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RESEARCH ARTICLE

EFFECT OF BERBERINE ON WOUND HEALING IN EXPERIMENTAL ANIMAL MODEL

Patil T.R* and Limaye R.P

Department of Pharmacology, Bharati Vidyapeeth Deemed University Medical College & Hospital, Sangli-416 416

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ABSTRACT

Introduction: Lack of effective drugs for wound healing necessitates the need to study the potential of herbal drugs in wound healing. Berberine has antimicrobial, antiinflammatory, antiplatelet, antioxidant and thrombolytic properties which would be supplementary in wound healing mechanism. Hence, berberine deserves a study to confirm its effect on wound healing in experimental animal.

Materials and methods: Wistar rats were divided into two groups, (n=6 each group) and a wound was created which was treated with petroleum jelly in group A and petroleum jelly based berberine cream in group B. The wound healing and epithelisation of wound was evaluated at day 4,8,12,16 and 20.

Results: Group A showed an average wound size of 348±13.0 on day 4, 278±10.5 on day 8, 119±9.8 on day 12, 86±9.2 on day 16 and 100% wound healing was observed day 20 onwards. Group B showed an average wound measurement of 296±10.0 on day 4, 192±10.0 on day 8, 86±9.6 on day 12 and complete wound healing was observed day 16 onwards in the test group.

Conclusion: Berberine can be the potential drug for enhancing wound healing in normal being and also in diabetic. Due to its antidiabetic potential it can be more promising for diabetic wounds.

INTRODUCTION

Lack of effective drugs for wound healing necessitates the need to study the potential of herbal drugs in wound healing.[1,2] Berberine is a plant alkaloid with long standing history of medicinal use in Chinese and indigenous Indian medicines. It is a bright yellow coloured isoquinolone alkaloid which is a chief alkaloid found in roots, stem and bark of berberine species. It has been found to have wide spectrum of pharmacological actions like antihypertensive, antiiflammatory, antioxidant, antidiarrheal , hepatoprotective , cholegogue, anticancer, antidepressant, antimicrobial and in CHF. Effect of berberine in diabetes mellitus has been studied extensively.[3]

Inflammation is a protective response to cell injury, trying possibly to eliminate the cause and to initiate the process of repair.[4] But, unregulated excessive response arising due to enhanced activation and dysregulation of macrophages results into the release of multiple mediators of inflammation, enhancing tissue injury.[5] Wound healing, despite the availability of multiple therapeutic modalities still poses many challenges. Patients of diabetes mellitus are prone for skin wounds due to underlying complications like vasculopathy or neuropathy. Berberine has been extensively tried in diabetes mellitus to treat the diabetes and its complications.[6]

Mansoureh Pashaee *et al* studied the effect of berberine on wound healing in diabetic rats which had showed improved wound healing in the berberine treated group than control group There is a lack of evidence in the literature regarding any possible wound healing effect of berberine in normal animals or human beings, despite its antiinflammatory, antiplatelet, antioxidant and thrombolytic properties which would be conducive in wound healing mechanism.[7]

Hence, berberine deserves a study to confirm its effect on wound healing in experimental animal. So this study was conducted to assess the wound healing property of berberine among the normal non diabetic experimental animals.

MATERIAL AND METHODS

This study was carried out in the department of pharmacology and central animal house Bharati vidyapeeth deemed university medical college and hospital Sangli after getting approval from institutional animal ethical committee.[IAEC]

Study was conducted in two groups of wistar rats, randomly selected, each consisting of 6 animals and individual rat weighing between 200-250gms.Two groups of six rats each were selected, one as control group which received only

petroleum jelly and second for test drug berberine cream, to be applied locally. Each animal was housed separately in an individual cage. Light and dark cycle was maintained. They had free access to standard pellet diet and water ad libitum except 12 hours prior to the creation of wound and until rat regained full consciousness after the wound creation. Experiments were carried out between 9.00 to 16.00 hours.

Back of rats were shaved and on the next day surgical intervention was carried out under general anaesthesia with Thiopental sodium in the dose of 25mg/kg body weight. 500mm^2 full thickness circular skin was excised with scalpel blade over the nape of neck. Control animals were treated with local application of petroleum jelly and test animals received local application of 1% berberine cream. 1% berberine cream was prepared by adding 1gm of berberine powder in 99gm of petroleum jelly. Drug and petroleum jelly local application was carried out in the respective group, from the next day of wounding and was continued till the epithelisation with no raw area left behind. Wound area was traced on polythene paper and was measured with the help of planimeter.

Two parameters studied were wound measurement and period of epithelisation. