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Efficacy of *Terminalia Brownii* Bark Extract in Treating Induced *Staphylococcus aureus* Mastitis in Nubian Goats

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Abstract

Mastitis was experimentally induced in 3 groups of lactating goats each of 5 goats; by inoculation of pathogenic *Staphylococcus aureus* into each teat canal at doses of 0.1ml of an over night broth culture containing 9^8-10^9 viable bacterial count per ml, $1x10^{-3}$ and $1x10^{-5}$ dilution of the same culture into the 3 groups respectively. Methanolic extract of *Terminalia brownii* bark, prepared at a concentration of 25 mg/ml of normal saline was infused at doses of 1.5ml daily for 12 days into each teat canal of the infected udders. Leucocyte count in the milk of goats was used to assess the *in vivo* efficacy of the bark extract in treating induced *Staphylococcus aureus* mastitis. Leucocytes were counted every 3 days during the 12 days of therapy. On the 13th day, milk specimens were collected aseptically from each teat of the infected udders and cultured on blood agar medium for detecting the presence of *Staphylococcus aureus* colonies. The extract of bark treated mastitis in ($^2/_5$) 40% of the goats of group (2). Goats of group 3 and 4 mastitis were treated in 60% ($^3/_5$) each group. That was apparent by the drop of leucocyte counts in the milk to normal levels and the absence of *Staph. aureus* colonies on cultures of milk specimens. The data presented in this study shows that *T. brownii* extract of the bark can treat induced *Staph. aureus* mastitis in goats.

Keywords: *T. brownii* bark extract; *Staph. aureus* mastitis; lactating goats.

Introduction

Terminalia brownii (Combretacaea) is known in Sudan as *shagarat elsobag*. It is a widely spread tree in the Sudan. Details of its botanical description were given by Elghazali *et al*.,(1997); Omer and Elnima, (1999). The maceration of the bark of the plant has been used in traditional medicine for the treatment of cough and bronchitis in west and East Sudan (Elghazali *et al*., 1997) and for treatment of diarrhoea and gonorrhea (Zakaria *et al*., 2007).

Chemical analysis of different parts of the plant revealed abundance of tannins, flavonoides and saponine (Omer and Elnima, 1999) in which the antimicrobial activity reside. The mode of antimicrobial action of tannins may be related to their ability to inactivate microbial adhesions enzymes and bacterial cell envelope transport protein (Scalbert, 1991 and Haslam, 1996).

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Flvonides, on the other hand, are antioxidants which synthesized by plants in responds to microbial infection (Dixon *et al.,* 1983). Their activity is probably due to their ability to complex with extracellular bacterial proteins and bacterial cells walls and disrupting microbial membranes (Tsuchiya *et al*., 1996). Whereas, *in vitro* studies revealed a high antimicrobial activity residing of the bark and leaves of the plant against pathogens (Omer and Elnima, 1999, Thoria, 2007, Zakaria *et al*., 2007).

A marked sensitivity to the bark and leaves extracts of the plant was exhibited by the clinical bacterial isolates, *Staph. Aureus* (a coagulase-positive strain and *Pasteurella multocida* Strain B_2, as shown by disc diffusion methods (Thoria, 2007). However, the therapeutic value of this plant extracts in treating clinical cases has not been investigated.

In the present study, experiments were designed to assess the efficacy of *T.* bark extract in treating local infection experimentally induced in the udder of lactating Nubian goats by *Staph. aureus* a Coagulase-positive strain.

Material and Methods

Animals used

Twenty five female Nubian goats 1-1.5 years old were used in these experiments. All goats were at the first lactating period. They were bought from Soba market, Khartoum and kept in pens at the Central Veterinary Research Laboratory at Soba. The goats were healthy and free from external parasites. The goats were fed on Lucerne and water *ad-libitum* and allowed one week for adaptation. Milk from each goat was examined daily for any physical, chemical or bacteriological changes as described by Blood and Rodostitis (1989).

Experimental design

The goats were allotted at random into 5 groups, of 5 goats each.

Goats in group (1) were kept as control.

Goats in group (2) were infected each with an over night (24 hours) nutrient broth culture of *Staph. aureus* a Coagulase-positive strain containing 9^8-10^9 viable bacterial count per ml, 0.1ml of this culture was injected via each teat canal.

