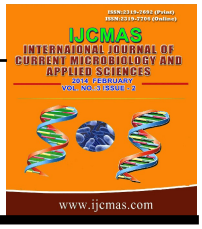
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 2 (2014) pp. 520-526**
http://www.ijcmas.com



Original Research Article

In vitro anti bacterial study of ayurvedic ointment used for post surgical dressing of haemorrhoids

Kruti Pandya*, Ghanshyam Patel, Vidhi Parikh, Krunal Ukani, Sandeep Kargathiya

Vasu Research Centre (A Division of Vasu Healthcare Pvt Ltd.)
896/A, GIDC, Makarpura, Vadodara, Gujarat
**Corresponding author*

Keywords

Kulon Ointment; *Staphylococcus aureus; Streptococcus pyogenes,* wound healing; surgical site infections etc;

A B S T R A C T

Haemorrhoids or internal piles are an extremely common condition encountered in surgical practice. Post surgery infections are one of the most common complications encountered in surgery and are a major cause of concern. This short paper is meant to be a preliminary assessment of the results carried out on one of the Ayurvedic Ointment formulated and manufactured by Vasu Healthcare Pvt Ltd., Vadodara. The ointment was tested against two gram positive bacteria viz; *Staphylococcus aureus* and *Streptococcus pyogenes* that are known to cause infection at the site of wounds or infection during surgery. The product was compared in two different ways; firstly it was compared with standard anti-bacterial discs Chloramphenicol 30mcg/disk and Ciprofloxacin 5mcg/disk. Secondly they were also screened against cream and ointment available in the market; the skin cream containing Framycetin Sulphate IP 1% w/w and the ointment containing Providone Iodine Ointment IP 5% w/w. The results of Kulon Ointment in comparison with the standards used showed promising results and thus can be used effectively during post surgical dressing of haemorrhoids.

Introduction

Haemorrhoid may be defined as a varicose dilatation of one or more of the veins in either the superior or the inferior haemorrhoidal plexus or both. The dilated veins of the superior haemorrhoidal plexus are called internal haemorrhoids. They originate above the dentate line and are covered by mucous membrane. Those of the inferior haemorrhoidal plexus are called external haemorrhoids. They are situated below the dentate line and are covered by the modified skin (Rangnekar et al., 1974).Treatment of haemorrhoid depends on the degree and symptomatology. Conservative treatment with diet and drugs is of preventive and to a lesser degree of curative value (Mukherjee, et al., 1976). Hence one of the treatments includes a surgical process to treat haemorrhoids.

Post surgery infections are a major cause of concern. Despite modern surgical

techniques and the use of antibiotic prophylaxis surgical site infections (SSI) is one of the most common complications encountered in surgery (Wilson et al., 2004). The development of SSI depends on the pathogenicity and level of bacterial load present in a wound following a surgical procedure, balanced against the host response (Accolla, 2006).

Infections occur when microorganisms overcome the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provoke a series of local and systemic host responses (Wound Microbiology; John G Thomas) . Wound infection is the commonest and most troublesome disorder of wound healing (Pea et al., 2003). Infection in a wound is a manifestation of disturbed host bacteria equilibrium that is in favour of the bacteria. This not only elicits a systemic septic response but also inhibits the multiple processes that are involved in the wound healing i.e. each of these processes is affected when bacteria proliferate in a wound (Robson, 1997).

Since wound colonization is most frequently polymicrobial (Bowler, P. G. 1998; Bowler, P. G., and Davies. 1999; Brook, and Frazier. 1998; Mousa, 1997. Summanen et al., 1995), involving numerous microorganisms that are potentially pathogenic rendering any wound at some risk of becoming infected. Healing of an acute wound follows a predictable chain of events. This chain of events occurs in a carefully regulated fashion that is reproducible from wound to wound. The phases of wound healing are overlapping, but are described in a linear fashion for the purpose of clarity. The five phases that characterize wound healing (Cohen et al., 1999 include hemostasis (Lawrence, 1998) inflammation (Cines et al., 1998), cellular migration and proliferation (White et al., 1976) protein synthesis and wound contraction, and (Hynes, 1976) remodeling.

Infection is considered a major factor in delayed wound healing, reduced tissue tensile strength gain, formation of exuberant granulation tissue and dehiscence following wound closure. Pathogenic bacteria may have or do the following (Ted S Stashak DVM, MS, Diplomate):

Adhere/bind to extracellular matrix proteins of exposed tissue which may have a direct effect on wound healing. Binding makes the protein unavailable for promoting tissue adherence.

