altered the toxicity however, pH of 10 only had a partial toxic effect.

Key words: Sarcotoxin, properties, rats, pH, heat, enzyme

INTRODUCTION

Sarcocystosis is azoonotic disease, which caused by species of *Sarcocystis*, an intracellular protozoan parasite in the phylum Apicomplexa, family *Sarcocystidae*. *Sarcocystis* are oval, whitish cysts that vary in size from microscopic to visible. They are filled with hundreds to thousands of bradyzoites (Dubey *et al*., 1989).

Sarcocystis sp. follow a two-host life cycle. Cattle sheep, goats and other animals may act as intermediate hosts and become infected by ingesting sporocysts or oocysts from the feces of definitive hosts (dogs, cats, etc.). The final hosts acquire the infection by ingesting sarcocysts (muscle cysts) with the tissue of the infected intermediate hosts. *Sarcocysits* are common in the musculature of many species of livestock, including sheep (Al-Hyali and Daoud, 2002; Kia, 2003).

Abattoir surveys had recorded infections by macroscopic cysts of *Sarcocystis gigantean* in 94.8% of examined ovine in Mosul city (Al-Hyali and Daoud, 2002).

The hypothesis that the wall of some macrosarcocysts rupture, being leaky and release toxin (Saha *et al*., 1985). *S. gigantean* sarcocysts contains very powerful toxin called sarcocystin or sarcotoxin, when it injected IP to rats is lethal in dose as low as 1 mg kg^{-1} BW (Al-Taee and Al-Hyali, 2007). The purposes of the current study were to characterize some properties depending upon physicochemical characters of sarcotoxin like effect of heat pH level, precipitation, dialysis and enzymes of *S. gigantean* obtained from sheep experimentally inoculated in the rats.

MATERIALS AND METHODS

Preparations of sarcotoxin: Esophagus of ovine slaughtered at Mosul abattoirs were macroscopically examined and those found heavily infected with macrosarcocysts of *S. gigantean* is collected (ME) were removed and processed as described by Saleque *et al*. (1991) and Al-Hyali *et al*. (2009). The crude proteins of (ME) is obtained by precipitating with Ammonium sulfate and the proteinous fractions obtained by gel filtration chromatography using 25 g of sephadex G-50 gel. A column of dimensions (126×1.3 cm) was used in packing of gel slurry to 120 cm or a volume of 159 mL. The sarcotoxin (fraction a) was then dried in a powder by lyphilizer and stored at 4°C before use in experiments (Robyt and White, 1987; Al-Hyali *et al*., 2009).

Animals: Six experiments were performed. The total number of Albino rats used in all experiments where fifty one, weighting 95-200 g of 3-4 months old. The rats were kept in wire cages fed on standard diet, given tap water, and housed in thermally regulated cages of 22-25°C light was controlled by 12 h light and 12 h dark cycle. Rats were randomly divided into six groups.

Experimental design

Experiment 1: Twelve rats, three rats were used for each temperature degree.

Experiment 2: Nine rats, three rats were used for each enzyme.

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Experiment 3: Twenty one rats were divided into seven groups of three rats each for each pH level.

Experiment 4: A group of three rats, for precipitation of dialysis.

Experiment 5: Three rats were acted as untreated sarcotoxin injected controls (control +ve).

Experiment 6: Three rats were inoculated with only 1 mL of PBS (control –ve).

In the above experiments, all the rats were injected IP with 1 mg mL^{-1} of protein from peak a.

In the first experiment, the effect of heat on the sarcotoxin was tested by its treatment at 40, 50, 60 and 70°C in a water bath for 30 min. The samples were rapidly cooled in an ice bath and were kept at 4°C before its inoculating (IP) into twelve rats (Saleque *et al*., 1991).

In the second experiment, the effect of proteolytic enzymes (protease, trypsin and papain) on sarcotoxin were tested in nine rats. The enzyme treatment was carried out following the methods of (Jacks and Wu, 1974). The enzyme were dissolved in 0.03 MPBS (pH 7.4) at 1 mg mL^{-1} then added to the sarcotoxin in a ratio of 1:10. This mixture was incubated at 37°C for 24 h after covering with 0.5 mL toluene before use.

In the third experiment, the effects of pH changes on sarcotoxin toxicity were studied. Sarcotoxin was subjected to seven different pH levels (pH 4-10) as mentioned by Saleque *et al.* (1991).

In the fourth experiment, the effect of precipitation and dialysis on sarcotoxin toxicity was tested. A group of six rats was inoculated IP with sarcotoxin, which was precipitated and dialysed using 80% ammonium sulphate as recommended by Koupal and Deibel (1975).

Criteria for evaluation of results: The main criteria for evaluation and determining the toxic effects of the sarcotoxin included clinical signs changes, occurrence of local reactions and death numbers of rats experimentally injected with the sarcotoxin.

RESULTS

The result of gel chromatography showed in (Fig. 1 and 2). Table 1 shows the effect of different temperatures on sarcotoxin stability. Its was found that the sarcotoxin remained stable up to 60°C for 30 min. however, the lethal effect of sarcotoxin was completely destroyed at ≥70°C. in comparison with –ve control group, which manifested normal.

The effect of enzymes on sarcotoxin inactivation is shown in Table 2. Its clear that trypsin and protease inhibit sarcotoxin, while papain has no any effect on sarcotoxin toxicity.

The results reveal that various level of pH (6-9) have no effect on the toxicity of sarcotoxin, on the other hand pH 4 completely inactivates its toxic effect. The effect of pH 10 was found to have partial toxicity due to the sole occurrence of clinical signs with no deaths among the

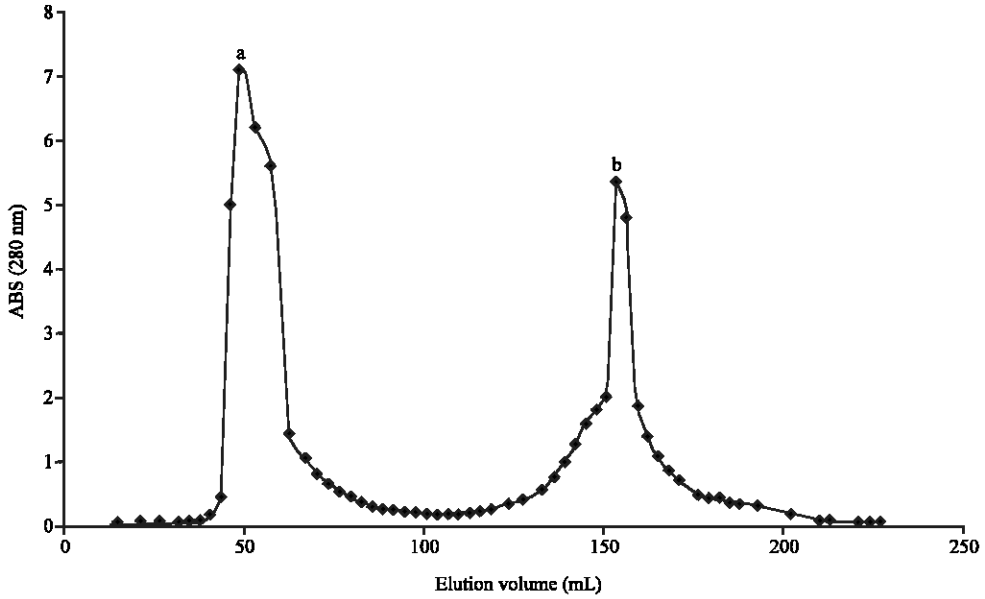

Fig. 1: Elution volume of the proteinous materials of macrosarcocysts extract in sephadex G-50

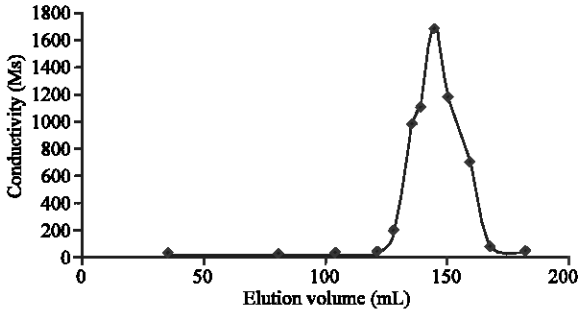

Fig. 2: Elution volume of the ammonium sulfate in sephadex G-50

Table 1: Effect of different degree of heat on sarcotoxin toxicity of *S. gigantean* injected to rats

