

The Efficacy of Ethanolic Extract of Lemon Verbena on the Skin Infection Due to *Staphylococcus aureus* in an Animal Model

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Abstract: Daily increasing of *Staphylococcus aureus* resistance to various antibiotics in particular penicillin and Methicilin has led the scientist to look fore new medicines in this area. In an *in vitro* laboratory studies, it has been demonstrated that ethanolic extract of *Lemon verbena* can prevent the growth of *Staphylococcus aureus*. In this study the efficacy of ethanolic extract of Lemon verbena against *Staphylococcus aureus* skin infection were assessed in an *in vivo*, in animal model. 200λ of *Staphylococcus aureus* suspension, were inoculated intradermally on the shoulder of 63 laboratory 20-30 g mice. the mice were divided in to 4 groups, 2 control groups: Negative (without treatment) and positive(treated with Mupirucin) and 2 test groups that treated for 7 days by ointment prepared from ethanolic extract of Lemon verbena (group3), or injection of Lemon verbena solution (group 4). The status of wounds, the rate of recovery was studied and the presence of local pus after dissection of mice on day 8 recorded and compared with each other. The wound appearance in the second day, on the injection site of *S. aureus*, in Group 1, 4, 3 and 2 were 84.2, 66.7, 46.2 and 23.1%, respectively. In the final day, the lesion still was remained in 78.9, 23.1, 92.3 and 77.7% in groups 1 -4, respectively. The necrotic and wide wounds were more observed in groups 1 and 3 vs two other groups. The results from this investigation indicate that the ointment prepared from ethanolic extract of *Lemon verbena* is a proper medication to prevent the skin infection by *Staphylococcus aureus* in early phase.

Key words: *Staphylococcus aureus*, *Lemon verbena*, ethanolic extract, animal model, skin infection

INTRODUCTION

The World Health Organization (WHO) estimates that 4 billion people (80% of the world population) use herbal medicine for some aspect of primary health care (Farnsworth *et al.*, 1985). Aromatic plants and spices have great importance for food, cosmetics and pharmaceutical industries The world wide spending on finding new anti infective agents is expected to increase 60% from 1993 and plant sources being especially investigated (Cowan, 1999). Thousands of phytochemical with inhibitory effect on microorganisms have been found to be active *in vitro*.

The genus *Lippia* belongs to the Verbenaceae family and contains about 200 species and are extensively used in folk medicine (Mey *et al.*, 2007). Lemon verbena is an aromatic shrub native to Argentina and Chile (Carnat *et al.*, 1999). The deciduous plant is commonly cultivated in the other countries such as Iran.

Their leaves are used to flavor drinks and fruit and sweet dishes and to make herb tea. The tea is refreshing

and mildly sedative. The leaves also yield a green coloring and essential oil and flowering tops are used to lower fevers, to relieve gas and indigestion. Lemon verbena is claimed to be, a sedative for the nerve system.

The plants are characterized by fragrant, lemon-smelling, narrow leaves and small white flowers borne in terminal panicles. Lemon verbena can be used internally to treat feverish colds. Its anti pyretic properties are great to bring down fever and to fight colds and ease asthma. It helps with indigestion, colic, flatulence, as well as diarrhea and externally, the herb can be used to treat acne, boils (Mojab *et al.*, 2002) and it is commonly used in South Africa against various chest ailments, influenza, measles, rashes, stomach problems, headaches. (Manenzhe *et al.*, 2004).

The hydro alcoholic extracts of this plant are largely used for treatment of skin wounds as a mouth antiseptic and in liquid soap presentation to treat and prevent general fungal infection of the body (Montario *et al.*, 2006).

Its antibacterial effect were shown in some studies, its inhibitory effect were shown against *Staphylococcus aureus*, *E. coli* and *Mycobacterium tuberculosis*. It contains terpenoid which is effective against different bacteria. (Cowan, 1999; Sartoratto, 2004).

Essential oil has also been found to have good antimicrobial activity. In Bostwana it is used as a caffeine free tea, a nerve tonic and was found to inhibit gram-positive, gram-negative bacteria and Fungi (Manenzhe *et al.*, 2004).