Produce cytotoxic exotoxins (e.g. *Clostridium spp, S pyogenes, S aureus*) causing more tissue damage and creating a microenvironment conducive to their survival.

Those pathogens with thick capsules body (e.g. *S.pyogenes, S aureus and K. pneumoniae*) are more resistant to phagocytosis by leukocytes (Davis, 1965; Melly et al., 1974).

However, to date, widespread opinion among wound care practitioners is that aerobic or facultative pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and beta-hemolytic streptococci are the primary causes of delayed healing and infection in both acute and chronic wounds (Daltrey et al., 1981; Danielsen et al., 1998; Gilliland et al., 1988; Halbert et al., 1992; MacFarlane et al., 1986; Pal'tsyn et al., 1996; Schraibman, 1990; Sehgal, and Arunkumar**.** 1992; Twum-Danso et al.,

1992). Despite the frequency and prevalence of endogenous anaerobes in surgical wound infections, the Centers for Disease Control and Prevention guideline, for the prevention of surgical site infection has recognized *S. aureus*, coagulase-negative S*taphylococci*, *Enterococcus* spp., *Escherichia coli*, *P. aeruginosa*, and *Enterobacter* spp. as the most frequently isolated pathogens (Mangram et al., 1999). *Staphylococcus aureus* is the most commonly cultured organism from SSIs (Ruden et al., 1995). Hence in this study, the two pathogens chosen include *Staphylococcus aureus* and *Streptococcus pyogenes*.

Resistance to antimicrobial agents have been an age old discussed problem, hence one needs to come up with better treatments with less or no side effects.

Plants are known to possess several properties which make them effective for the treatment of Haemorrhoids, like anti-oxidant, anti inflammatory, and anti bacterial. Botanicals used internally or topically, can treat early stages of hemorrhoids effectively and can be used as adjuncts in higher stages of hemorrhoids, where surgical treatment is necessary (Brisinda, 2000).

Traditionally used botanical treatments are safe and effective for haemorrhoids. Several botanical extracts have been shown to improve wound healing conditions by enhancing microcirculation, capillary flow, vascular tone and by strengthening the connective tissue of the perivascular amorphous substrate (Sarah Khan and Michael J. Black, 2001) although they have been poorly researched (Douglas Mackay, 2001).

Hence an Ayurvedic Ointment namely "Kulon Ointment" was formulated at the R&D dept. of Vasu Research Centre (A Division of Vasu Healthcare Pvt Ltd).

This Ointment contains Nirgundi Tailam (Aryabhishak et al., 2006) and Jatyadi Tailam Aryabhishak et al., 2006) (Ayurvedic classical oil formulations), *Azadirachta indica* (Neem) Oil (Bhavprakash Nighantu of shri bhavamisra, 2002), *Pongamia pinnata* (Karanj) Oil ((Bhavprakash Nighantu of shri bhavamisra, 2002), *Cinnamomum camphora* (Karpoor) Oil((Bhavprakash Nighantu of shri bhavamisra, 2002), *Shorea robusta* (Ral) Gum resin((Bhavprakash Nighantu of shri bhavamisra, 2002), *Curcuma longa* (Haridra) Oil ((Bhavprakash Nighantu of shri bhavamisra, 2002) and micronized powder of Pushpanjan (Zinc oxide) and Shuddh Tankan (Boric acid)((Bhavprakash Nighantu of shri bhavamisra, 2002).

Materials and Methods

Sample preparation:

The sample (Kulon Ointment) was first determined for its solubility property. Different solvents were used in order to test the solubility. 0.5g of the ointment was accurately weighed and dispensed in different test tubes containing the solvents to be checked for. The results are as tabulated in Table 1.

Based on the solubility properties DMSO was chosen as the solvent and the ointment was dissolved in 5mL of DMSO taken in sterile screw capped containers. The mixture was then sonicated to get a uniform suspension and stored for not more than 24h at 4°C till use.

Bacterial Culture:

Streptococcus pyogenes ATCC 14289 and *Staphylococcus aureus* ATCC 6538 was used. The culture was revived in Nutrient Broth and incubated at 37°C for 18-24h after which this was used as the inoculum.