Types of treatment (heat °C)	No. of animals	Time of treatment (min)	Observation
40	3	30	S+D
50	3	30	S+D
60	3	30	S
70	3	30	N

S = Showing singe, D = Death, N = Normal

Table 2: Effect of different enzymes treatment on sarcotoxin of *S. gigantean* toxicity in rats

Types of treatments (enzymes)	No. of animals	Time of treatment (h)	Observation
Trypsin	3	24	N
Protease	3	24	N
Papain	3	24	S+D

N = Normal, S = Showing singe, D = Death

Table 3: Effect of different pH level on sarcotoxin of *S. gigantean* toxicity injected IP in to rats

Types of treatments (pH levels)	No. of animals	Time of treatment (h)	Observation
4	3	4	N
5	3	4	S
6	3	4	S+D
7	3	4	S+D
8	3	4	S+D
9	3	4	S
10	3	4	S

Table 4: Effect of precipitation and dialysis on sarcotoxin of *S. gigantean* toxicity injected in to rats

Treatments	Type of treatment	No. of animals	Observation
Precipitation and dialysis	PDP	3	S+D
Untreated sarcocystin (+ve control)	Nil	3	S+D
Uninfected control (-ve control)	PBS	3	N

injected rats (Table 3). Regarding to the perceptibility and dialyzability of the toxin it was found to be perceptible, but not dialyzable (Table 4).

Clinical signs were not noticed until about 30 min after inoculation with sarcotoxin when animals exhibited loss of appetite, loss of activity, feeling dullness, weakness, itching at injection site, rough hair, nose itching with redness, hypersensitivity reaction of ears. However, other signs included increase heart and respiratory rates, abdominal pain with colic posture, impaired movement due to severe pain, muscle tremors. At progressive stage, signs of central nervous system may be involved such as saw like position, paresis of hind limbs, rolling and frequent pawning fallowed by convulsion and death.

DISCUSSION

Sarcocystosis is one of the most widespread muscle parasitoses of domestic as well as wild herbivorous and omnivorous animals in the world and cab be an important factor limiting animals production, losses occur from abortion, reduction milk yield and growth (Kia, 2003).

Intraperitoneally injection of rats with 1 mg mL^{-1} of sarcotoxin, for 30 min, showed severe clinical signs followed by death during the 24th h of injection. It is evident from the results that injection of 1 mg mL^{-1} of *S. gigantean* sarcotoxin is lethal to rats. The finding are in agreement with previous study reported by (Al-Taee and Al-Hyali, 2007). It is been suggested that rat could be very susceptible animal to the toxic effect of sarcotoxin of *S. gigantean*. On the contrary (Brose *et al*., 1989) that rabbits were susceptible to the extract of *S. gigantean* but not mice, also the result are different with those of (Saleque *et al*., 1991), who found that *S. fusiformis* extract in a concentration of 2 mg mL^{-1}, is lethal to rats and mice. Such discrepancy might be due to the variable nature of the toxin obtained from different species of *Sarcocystis*.

Physical character of sarcotoxin was evaluated by its exposure to different degree of temperatures. It was found that sarcotoxin was thermolabile. A proportional losing or reducing toxicity and or antigenicity is linked to increased exposure to the heat. Similar observations were reported by many researchers as Srivastava *et al*. (1986).

Result indicated that trypsin and protease, but not papain, altered the toxicity indicating the proteinous nature of the sarcotoxin. The present observations were quit analogous to the findings of (Saleque *et al*., 1991) relating to the effect of heat and enzyme on toxic effect of sarcotoxin.

The results of this experiment revealed that sarcotoxin was active within pH range of 6-9. The results were in agreement with previous study (Saleque *et al*., 1991).

Results of group four associated with (precepitation and dialysis) explained that sarcotoxin of *S. gigantean* is perceptible and dialyzable, possibly indicating its higher

molecular weight. These findings were in accordance with that reported earlier by Al-Taee and Al-Hyali (2007). The later researchers separated a peptide from the toxin of *S. gigantean* with a molecular weight of 27410 dalton. The current observation differs from the results of (Montag *et al*., 1989), who found that the toxin of *S. gigantean* with a molecular weight of 8000 dalton. These findings are acceptable and may be due to the difference in the techniques of separation, species of *Sarcocystis* and host specificity.

