

Dept. of Biochemistry,
Faculty of Vet. Med., Alexandria Univ.,
Head of Dept. Prof. Dr. B.R. Nemetallah.

**THERAPY OF MASTITIS AND BEHAVIOUR OF LIPID
PEROXIDATION AND ANTIOXIDANT ENZYMES PRE
AND POST TREATMENT**
(With 4 Tables)

By

L.F. HASSAN and A.A. MAHMOUD*

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علاج التهاب الضرع وسلوك تزنج الدهون والانزيمات المضادة للأكسدة
قبل العلاج

ابراهيم حسن ، عبدالكريم عبدالقواب

في هذا البحث قد استخدم البنسلين أستربتوميسين والفالوسبورين مع الفاكيموتريسين في ٧ حالات التهاب ضرع في الأبقار وقد تم عزل الميكروب السبحي الاجلكتي والعنقودي الذهبي من هذه الحالات . وقد اتضح من هذه الدراسة أن الفالوسبورين مع الفاكيموتريسين أحسن علاج ضد حالات التهاب الضرع الناتجة عن الإصابة بميكروب السبحي الاجلكتي والعنقودي الذهبي في هذه الحالات . وقد أسفرت الدراسة أيضا أن هناك زيادة ملحوظة في تزنج الدهون والانزيمات المضادة للأكسدة في الحالات المرضية عنها في الحالات السليمة .

SUMMARY

The results reported in this paper are concerned with a trial of penicillin streptomycin, cephalosporin and chemotrypsin in treatment of 47 clinical case of cattle mastitis with a distribution of infection in 165 quarters. The disease was due to strept. agalactiae in 5.4 percent, staph. aureus in 2.4 percent and strept. agalactiae and staph. aureus mixed infection in 92.1 percent.

Cephalosporin plus chemotrypsin were found to be an effective chemotherapeutic agent against mastitis caused by strept. agalactiae and staph. aureus.

A significant increase in the values of lipid peroxidation and antioxidant enzymes in mastitic cows than normal ones.

* Dept. of Vet. Med., Faculty of Vet. Med., Alexandria University.

HASSAN and MAHMOUD

INTRODUCTION

The prevalent occurrence of mastitis in dairy herds is evidence that this disease continues to constitute a national herd problem. It is mainly treated with a wide range of antibiotic formulation administered intracysternally. Frequently however, poor or no response to these modern treatments was noticed despite of their activity in high concentration in vitro. This had been attributed to several pathological, mechanical and physiological factors (PACKER, 1948). Moreover, PLANTONOW and BLOBEL (1963) reported that the principal cause of therapeutic failure was tissue barriers rather than drug resistance.

The use of hyaluronidase to promote the diffusion of an infused agent has not been generally adopted but the use of enzymes to break down necrotic tissue such as stablized trypsin 50 mg daily for 3 days together with antibiotics appear to be safe and beneficial (JORDAN, 1957) with day's advancing technologies, the clinician has his disposal increasingly sophisticated equipment and procedures to aid him in diagnosing a disease or estimating its severity. Not least among these valuable tools are the various laboratory methods or analyses which establish critical indices of components such enzymes in blood (BOYD, 1962). The present position of clinical enzymology in Veterinary Medicine has been reviewed, but it is quite apparent that data are meager on changes in enzyme profile which are associated with many commonly encountered diseases notably mastitis.

The purpose of the present study was to:

- 1 - Attempts to treat the clinically mastitic cattle with different chemotherapeutic agents.
- 2 - Investigate the changes in lipid peroxidation and antioxidant enzymes in mastitis pre and post treatment.

MATERIAL and METHODS**Animals:**

A mastitic proplem attacking a freizian dairy herd at Elmenia Village of Kafr-El-Sheekh Governorate. The proplem was an increasing rate of infection of clinical mastitis showing poor or no response to different treatments applied (mastolon Pfizer, sulphademinine 33% El-Nile, Bykocillin, Byko, and cortzone ointment).

On the primary visits to the farm, the following predisposing factors to this problem were taken into consideration. These were absence of programme to control the disease, fair managements and sanitation. 50 cows, were suffering from clinical mastitis and the herd free from tuberculosis, brucellosis and parasitic infestation.

MASTITIS, PEROXIDATION & ANTIOXIDANT

Clinical examinations

Clinically mastitic quarters were subjected to physical examination included careful observation of the milk or discharge and palpation of each quarter to determine the degree of fibrosis following the (UDALL and JOHNSON, 1931).

Milk samples

Ten milliliters milk samples were taken aseptically in sterile test tubes and incubated at 37°C for 12 hours, then centrifuged for 5 minutes at 1500 rpm.

