International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 1(K); January 2018; Page No. 9467-9471
DOI: http://dx.doi.org/10.24327/ijcar.2018.9471.1567

Research Article

THE EFFECT OF SESAME OIL AS TRIGLYCERIDES ON STAPHYLOCOCCAL FATTY ACID MODIFYING ENZYME (FAME) AND ABSCESS FORMATION AND COMPARE ITS EFFECT WITH TRUE TRIGLYCERIDES

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A R T I C L E I N F O

Article History:
Received 9th October, 2017
Received in revised form 12th November, 2017
Accepted 26th December, 2017
Published online 28th January, 2018

Key words:
Sesame oil; Staphylococcus; fatty acid modifying enzyme (FAME);abscess formation

A B S T R A C T

Staphylococcus aureus(*S. aureus*) *subsp. anaerobius* was isolated from sheep abscess for extraction of FAME by culturing it on trypticase soy broth medium.
Eight sheep, divided into four groups of two animals each, were inoculated subcutaneously (S/C) with different protocols and doses. Inocula included: *S. aureussubsp. anaerobius* plus sesame oil; orplus sesame oilandFAME; or plus triglyceride; orplustriglyceride andFAME. The control groups (two groups of two animals each) were inoculated with the enzyme FAME, sesame oil, triglycerides and *S. aureussubsp. anaerobius.*
Triglycerides (true or sesame oil) were found to inhibit the formation of abscess when injected with abscess-producer Staphylococci (*S. aureus subsp. anaerobius*), but when FAME was added and when the concentration of the organism was increased, abscess was formed in case of sesame oil, but abscess was small and its nature was changed, whilesites injected with *S. aureussubsp. anaerobius* plus triglycerides showed slight inflammatory reaction.
Control group, injection of *S. aureussubspanaerobius*alonegrossly showed abscess formation, while the inoculation of FAME, sesame oil and triglycerides produced no lesions at the inoculation sites. All injected sites were confirmed histopathologically.

INTRODUCTION

According to the studies conducted by Khidir (1997) in Sudan, sesame oil contains 85% unsaturated fatty acids (oleic and linolenic acids). Therefore, it can be used as triglycerides and this study seems to be the first of its type.

Sudan possesses a large animal wealth estimated to be 128.523.000 of which sheep constituted 48.136.000 (MARF, 2002). Sheep export is one of the most important public incomes. However many diseases cause great economic losses due to return back of sheep to Sudan, one of these sheep diseases is abscess disease which is characterized by superficial enlargements. The disease is caused by *S. aureus subsp. anaerobius*. It is endemic in nature with high morbidity and no mortality (Bajmocy*et al.,* 1984). It seems to affect all age groups causing more than two abscesses in one animal. Any *S. aureus subsp. anaerobius* survives and multiplies in the host tissues and causes abscess must produce an esterifying enzyme known as fatty acid modifying enzyme (FAME).

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Fatty acid modifying enzyme inactivates the bactericidal fatty acids which is produced by the host in the abscess. This enzyme itself is inhibited by glycerides and the host delivers large amounts of triglycerides into the core of the abscess which keep the enzyme non-functional (Long *et al.,* 1992).These triglycerides are overcome by lipase. Many researchers worked in this syndrome so as to solve export sheep problem and improve the statement. Previous works studied the mechanism of abscess formation and the factors contributed with it, this work was performed to study the effect of sesame oil as triglycerides on FAME and abscess-producer Staphylococci and to compare the effect of sesame oil with the effect of true triglycerides.

MATERIAL AND METHODS

Experimental animals

Eighthealthy fattened Hamari and Kabashi sheep aged six months to one year were purchased from Omdurman Local Market. They were identified by numbered ear-tags and divided into four test groups (two animals each) plus control ones (four animals). The animals were kept in convenient open sided-pens under strict healthy and sanitary condition. All animals were fed on roughages and concentrates.

Experimental design

Preparation of inocula

Preparation of the organisms

Abscesses were collected from sheep for isolation of *S. aureus subsp. anaerobius.* Impression smears were first made from each pus sample, fixed, stained and examined microscopically. Samples showing staphylococcal shape-like were streaked onto blood agar plates then incubated aerobically and under microaerophilic condition at 37^{o}C. Growth was examined after 48 hours and the isolates were identified according to Barrow and Feltham (2003) and to scheme for identification of Staphylococcal species (Saeed and El-Sanousi, 1995).

Isolated *S.aureus subsp. anaerobius* was grown microaerophilically in 10 ml nutrient broth at 37^{o}C overnight. Serial ten-fold dilution from 1 ml of culture was made. Inoculafrom dilutions 10^{-1}(containing about 10^{4}bacterial cells) and10^{-3}(containing about 10^{3}bacterial cells) were used.