Goats in group (3) were infected each with 0.1ml of the same broth culture of *Staph. aureus* diluted $1x10^{-3}$. The diluted culture was installed in each teat.

Goats in group (4) were given 0.1ml each via the teat canal of $1x10^{-5}$ *Staph. aureus* over night culture According to the method described by Thoria (2007).

Goats in group (5) were kept as uninfected, controls but each one was infused daily in each teat canal with 1.5ml of the methanolic extract of *T. brownii* bark for 12^{th} days.

Milk samples were collected thereafter every 3 days until the 12^{th} day of therapy. White blood cells in the milk were counted using Neubaur haemocytometer according to the method of Schalm (1970).

Treatment

Plant collection and identification

The fresh bark of *T. brownii*, was collected from Khartoum National botanical garden. The plant was identified by the botanists in Medicinal and Aromatic plant Research institute, and was shade dried and crudely powdered.

Plant barks methanolic extraction and preparation:

Five hundred (500) gram of the crude powdered bark was extracted with methanol using soxhlet extractor. The extract was filtered and the filterate was evaporated to dryness under reduced pressure at 40^oC using a rotary evaporator. The methanolic extract was given to each goat in group 2, 3, 4 and 5 by infusing each teat canal with 1.5ml of the extract at concentration of 25 mg/ml(Philipot and Nickerson, 1991). The treatment was started on day 3 after infection and usually after milking the goats.

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Bacterial detection

On the 13th day after infection milk from each udder of the survived goats was collected aseptically and cultured on blood agar for detecting the presence of *Staph. aureus* colonies.

Stactical analysis:

Statistical analyses were done according to the method descrbed by Mead and Gurnow (1983).

Results

Group (2) goats showed dullness, inappetence and decrease in milk yield on the next day after infection. The milk of all goats in the group was dark red, watery and the udder was swollen, hard and changed in colour on the 3rd day of infection. Three goats died on day 2, 4 and 10 after infection (Table 1).

Table 1: Fate of lactating Nubian goats infected in different groups in the mammary glands with *Staphylococcus aureus* and treated locally with *Terminalia brownii* bark methanolic extract

Group No.	Goat No.	Age (year)	Dose	Fate of goats
Group (1) control	1	1-1.5	Nil	Healthy
	2	"	"	"
	3	"	"	"
	4	"	"	"
	5	"	"	"
Group 2	6	1-1.5"	Each one was infected with 0.1ml of an over	Died on day 2
	7	"	night broth culture of *Staph. aureus*, injected	Cured
	8	"	in each teat, and treated locally with	Died on day 4
	9	"	*Terminalia brownii* bark methanolic extract	Cured
	10		for 12 days.	Died on day 10
Group 3	11	1-1.5"	Each goat was infected with 0.1ml of $1x10^{-3}$	Cured
	12	"	diluted over night culture of *Staph. aureus*,	Cured
	13	"	injected in each teat, and treated with	Died on day 4
	14	"	*Terminalia brownii* bark for 12 days	Died on day 7
	15			Cured
Group 4	16	1-1.5	0.1ml of $1x10^{-5}$ diluted over night culture of	Cured
	17	"	*Staph. aureus* coagulase positive injected in	Died on day 6
	18	"	each teat, and treated locally with	Died on day 4
	19	"	*Terminalia browii* bark methanolic extract	Cured
	20	"	of 12 days.	Cured
Group 5	21	1-1.5	0.1 ml of suspension containing 25mg/ml of	Healthy
	22	,,	*Terminalia browii* bark methanolic extract	
	23	,,	for 12days	
	24	,,		
	25	,,		

There was a significant increase in the white blood cells count in the milk of the mastitic goats three days after infection compared to the control ones ($P<0.05$), but these counts started to decrease after treatment with the methanolic extract of the bark

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Group (3) goats showed swollen and enlarge tender udder on the 3rd day after infection, watery secretion containing small clots and flakes came down the teat canal during milking. Two goats died on days 4 and 7 after infection (Table 1) white blood cell count in the milk of the survived goat increased significantly on the 3rd day after infection compared to the control goats ($P<0.05$). These counts decreased significantly during treatment with the bark extract (Table 2).

In group 4 goats, there was watery secretion coming down the teat canal during milking. The goats showed dullness, inapetence and decrease in milk yields. Two goats died on days 4 and 6 after infection (Table 1). White blood cell counts in the milk increased significantly on the 3rd day after infection. These counts started to decrease after treatment with the bark extract (Table 2).