It was clear that several clinical signs were manifested after 30 min post-inoculated of the sarcotoxin into the rats. Such sings included listlessness, lethary, paresis, the abdominal discomfort followed by recumbence. Loss of nervous system functions were these involve, while were terminated by death, which was preceded by brief convulsive seizure. These results were in correspondence to others (Al-Taee and Al-Hyali, 2007), who found that sarcotoxin had a toxic affect on the central nervous system, heart, lungs and intestines.

CONCLUSION

Being authrozoonosis and due to its high incidence rate in the meat of slaughtered livestock at Mousl abattoir. It can be concluded that heating of the flesh at 70°C is effective and necessary to render the meat safe for human consumption.

REFERENCES

Al-Hyali, N.S. and M.S. Daoud, 2002. Study the occurrence of ovine Sarcocystosis in the abattoir of Mousl city. Iraqi J. Vet. Sci., 16 (2): 125-132.

Al-Hyali, N.S., L.Y. Khalil and M.A. Aljawady, 2009. Sarcotoxin effect on leukocytic finding and phagocytic activity in mice. J. Anim. Vet. Adv., 8 (1): 2395-2398. http://medwelljournals.com/fulltext/java/2009/2395-2398.pdf.

Al-Taee, A.F. and N.S. Al-Hyali, 2007. Toxicity of Sarcocystis gigantea detected from sheep in nineveh governorate. Iraqi J. Vet. Sci., 21 (2): 224-243.

Brose, E.E., H.Z. Tietz, T. Montag and P. Widera, H.D. Volk *et al*., 1989. Sarcocystis gigantea influences host reaction. Presented at the 13th Conference of World Association for the Advancement of Veterinary Parasitology, Aug. 7-11. Berlin, GDR, pp: 71.

Dubey, J.P., C.A. Speer and F. Fayer, 1989. Sarcocystis of animals and man. CRC Press/Boca Raton, Florida, pp: 113-120. www.bioone.org/doi/abs/10.1645/0022-3395.

Jacks, T.M. and B.J. Wu, 1974. Biochemical properties of Escherichia coli low molecular weight heat-stable enterotoxin. Infect. Immun., 9 (2): 342-347. http://Iai.asm.org/cgi/conent/abstract/9/2/342.

Kia, E.B., 2003. A simple and large scale performing protocol for the Detection of Sarcocystis in Muscles of some small mammals. Iranian J. Publ. Health, 32 (2): 28-30. http://www.ijphir/home.php?archive&year=2003& issueld=16&issueyear=2003.

Koupal, L.R. and R.H. Deibel, 1975. Assay, Characterization and localization of an enterotoxin produced by salmonella. Infect. Immun., 11 (1): 14-22. http://Iai.asm.org/cgi/conent/abstract/11/1/14.

Montag, T., E. Brose, S. Kiessing, F. Weichold and J. Coupek, 1989. Isolation and partial characterization of a lectin and other protein of Sarcocystis gigantea. Presented at the 13th Conference of World Association for Advancement of Veterinary Parasitology, Aug. 7-11. Berlin, GDR, pp: 71.

Robyt, F.J. and J.B.I. White, 1987. Gel Permeation chromatography. II. Organic solvent protein precipitation. In. Biochemical techniques, theory and practice. Wads Worth Inc. USA, pp: 88-95, 268.

Saha, A.K., P.S. Srivastava and S.R.P. Sinha, 1985. Toxic effect of the extract of Sarcocystis fusiformis to laboratory mice. Indian J. Anim. Sci., 55: 656-658.

Saleque, A., B.B. Bhatia, P.D. Juyal and H. Rahman, 1991. Toxicity of cyst extracts of Sarcocystis fusiformis from buffalo in rabbits and mice. Vet. Parasitol., 38: 61-65. DOI: 10.1016/0304-4017(91)90009-K.

Srivastava, P.S., A.K. Saha and S.R.P. Sinha, 1986. Effect of heating and freezing on the viability of Sarcocysts of Sarcocystis levinei from cardiac tissues of buffaloes. Vet. Parasilol., 19: 329-332. DOI :10.1016/0304-4017(86)90080-4.