Bacteriological examinations

Loopfuls of the sediment milk samples were streaked on blood agar, Macconkey agar and Edward's media and incubated at 37°C for 24 hours to 5 days, pure colonies were subcultured and identified according to CRUICKSHANK, et al. (1975).

Treatment:

History of the different mastitic treatments including methods of their application together with the bacterial status of the herd udder were the basis used in selecting a chemotherapeutic agents to be appropriately administered for treatment.

Drugs

The animal suffering from clinically mastitic quarters were divided into four groups according to type of drug used.

- Group A:** Consists of 9 cases of 32 infected quarters, received penicillin streptomycin produced by El-Nile (Egypt), the vial contain 300,000 i.u. penicillin procaine G, 100,000 crystalline penicillin G sodium and 1/2 gram streptomycin. The powder dissolved in normal saline solution and infused intramammary at rate dose of 200,000 IU penicillin, and 250 streptomycin twice daily for 3-5 days.
- Group B:** Include 11 cows, of 37 infected quarters received the treatment as group A in addition to 10 mg chemotrypsin leurquin dissolved in 10 ml pyrogenic saline and infused intrammary twice daily for 3-4 days. Chemotrypsin leurquin 5 mg supplied by laboratories leurquin.
- Group C:** Include 12 cases of 40 infected quarters, infused intrammary twice daily for 3-4 with 250 mg velosef produced by squibb, the vial contain 250 mg cephadrine which is a semisynthetic cephalosporin antibiotic.
- Group D:** Consists of 15 cows of 56 infected quarters, treated as group C but in addition of 10 mg chemotrypsin infused intrammary twice daily. The treatment continue for 3 days.

All infected quarters in all groups suffering from acute mastitis were infused with appropriate chemotherapeutic agent subsequent to thorough evacuation of their content.

HASSAN and MAHMOUD

Post treatment bacterial examination of milk samples were made 2 weeks after the completion of the treatment procedure. Incurable mastitic quarters were dired of by intrammary infusion of 3% silver nitrate 30-60 ml per quarter.

Biochemical analysis:

Venous blood samples about 4 ml were collected from 30 clinically mastitic cows before and after treatment as well as from 10 normal ones, the blood placed on EDTA as anticoagulant, and centrifuged for 10 minutes at 3000 rpm and plasma removed, the erythrocytes were then washed three times with cold isotonic saline 0.9% sodium chloride. Erythrocytes lysate were prepared 0.1 ml cell suspension to 0.4 ml distilled water followed in 10 minutes by freezing. Enzymatic activities were determined using the method of PLACER, *et al.* (1966) for lipid peroxidation and that of MISRA and FRIDOVICH (1972) for superoxide dismutase. Glutathione peroxidase and catalase were measured according to the method described by SEDLAK and LINDSAY (1968) for the former enzyme and by the method of BEER'S and SIZER (1952) for the later.

RESULTS

Results of physical examination of the clinically mastitic quarters is recorded in table (1).

Microorganisms isolated from clinically mastitic quarters is recorded in table (2).

Response of cows with clinically mastitic quarters to treatment with different chemotherapeutic agent is recorded in table (3) changes of lipid peroxidation and antioxidant enzyme in erythrocytes lysate of normal and clinically mastitic cows pre and post treatment are recorded in table (4).

DISCUSSION

Results obtained from studies on mastitis therapy, emphasized the essential need for the proper treatment to control mastitis and approach vertical expansion in milk production of the herd. On the long run of the problem, Failure to different treatments improperly applied to mastitic cows led to serious economic losses among the herd. The culling of 3 cows for permanent induration of more than two quarters per udder in addition to 3 cows each had two fibrosed quarters and 5 cows each had one fibrosed quarters (table 1) was a major problem.

The physical examination of the udder by UDALL and JOHNSON system (1931) had proved to be valuable as a basis for culling mastitic cows that are unlikely to respond to treatment. Moreover, it gave information on the udder condition necessary to determine the proper administration of the drugs.

MASTITIS, PEROXIDATION & ANTIOXIDANT

Different isolates from quarter milk samples, were identified before carrying the treatment 152 samples showing mixed infection with staph. aureus and strept. agalactiae, 9 samples infected with strept agalactiae and 4 samples infected with staph. aureus (table 2).