Preparation of Mc Farland standard Turbidity Standards

Mc Farland standard 0.5 was prepared by adding specific volume i.e. 1.174% barium chloride into 1% sulphuric acid. 10mL of Mac Farland 0.5 standard was used in this study, which was containing 9.95mL of 1% sulphuric acid and 0.05mL of 1.174% barium chloride. Standard solution was dispensed into a tube to attain comparable turbidity to that of the inoculum used. The Mc Farland 0.5 standard provides turbidity comparable to a bacterial suspension containing 1.5 x 10^8 cfu/ml (NCCLS 1993).

Media preparation

Nutrient Broth and Mueller Hinton agar (MHA) agar medium were used for determining the anti bacterial activity. Media was prepared according to the Manufacturer's instructions. The pH of the same was adjusted as per requirement. The media was then autoclaved at 15lbs pressure for 20minutes. Nutrient Broth was allowed to cool down to room temperature and kept at 4°C till use whereas the agar medium was allowed to cool down to about 45°C and then poured in sterile petri plates. The plates were then allowed to solidify overnight after which they were kept at 4°C till use.

MHA plates were used for determining the anti-bacterial activity of *S.aureus*. In order to study the anti bacterial activity of *Streptococcus pyogenes*, Mueller Hinton Agar Plates with 5% Sheep Blood were used.

The agar well diffusion method was employed to study the antibacterial activity (Mbata et al., 2006; Panda et al., 2011b).

0.1mL of the culture was added into the plates. Lawn culture of the test strain was prepared by swabbing with the help of a sterile cotton bud to give a uniform inoculum to the entire surface. The plates were allowed to dry, after which wells were bored with the help of a sterile cork borer and 0.1mL of the sample was loaded into the well. The test was carried out in triplicates to get a mean value. The plates were then incubated at 37°C for 18-24h.

Standards used:

A proprietary Ayurvedic formulation – Kulon Ointment was provided by Vasu Healthcare Pvt. Ltd. Vadodara, Gujarat, India. The ointment was checked against two well known cream/ointment available in the market such as [A] skin cream containing Framycetin Sulphate IP 1% w/w and [B] ointment containing Providone Iodine IP 5% w/w (0.5% w/w available Iodine). Apart from these two standard formulations two known antibiotic disks of known concentration such as Chloramphenicol (30mcg/disk) and Ciprofloxacin (5mcg/disk) were also screened.

The well diffusion method (Mbata et al., 2006; Panda et al., 2011b) followed above was used for the marketed standards whereas the disk diffusion method (Kirby-Bauer Method)(Bauer et al., 1966) was followed for testing the standards antibiotic disks against the bacterial species.

0.1mL of the culture was added into the plates. Lawn culture of the test strain was prepared by swabbing with the help of a sterile cotton bud. The plates were allowed to dry. With the help of a sterile forceps the antibiotic disk was gently placed in the centre of the plate to ensure full contact with the media. The plates were then inverted and incubated at 37°C for 18-24h after which the Zone of Inhibition was read.

Results and Discussion

Despite modern surgical and sterilization techniques and prophylactic use of good antibiotics, postoperative wound infection remains a major contributory factor of patient's morbidity. Conventional Ayurvedic Therapy is being used to treat haemorrhoids with satisfactory results as anti-microbial resistance is a major problem faced in the management of patients with infectious diseases.

This study thus mainly focused on an Ayurvedic formulation – Kulon Ointment that was designed to treat the wounds which posed a major problem in post operative surgeries. Two major gram positive bacteria; viz *Staphylococcus aureus* and *Streptococcus pyogenes* that are known to colonize in wound infections were considered in this study. All the raw materials and the Kulon ointment were checked against these two bacteria in comparison with modern available standards.

Table.1 Solubility Test of Kulon Ointment

Sr No	Observation Time	Solubility in different solvents					
		Water	DMSO	Hexane	Chloroform	Acetone	Methanol
1	2 min	+	+++	++	++	+	+
2	10 min	+	+++	++	++	+	+

Keys: + (weakly soluble), ++ (Partially soluble), +++ (soluble), DMSO (Di Methyl Sulphoxide), Based on the above data DMSO was chosen as the solvent for test.

Table.2 The ZOI of the product under study against both the organisms

Ingredients	*S.aureus*		*S.pyogenes*	
	0.5g + 5mL DM	1.0g + 5mL DM	0.5g + 5mL DM	1.0g + 5mL DM
NTO	19mm	22mm	11mm	12mm
JTO	No ZOI	18mm	11mm	12mm
NO	12mm	15mm	11mm	12mm
KO	No ZOI	16mm	12mm	13mm
KAO	14mm	20mm	11mm	12mm
HO	17mm	19mm	10mm	11mm
PP	18mm	21mm	No ZOI	No ZOI
ST	18mm	22mm	11mm	12mm

NTO: Nirgundi Tailam Oil; **JTO**: Jatyadi Tailam Oil; **NO**: Neem Oil; **KO**: Karanj Oil; **KAO**: Karpoor Oil; **HO**: Haridra Oil; **PP**: Pushpanjan; **ST**: Shuddh Tankan; **DM**: Di Methyl Sulphoxide (DMSO); **ZOI**: Zone of Inhibition.

Table.3 The ZOI of Kulon Ointment and the standards checked against.

Name of Drug	***S.aureus***		***S.pyogenes***	
Kulon Ointment				
KO (0.5g + 5mL DM)	18mm		10mm	
KO (1.0g + 5mL DM)	20mm		12mm	
Standard Antibiotic Disks				
Ciprofloxacin 5mcg/disk	20mm		19mm	
Chloramphenicol 30mcg/disk	18mm		27mm	
Marketed Brands				
	0.5g+5mL DM	1.0g+5mL DM	0.5g+5mLDM	1.0g+5mLDM
[A] Framycetin Sulphate IP 1% w/w	27mm	28mm	No ZOI	10mm
[B] Providone Iodine IP 5% w/w	17mm	19mm	10mm	12mm

DM: Di Methyl Sulphoxide (DMSO); **[A]** skin cream containing Framycetin Sulphate IP 1% w/w and **[B]** ointment containing

As a standard, two antibiotic disks of known concentrations viz; Chloramphenicol 30mcg/disk and Ciprofloxacin 5mcg/disk were used against both the bacteria; also two standard and well known creams / ointment commercially available in the market were also checked. The results of Kulon Ointment were found to be at par with the standards used.

The study can thus be concluded that Kulon Ointment manufactured and marketed by Vasu Healthcare Pvt Ltd is safe and effective in treating wounds and skin infections involved during post surgery.

Acknowledgement

The authors would like to express their gratitude to Vasu Research Centre, A Division of Vasu Healthcare Pvt. Ltd, Vadodara, India, for providing state of the art testing facility and financial support to carry out this research work.

References

Box, G. E., W.G. Hunter and Hunter, J.S.2005. "Statistics for Experimenters: Design, Innovation, and Discovery", 2nd Edition, Wiley.

Box, G. E. P., W.G. Hunter and Hunter, S. J.1978. "Statistics for Experimenters", John Wiley & Sons, Inc, New York, 1978.

Choi, H., S.R. Al-Abed, D.D. Dionysiou, E. Stathatos and Lianoss, P.2010. "TiO2 Based Advanced Oxidation Nanotechnologies for Water Purification and Reuse", Sustainability Sci. Engineer. 2: 229-254.

Jun Wang, W., Z. Zhaohong Zhang , Y. Rui Xu and Xiangdong, Z.2007. "Treatment of nano-sized rutile phase TiO2 powder under ultrasonic irradiation in hydrogen peroxide solution and investigation of its sonocatalytic activity", ultrasonic sonochemistry.

Manmohan Lal Kamboj, 2000. "Studies on the degradation of industrial waste water using heterogeneous photocatalysis", Department of biotechnology and environmental sciences, Thapar University, Patiala, pp.1-67.

Naresh, N., Mahamuni and Aniruddha B. Pandit. 2006."Effect of additives on ultrasonic degradation of phenol", Ultrasonic Sonochem.13:165–174.

Rajeshwar, K., J.G. Ibanez and Swain, G.M.1994. "Electrochemistry and the Environment", J. Appl. Electrochem. 24:1077.

Sandra Contreras Iglesias.2002. "Degradation and biodegradability enhancement of nitrobenzene and 2, 4-dichlorophenol by means of advanced oxidation processes based on ozone", Barcelona, pp.1-22.

Suneetha Parameswarappa, Chandrakant Karigar and Manjunath Nagenahalli. 2008. "Degradation of ethylbenzene by free and immobilized", Biodegrad.19:137–144.

US EPA.,1996. "Priority pollutants", Code of Federal regulations, Title 40, Chapter 1, Part 423, Appendix A. Environmental Protection Agency, Washington, DC.