Table 2: Changes in white blood cell counts/µl of milk of goats in different groups infected locally in the mammary glands with *Staphylococcus aureus* and treated locally with *Terminalia brownii* bark methanolic extract

Days	Groups				
	G_1	G_2	G_3	G_4	G_5
0	6.440 ± 0.73^{k}	6.580 ± 0.58^{k}	6.570 ± 0.61^{k}	6.840 ± 0.57^{k}	5.880±0.93c
3*	6.450 ± 0.71^{k}	22.575 ± 2.39^{a}	18.560 ± 1.34^{b}	14.700 ± 3.01^{d}	6.11 ± 0.93^{b}
3**	6.440 ± 0.72^{k}	18.500 ± 1.40^{b}	17.980 ± 2.79^{c}	13.640 ± 2.99^{e}	6.321 ± 0.92^{b}
6	6.451 ± 0.80^{k}	16.625 ± 1.63^{d}	15.560 ± 1.13^{f}	12.520 ± 0.52^{f}	6.690 ± 0.76^{b}
9	6.443 ± 0.72^{k}	14.125 ± 1.0^{e}	11.680 ± 1.05^{g}	10.000 ± 0.14^{h}	6.900 ± 0.72^{b}
12	6.453 ± 0.82^{k}	10.900 ± 0.73^{h}	8.780 ± 1.26^{j}	8.220 ± 0.33^{i}	7.24 ± 0.66^{a}
15	6.444 ± 0.75^{k}	7.825 ± 0.86^{j}	6.760 ± 0.50^{k}	7.220 ± 0.40^{j}	7.680 ± 0.58^{a}

Key:
G_1: Control
G_2: Overnight broth culture
G_3: Overnight broth culture from *Staph. aureus* diluted $1x10^{-3}$.
G_4: Overnight broth culture diluted $1x10^{-5}$.
G5 uninfected treated with *Terminalia brownii* bark methanolic extract
3*: 3 days after infection and day (1) of treatment
3**: 3 days after treatment
Mean values (±SD) having different superscript letters in columns and rows (between groups and days) are significantly different ($P\leq0.05$).
These counts were the average of cells present in the milk from the two teats.

Group (5) goats were kept uninfected but they were infused with the methanolic extract of *T. brownii* bark in doses of 1.5 ml daily into each teat for 12 days. In this group there was slight increase in white blood cell counts in milk compared to the control goats but the difference was significant ($P<0.05$) (Table 3).

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Table 3: Changes in white blood cell counts in milk of goats dosed locally in the mammary glands daily for 12 days with *Terminalia brownii* bark methanolic extract compared to control group

Days	Groups	
	G_1	G_5
0	6.440 ± 0.73^{b}	5.880 ± 0.93^{c}
3	6.450 ± 0.85^{b}	6.320 ± 0.92^{b}
6	6.445 ± 0.76^{b}	6.690 ± 0.76^{b}
9	6.453 ± 0.85^{b}	6.900 ± 0.72^{b}
12	6.443 ± 0.73^{b}	7.240 ± 0.66^{a}
15	6.442 ± 0.72^{b}	7.680 ± 0.58^{a}

Key:
G_1: Control
G_5: Given the extract daily for 12 days
Mean values (±SD) having different superscript letters in columns and rows (between groups and days) are significantly different ($P\leq0.05$).

There was no significant change in the count of white blood cells in the milk of the control goats of group (1).

Culture of milk on blood agar from each teat of the survived goats showed absence of *Staph. aureus* colonies in the milk of goat No. 7 (group 2). However, there are a few colonies in the milk of the left teat of the goat No. 9 same group. Group 3 survived goats showed absence of *Staph. aureus* colonies in the milk of goats no. 11 and 13 but there was scanty growth in the milk of goat no. 14 from both right and left teats.

In group 4 survived goats there was absence of *Staph. aureus* colonies in the milk of both quarters of the udder.

Discussion

The antimicrobial activity of methanolic extract of *T. browni* bark was assessed by the treatment of induced *Staph. aureus* mastitis in Nubian goats.

Staph. aureus were regarded as an important pathogen causing mastitis in goats (Peterson, 1981). They are implicated in both clinical and subclinical mastitis (Guss, 1997). It is observed in the bovine species that *Staph. aureus* mastitis is difficult to eradicate with the available antibiotics (Haile, 2004). The resistance of *Staph. aureus* to several antibiotics and it's capacity to survive intracellularly has been proposed as factors contributing to its persistence in infected mammary glands (Brouillette *et al*., 2004).

In vitro experiments showed that *Staph. aureus* a coagulase positive strains, was highly sensitive to the methanolic extract of bark and leaves of *T. brownii* (Omer and Elnima, 1999; Thoria, 2007; Zakaria *et al*., 2007).

In the present study there was complete absence of *Staph. aureus* colonies in the milk culture of all goats of group 3 and 4 after infusing the udder with the bark extract of *T. brownii*. This may indicate that the crude bark extract contains antibacterial agent capable of killing or inhibiting the growth *Staph. auresu* existing extracellularly or intracellularly within the caprine mammary glands.

Staph. aureus was known to be resistant to many antibiotics (Sleigh and Timbury, 1989). However, the efficacy of this crude extract in cleaning off the udder from *Staph. aureus* was evident in many goats in our experiments. This may suggest presence of more potent antibacterial compound or a synergy occurring at molecular level between antibacterial components existing in the bark of this plant. Such a synergy between components at molecular level was detected in other medicinal plants (Stermitze *et al*., 2002). However, a few goats died earlier during the course of infection. This may indicate that the intramammary infused bark

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extract can not cure systemic infection disseminating from the udder (Brouillette *et al*., 2004) which may be due to individual variation, and stage of lactation and number of kidding (Suheir *et al*., 2005).

In the present study leucocyte counts in goat's milk were used to assess the *in vivo* efficacy of the bark extract in treating *Staph. aureus* mastitis, a good correlation was found between high milk leucocyte counts and persistence of pathogens in the mammary gland (Obeid, 1983). Leucocyte counts were high in the milk during the first days following infection, and then started to drop gradually during the intramammary therapy. Hence, leucocyte counts appear to be of value in predicting the infectious status of the udder (Hinchkley and leander, 1983; Abdu-ulrahman, 1996).

It appears that the infused bark extract was not uniformly distributed in the different parts of the udder tissues although, standard procedure was used. That was demonstrated by culture of milk specimens of a few goats showing sterile milk from one teat and scantly bacterial growth in milk of the other teat.

On conclusion, the data presented in this study showed that the methanolic extract of the bark of *T. Brownii* can treat induced *Staph. mastitis* in Nubian goats. Yet, clinical application of this extract in curing goats mastitis is far from speculation, unless the antimicrobial ingredients in this plant extract are well defined and their side effects are precisely monitored.

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References

Abdu-ulrahman, O.Sh. (1996). The detection of subclinical mastitis in the Bactrian camel by somatic cell count and California test *Veterinary Research Communication*, **20**(1): 9-14.

Blood, D.C. and Rodostitis, O.M. (1989). *Veterinary Medicine* 7th ed., Bailliere Tindall, P. 501-559.

Brouillette, E., Grondin, G., Lefebvre, C., Talbot, B.G. and Malouine, F. (2004). Mouse mastitis model of infection for antimicrobial compound, efficacy for antimicrobial compound, efficacy studies against intracellular and extra cellular forms of *Staphylococcus aureus*. *Veterinary Microbiology*., **101**(4):253-262.

Dixon, R.A, and Lamb, C.G. (1983). Phytoalexin: enzymology and molecular biology. *Advances in Enzymology*, **55**:1-69

Elghazali, G.E.B., Eltohami, M.S., Elegami, A.A.B., Abdalla, W.S.and Galal, M.A. (1997). Medicinal plants of Sudan: Medicinal plants of Northern Kordofan Part IV. Medicinal and Aromatic Plant Research Institute, Khartoum, Sudan. pp. 119.

Guss, S.B. (1977). Management and diseases of dairy goats. Dairy goats publishing corporation. pp. 116-126.

Haile, T. (2004). Prevalence and factors associated with bovine mastitis in South Wollo, Ethiopia, *Bulletin of Animal Health. Production in Africa.*, 52:1-6.

Haslam, E. (1996). Natural polyphenol (vegetable tannins) as drugs: possible modes of action. *Journal of Natural Product*. 59(2):205-215.

Hinckley, L.S., Leander W.F. (1981). Diagnosis of mastitis in goats, Agri-Practic, p. 1267-1271.

Hinckley, L.S., Leander, W.F. (1983). Somatic cell count in relation to caprine mastitis. Agri-Practic, p. 1267-1271.

Mead, B and Gurnow, R (1983). Statistical methods in agricultural experimental

biology, London, New York, Chapman and Hall p.111-222

Obeid, A.I. (1983). Field investigation, clinical and laboratory findings of camel mastitis, M.Sc. Thesis, University of Khartoum, Sudan.

Omer, M.E.A. and Elnima, E.I. (1999). Antimicrobial activity of *Terminalia brownii*. *Azhar Journal of Pharmacology and Science*, 24:207-215.

Peterson, K.E. (1981). Cell content in goat's milk. Acta Veterinaria Scandinavia, 22:226-237.

Philipot, W. and Nickerson, S.(1991).Mastitis : Caounter attack, Babson, Bross CO.P 96.

Scalbert, A. (1991). Antimicrobial properties of tannin. *Phytochemstry*, 30:3875-3883.

Schalm, J.W. (1970). Bovine mastitis, somatic cell count. Pub. Tandel, London-New York, P. 271-274.

Sleigh, J.D. and Timbury, M.C. (1998). Notes on medical bacteriology 5th ed., Churchill Livingstone London. P.85.

Stermitze, F.R., Lorenze, P., Tawara, J.N., Zenewiz, L.A. and Lewis, K. (2002). Synergy in medicinal plant: Antimicrobial action of berberin potentiated by 5-methoxy hydroca-rprin, a multidrug pump inhibitor. *PNAS*, 97(4):1433-1437.

Suheir, I.A., Salim, M.O. and Yasin, T.E. (2005). Bacteria, Mycoplama, and fungs Associated with sub-clinical mastitis in camel. *The Sudan Journal of Veterinary Research*, 20:21-28.

Thoria, O.O. (2007) Biological and Toxico-Pathological Studies of *Terminalia brownii Tree*. Ph.D. Thesis, University of Khartoum, Sudan.

Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaks, S., Ohyama, M., Tanka, T. and Linuma, M. (1996). Comparative study on the antimicrobial activity of Phytochemical flavones against methicillin resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology*, 50:27-38.

Zakaria, H.M., Mainen, J.M., Pax, J.M., Modest. C.K. and Ramadhani, S. (2007). Antimicrobial activity and brine shrimp toxicity of *Terminalia brownii* roots and stem. *BMC Complementary and Alternative medicine*, 1(1): 7-9.

تأثير مستخلص لحاء شجرة الصباغ في علاج التهاب الضرع المحدث بواسطة المكورة العنقودية الذهبية

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المستخلص

لمعرفة مدى فعالية المستخلص المثيلى للحاء شجرة الصباغ في علاج التهاب الضرع التجريبى المحدث بواسطة المكورة العنقودية المنتجة الذهبية لانزيم التخثر أجريت تجارب لعلاج أغنام حقنت في اضرعها بجرثومة المكورة العنقودية الذهبية علي النحو التالي: المجموعة الاولي (5 اغنام) حقنت بـــــ 0.01 مل من مزرعة حديثة للبكتريا المتيحة لانزيم التخثر تحتوى علي 9^8–10^9 من البكتريا الحية، وقد خفف هذا الزرع السائل لدرجة $1/1000$، و$1/100.000$ للمجموعتين الثانية والثالثة علي التوالي.

وقد استعمل المستخلص المثيلي للحاء الشجرة بتركز 25ملجرام/مل محلول فسيولوجى لمعالجة هذه الاغنام المصابة بجرعات 1.5 مل كل 24 ساعة لمدة 12 يوم عن طريق الحقن داخل الضرع المصاب لتحديد فعالية المستخلص فى علاج التهاب الضرع. تم عد كريات الدم البيضاء كل ثلاثة ايام خلال فترة العلاج. في اليوم الثالث عشر من بداية العلاج اخذت عينات لبن من كل ضرع وزرعت فى آجار الدم للكشف عن وجود الباكتريا. أظهرت النتائج ان المستخلص قد عالج التهاب الضرع في 40% ($2/5$) من أغنام المجموعة الاولي60% ($3/5$) من اى من اغنام المجموعتين الثانية و والثالثة. وقد وضح ذلك بنقصان عدد كريات الدم البيض للمستوى الطبيعى في اللبن وكذلك بعدم ظهور اى نمو للبكتريا في مزراع عينات اللبن.

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