Lippia citriodora completely inhibited the growth of *Helicobacter pylori in vitro* whose responsible for many intestinal diseases in humans (Anold, 2003).

In an *in vitro* study we found anti *Staphylococcal* effect of ethanolic extract of *Lemon verbena* (unpublished data) and this present study has been designed to find out the effect of the above extract on the *Staphylococcal* skin infection in animal model.

MATERIALS AND METHODS

Lemon verbena ethanolic extract: The part of lemons-verbena which has a pharmaceutical application is the plant leaves. The shiny green leaves collected from herbal medicine garden in Gorgan the capital city of Golestan province located in south-east of Caspian Sea in the north of Iran during 2004. One complete sample of the dried plant was herbariomised. The leaves then were let to be dried gradually and the prepared powder was used for extraction procedure using ethanol by the Percolation method. Briefly, 100 g of prepared powder was added to 600 mL ethanol (70%) in a proper closed percolator for 48 h. This extract was concentrated using a vacuum Rotary in 45°C temperatures and was dried in 45°C oven afterward.

Preparation of bacterial strain: Minimum Inhibitory Concentration (MBC) of ethanolic extract of *Lemon verbena* against 10 clinically isolated *Staphylococcus aureus* in Gorgan (North of Iran) was assessed by Macro dilution method. One strain out of above 10 bacteria, isolated from skin infection and show the highest susceptibility to ethanolic extract of *Lemon verbena*, with MBC equivalent 25 mg mL⁻¹ were chosen for this study.

Laboratory animal: Altogether 63 white laboratory male mice of 20-25 g weight which was supplied by pasture Institute of Iran were gone under this study. The hair on the shoulder region was shaved; and the location was disinfected by ethanol. Two hundred microliter of the *Staphylococcus aureus* suspension equivalent to the 0.5 McFarland was injected in sub dermal rout.

The mice then divided in 4 groups as below:

Group 1: (Negative control). Include 19 mice, without any treatment. (No treatment was done on this group.)

Groups 2: (positive control) include 13 mice, under daily treatment by Mupirucin 2%, this drug is used in treatment of carriers and local infection of *Staphylococcus aureus* (Trish, 2002; Caelli *et al.*, 2000)

Groups 3: Include 13 mice which were under daily treatment of *Lemon verbena* ointment, the bases for this ointment was Vaseline with the concentration of 20% of Lemon extract.

Group 4: Include 18 mice, which were under daily treatment by injection of 200 µL of *Lemon verbena* extract with concentration of 200 mg mL⁻¹. The injection was carrier out through the tail of the mice.

In the three groups the treatment was carried out only once a day and in particular time for 8 days. The mice were checked every day prior to the treatment for presence and intensity of the wound on the site of injection. At the end of eight days, the mice were dissected and the injection site of bacteria, were studied for any sub dermal infection.

RESULTS

We did not notice any dermal change on the site of staphylococcus injection on day 1, but the site was infected in the second day in some of the mice. The highest rate of wound in day two belong to the negative and the lowest belong to the positive control groups, about the half of the mice treated with ointment prepared from of the extract and the majority of the mice treated by injected form of the extract had wound lesion on the site of injection in second day. As the time passed; the number of mice with the wound especially among the group treated with ointment were clearly increased, as such that in day eight, 92.3% of this group had wound lesion on the site of bacterial injection (Table 1).

The intensity of the wound in the mice were varied and include small wound, till disorientated wound and some with wide necrosis, which sometimes accompanied with skin perfusion (Fig. 1).

The findings from this investigation summarized in Table 2 indicated that the mice which were treated with ointment of *Lemon verbena* extract in case of wound incidence, has the bigger intensity.

The mice were dissected after they were scarified. Figure 1D show the sub dermal infected sites under the site of injection on the day 8, after the dissection.