On the basis of history of the previous treatment, applied on the farm and kind of pathogen isolated, the drug selected was a combination of penicillin streptomycin or cephalosporin with or without chemotrypsin. The results obtained from application of the treatment trials (table 3) showed that the use of cephalosporin with or without chemotrypsin gave superior results than penicillin streptomycin combination in treatment of mastitis. This may be due to seldom use of cephalosporin as udder treatment in the Egyptian field beside it has a broad spectrum bactericidal activity and not affected by penicillinase enzyme. Moreover, it has high activity against most strains of penicillinase producing staph. aureus. In the same time such findings is in conformity with CURTIS, *et al.* (1977). However, it is worthy to note that the treatment with cephalosporin, the antibiotic of choice, it is not available as mastitic formula in Egyptian market.

The combination of penicillin streptomycin gave satisfactory results in our present study (table 3). This combination has a synergistic effect and together they have a wide spectrum activity. Their combined use decreased the development of resistant organisms and it will keep the development of penicillin resistance to a minimum, SANDERSON (1966).

In our present study as shown in table (3), the group B and D gave superior results than group A and C respectively. This may be attributed to effect of chemotrypsin which possess a potent antiinflammatory properties that enables to hasten the resorption of inflammatory oedema furthermore, posses a proteolytic activity that enables to destroy any tissue barrier, hence the drug can reach to infected foci in the udder and approach cure.

With respect to biochemical analysis our results indicated that significant increase in lipid peroxidation in erythrocyte of mastitic group than normal one's and return to normal after treatment (table 4). Such increase may be attributed to the production of macrophages which increase during inflammatory process and generate considerable quantities of lipid peroxidation (SERFASS and GANTHER, 1975).

The erythrocytes antioxidant enzymes activities namely superoxide dismutase, Glutathione peroxidase and catalase significantly increased in mastitic animals as compared to control groups (table 4). Such finding indicated that three major antioxidant enzymes exert protective action against peroxidation in erythrocytes and preserve structural integrity NICHOLS (1972), PRYOR (1973) and KAPLAN and ANSAR (1984).

In conclusion, the present study showed that, cephalosporin plus ~~chemotrypsin~~ - chemotrypsin are drugs of choice in treatment of mastitis caused by strept. agalactiae and staph.

HASSAN and MAHMOUD

aureus. Moreover, differences in the values of lipid peroxidation and antioxidant enzymes were statistically significantly increased between mastitic cases and normal ones.

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MASTITIS, PEROXIDATION & ANTIOXIDANT

Table (1): Results of physical examination of clinically mastitic quarters.

Condition of the udders	No.
Udders with completely indurated quarters	1
Udders with three indurated quarters	2
Udders with two indurated quarters	3
Udders with one indurated quarters	5
Udder with healthy quarters	39

Table (2): Microorganisms isolated from clinically mastitic quarters.

No. of samples	No. of infected samples	Type of pathogens isolated		
		No. of samples with percent	Staph aureus	Mixed infection (Strept + staph)
177	165 (93.2) %	9 (5.4) %	4 (2.4) %	152 (92.1)

Table (3): Response of cows with clinically mastitic quarters to treatment with different chemotherapeutic agents.

Type of drug used	No. of treated quarters	No. of treatment	No. of recovered quarters	% of recovery
Group A: Penicillin streptomycin mixture	32	Twice daily for 3 - 5 days	24	75
Group B: Penicillin streptomycin mixture plus chemotrypsin	37	Twice daily for 3 - 4 days	31	83.8
Group C: Cephalosporin	40	Twice daily for 3 - 4 days	36	90.0
Group D: Cephalosporin plus chemotrypsin	56	Twice daily for 3 days	54	96.4

Table (4): Changes of lipid peroxidation and antioxidant enzyme in erythrocytes of normal and clinically mastitic cows pre and post treatment.

Parameter	Control		Before treatment		After treatment	
	Mean	SE	Mean	SE	Mean	SE
Lipid-peroxidation (nm MDA/ml. blood)	92.25 ± 1.62	(84.60 - 97.62)	110.64 ± 6.42**	(94.37 - 139.92)	85.36 ± 1.85	(78.10 - 89.16)
Superoxide dismutase (U/ml. blood)	227.49 ± 21.82	(162.26 - 300.37)	328.52 ± 17.99**	(272.56 - 403.25)	216.20 ± 47.84	(110.34 - 422.39)
Glutathione peroxidase (U/ml. blood)	1.40 ± 0.12	(1.08 - 1.89)	3.13 ± 0.45**	(2.13 - 5.44)	1.56 ± 0.13	(1.12 - 1.98)
Catalase (BU/ml. blood)	0.35 ± 0.02	(0.29 - 0.42)	0.50 ± 0.01**	(0.42 - 0.55)	0.38 ± 0.02	(0.36 - 0.42)

Significance values: ** = P < 0.05 *** = P < 0.01