Preparation of crude FAME

Crude FAME was prepared according to the method of Long *et al*. (1992).

S. aureus subsp. anaerobius was grown microaerophilically in 100 ml of Trypticase soy broth (TSB) at 37^{o}C for 18 hs. with continuous shaking. The culture was centrifuged at 3000 r.p.m. for 30 minutes using cold centrifuge and the supernatant fluid was sterilized by filtration using 0.22 µm. filter membrane. The culture filtrate was concentrated to half volume at 4^{o}C using dialysis tubes and carbowax.

Triacylglycerols (Triglycerides)

Sesame oil which contains 85% unsaturated fatty acids was used as triglycerides purchased directly from local producing factories. Pure triglycerides were also used for comparison.

Inoculation of experimental animals (sheep)

Group (1)

Right side: 0.5 ml of dilution $10^{-3}$of *S. aureus subsp. anaerobius*plus0.5 ml of sesame oil.

Left side: 0.5 ml of dilution $10^{-3}$of *S. aureus subsp. anaerobius* plus0.5 ml of FAME plus0.5 ml of sesame oil.

Group (2)

Right side: 0.5 ml of dilution $10^{-3}$of *S. aureus subsp. anaerobius* plus 0.5 ml of triglycerides.

Left side: 0.5 ml of dilution $10^{-3}$of *S. aureus subsp. anaerobius* plus0.5 ml of FAME plus0.5 ml of triglycerides.

Group (3)

Right side: 0.5 ml of dilution $10^{-3}$of *S. aureus subsp. anaerobius* was injected, then after of half an hour, followed by 0.5 ml of sesame oil in the same place of injection.

Left side: Before 0.5 ml of dilution $10^{-3}$of *S. aureus subsp. anaerobius* was added, 0.5 ml of FAME was incubated with 0.5 ml of sesame oil for six hours at 37^{o}C then the mixture was injected.

Group (4)

In this group the concentration of the organism was increased from dilution 10^{-3} to dilution 10^{-1} with constant amount of triglycerides.

Right side: 0.5 ml of dilution $10^{-1}$of *S. aureus subsp. anaerobius*plus0.5 ml of sesame oil.

Left side: 0.5 ml of dilution $10^{-1}$of *S. aureus subsp. anaerobius*plus0.5ml of triglycerides.

Control Group

Four sheep were injected as follows:

Group (1)

Right side: 0.5 ml of FAME.
Left side: 0.5 ml of triglycerides.

Group (2)

Right side: 0.5 ml of sesame oil.
Left side: 0.5 ml of dilution $10^{-3}$of *S. aureus subsp. anaerobius.*

All animals were inoculated S/C at the neck region, followed up and the observations were recorded.

Histopathology

Tissue sections were prepared for histological examination according to the method described by Durury and Wallington (1980).

RESULTS

The effect of external Triglycerides on abscess formation by abscess-producer Staphylococci (S. aureussubspanaerobius)

Addition of ½ ml of triglycerides (pure or sesame oil) to ½ml of dilution $10^{-3}$of *S. aureussubspanaerobius* resulted in inhibition of abscess formation.

Histopathological results revealed thataddition of pure triglycerides to*S. aureussubspanaerobius* showed no change, while sesame oil showed slight edema with deep eosinophilic staining fibres (Fig.1).

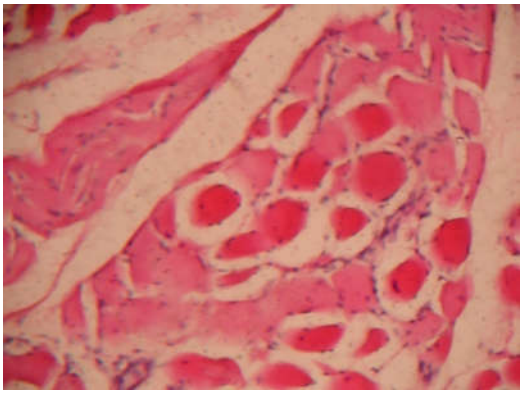

Fig 1 Subcutaneous tissue: Some muscle fibres staining deep eosinophilic (hyaline degeneration) (H and E x 250).(Sectionfromsite injected with*S. aureus subsp. anaerobious*plussesameoil).

Lymph node sections prepared from animals injected with *S.aureussubspanaerobius* plus triglycerides showed lymphoid hyperplasia and large lymphoid follicles with germinal centers.

Liver sections showed diffuse hepatocytes vacuolation (autolysis) and proliferation of kupffer cells.

Abscess was obtained when *S. aureussubspanaerobius* was increased from dilution 10^{-3} todilution $10^{-1}$and also when FAME was added to the inoculum (½ ml dilution $10^{-3}$of*S. aureussubspanaerobius* plus sesameoilplus FAME) butthe abscesses were small and some contained watery deep yellow pus. On incision the content oozed leaving empty cavity (Fig. 2).