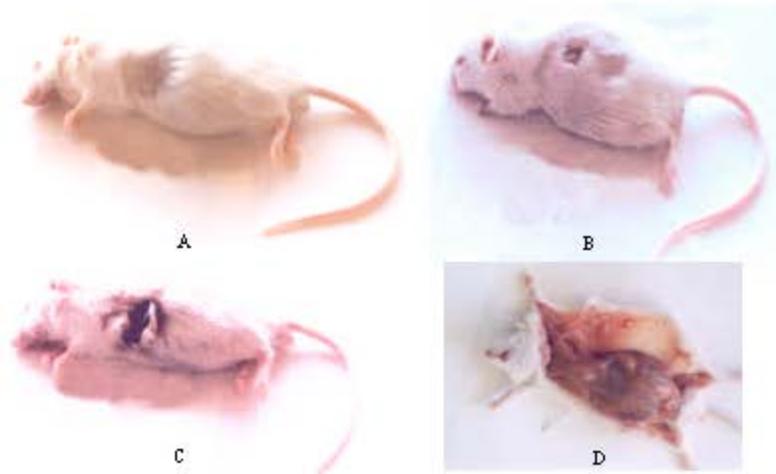


Fig. 1: Different skin lesions induced by the *S. aureus* sub dermal injection in mice. (A) small wound (B) Disorientated wound (C) wide necrosis and (D) infected lesion after dissection (8Th)

Table 1: The prevalence of wound lesion on the site of *Staphylococcus aureus* injection in the laboratory mice

Groups	Number	2nd day	5th day	8th day
Negative control	19	*16(84.2%)	15(78.9%)	15(78.9%)
Positive control	13	3(23.1%)	4(30.8%)	3(23.1%)
Ointment extract treatment	13	6(46.2%)	12(92.3%)	12(92.3%)
Injected extract treatment	18	12(66.4%)	14(77.7%)	14(77.7%)

*Cases and (%) percentage of wound lesion in each group

Table 2: The wound intensity, on the site of *Staphylococcus aureus* injection in the mice (during eight days)

Groups	Deep wound, wide necrosis	Limited light wound	Without wound
Negative control	52.6%	31.6%	15.8%
Positive control	7.7%	23.1%	69.2%
Ointment extract treatment	84.6%	7.7%	7.7%
Injected extract treatment	50%	27.7%	22.3%

The percentage of the sub dermal infection after the dissection in negative and positive control, ointment and injected extracts groups were 84.2, 30.8, 92.3 and 66.6, respectively.

DISCUSSION

Herbal medicine is playing an important role in trade industry. Yet alone in Europe in 2003 about 5 billions dollar was spend on this industry (Smet, 2005).

This research showed that injection of ethanolic extract of lemon Verbena dose not have considerable efficacy in controlling and preventing dermal infection, in spit of it's inhibitory effect on staphylococcus aureus growth *in vitro*. These differences between *in vitro* and *in vivo* experiment, may be due to some different reasons: 1) the destruction of extract within the body 2) inadequate dose of extract 3) improper method of injection.

The ointment extract of lemon Verbena was rather successful in delaying the wound occurrence till the 2nd day and also its efficacy was better from injected extract and negative control group of mice, but as the time passes its efficacy reduced and in the subsequent days, the wounds sizes in this group of mice, getting bigger than the negative control (the group with no treatment), the reason for this observation may be due to Vaseline which was used as basis for the production of ointment extract of lemon. Vaseline has a weak efficacy in spreading the chemical constituent of the herbal extract on the skin, therefore it is suggested that in further investigation other pharmaceutical basis such as Eucerin (Martindale, 1999) is to be used instead of Vaseline.

Due to some restriction ointment was only applied once a day to the site of bacterial inoculation, this can also be another reason behind the above observation, because this study also indicated that even mupirucin ointment when is applied once a day on the site of bacterial inoculation, can not prevent the wound healing in 23% of the mice and infected lesion remained at the end of eight days. Due to this reason it is suggested in future investigation to increase the daily ointment application to the site of inoculation.

We are suggesting either to increase the inoculum's dose or to use another animal type in future investigation, because even the injection dose equivalent of 1.5×10^8 bacteria (0.5 McFarland) could not cause lesions in 3 mice (15.8%).

The results from this investigation indicate that ointment extract of lemon verbena is effective in preventing skin infection and delaying the initiation of staphylococcal infection.

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