Fig 2 Skin: Cyst-like structure containing yellow watery fluid formed at the site injection of *S. aureus subsp. anaerobious* plus sesame oil plus FAME.

Histopathological examination revealed dermal edema, suppuration, fibrosis and pyogranulomas with bacterial colonies in deep dermis and S/C tissue (Fig. 3).

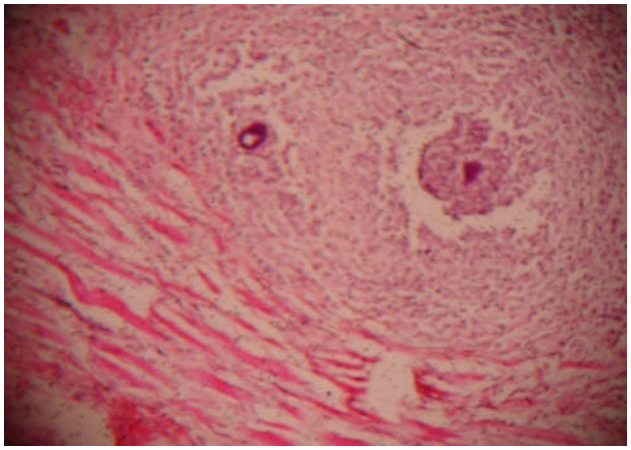

Fig 3 Skin: Subcutaneous tissue showing a pyogranuloma (H and E x 100). (Section from site injected with *S. aureussubspanaerobius* plussesameoilplus FAME).

Control group

Necropsy of the experimental sheep revealed the presence of abscess at the injection of *S. aureussubspanaerobius* alone (Fig.4 and 5) and histopathologically there was large abscess surrounded by connective tissue capsule and mononuclear cells infiltration in deep dermis (Fig. 6). Other sections showed large areas of suppuration with appearance of bacterial colonies and pyogranulomas. In some sections, chronic inflammatory reaction with S/C muscle degeneration and presence of giant cellswere seen.

Lymph node at *S. aureussubspanaerobius* injection site was grossly enlarged, lobulated and congested.Histopathologically there was lymphoid hyperplasia and well developed follicles, some having germinal centers. There was medullary edema and thickened medullary cords (Fig.7).

Fig 4 Subcutaneous abscess in sheep experimentally infected with *S. aureus subsp. anaerobious*alone.

Fig 5 Skin: Abscess formation at the site of *S. aureus subsp. anaerobious* injection.

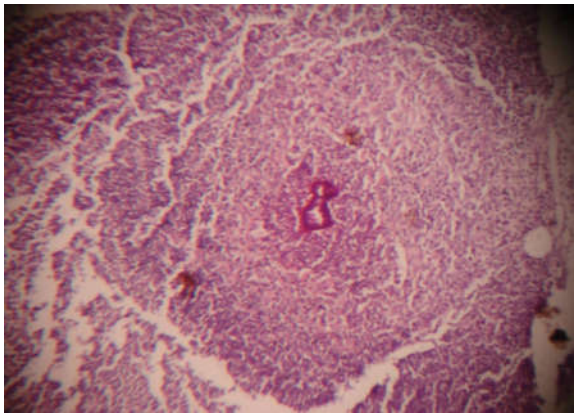

Fig 6 Large abscess showing club-shaped bacterial colonies (H and E x 250)(Sectionfromsite injected with*S. aureus subsp. anaerobious*alone).

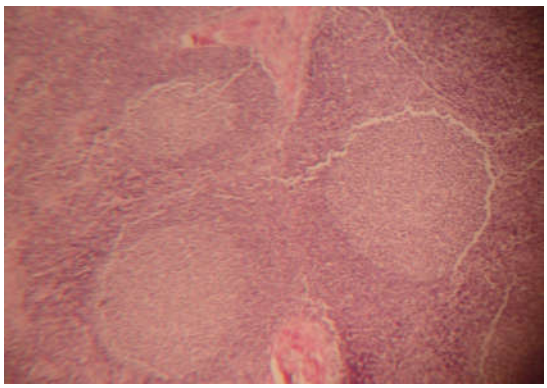

Fig 7 Lymph node: Exhibiting lymphoid hyperplasia with germinal centres (H and E x 250).(section fromlymph node fromsite injected with*S. aureus subsp. anaerobious* **).**

Liver showed hepatocyte swelling and vacuolation. Portal triads were density infiltrated by mononuclear cells, mainly lymphocytes. Some areas showed parenchymal necrosis and fibrosis and diffuse mononuclear cell infiltration.

The control sites which were injected with enzyme, FAME, triglycerides and FAME+triglycerides showed no swelling or caseation. Sections from the skin of inoculated sites showed no significant histopathological changes but in some sectionsthere was dermal edema (Fig. 8), subcutaneous muscles stained pale and some muscle fibres showed intense eosinophilia.

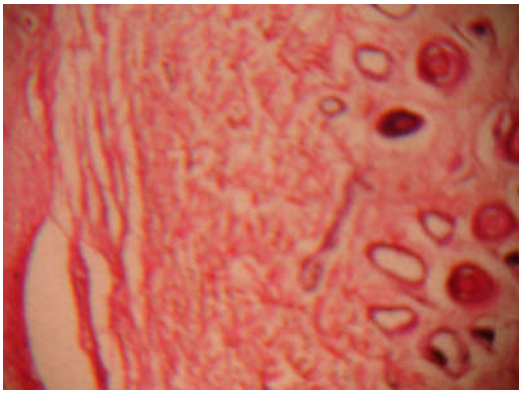

Fig 8 Skin section showing slight dermal edema (H and E x 100). (section fromsite injected withFAME alone).

DISCUSSION

This study was conducted on sheep abscess disease which isa very important one of the major economic loss. Therefore, many researchers investigated the problem from different aspects. Generally, the disease was discussed by many people in different part of the world (Aynaud, 1922;23;27; 28; Carre, 1923; Shirlaw and Ashford, 1962; Schleifer*et al*., 1979; Bajmocy*et al*., 1984; Fuente*et al*., 1985 and Moller *et al*., 2000).

In Sudan, studies revealed that the main causative agent of the disease in sheep and goats is *S. aureus subspecies anaerobius* (Hamad, 1989 and El-Sanousi, 1989). Then followed by other researchers such as Karamalla (1993; 97); Saeed (1995); Radwan (1996); Noura (1996); Hassan (1996;2001) Sara (2000) and Muna (2002).

Previous studies showed that for the organism to form abscess and cause this problem must possess two enzymes: Fatty acid modifying enzyme (FAME) and lipase. Within the abscess, there are bactericidal fatty acids, FAME, triglycerides and lipase and inside the abscess, the following occurs: Bactericidal fatty acids produced by the host act on the organism and control the multiplication and survival of staphylococci within abscess. FAME produced by the organism is capable of destroying the bactericidal fatty acids by esterfying them to alcohols. This leads to protection of the organism, which can survive and multiply to form abscess. Triglycerides produced by the host containing unsaturated fatty acids are very potent inhibitors to FAME, keep FAME non-functional and make the bactericidal lipids free to act on the bacteria. Lipase produced by the organism breakdown the triglycerides to glycerol and fatty acids; the latter are utilized by bacteria. High concentrations of free fatty acids kill the bacteria (Dye and Kapral, 1981) while, fatty acid esters are unable to kill Staphylococci.Studies done by Kapral *et al.* (1992) confirmed that FAME esterifies the fatty acids within the abscess and it inhibited by triglycerides. He also reported that triglycerides containing unsaturated fatty acids were more potent inhibitors than saturated ones.

As shown in this study, inhibition of abscess formation by triglycerides (sesame oil or pure triglycerides) when injected with *S. aureus subsp. anaerobius* (FAME and abscess producer) may be attributed to the inactivation of FAME by triglycerides rendering it non-functional, leaving the organism exposed to the bactericidal lipids. It should be stated that, the organism produces lipase to act on triglycerides, but when triglycerides level becomes too high and lipase is unable to breakdown all amount.Thus FAME remains to be inactivated and the bactericidal lipids are free to act on the organism. Another possible factorin the inhibition of the bacteria is the high concentration of fatty acids produced as a result of the breakdown of triglycerides by lipase; these fatty acids are known to be bactericidal. This result was confirmed histopathologically as no evidenceof abscess formation was seen in the sections.

CONCLUSIONS AND RECOMMENDATIONS

- Injection of *S. aureus subsp. anaerobius* with external triglycerides tended to inhibit abscess formation.
- Incorporation of oils containing unsaturated fatty acids may be considered in vaccine development.
- The microenvironment affecting the action of FAME, lipase and triglycerides in induction of abscesses e.g pH and temperature should be properly assessed.

Acknowledgment

Thanks are to the family of Microbiology and Pathology Departments, Faculty of Veterinary Medicine, University of Khartoum, and all members of the Dept. of Bacteriology, Central Veterinary Research Laboratory, Soba.

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How to cite this article:

Raga, I. O *et al* (2018) 'The Effect of Sesame Oil As Triglycerides On Staphylococcal Fatty Acid Modifying Enzyme (Fame) and Abscess Formation and Compare its Effect With True Triglycerides ', *International Journal of Current Advanced Research,* 07(1), pp. 9467-9471. DOI: http://dx.doi.org/10.24327/ijcar.2018.9471.1567

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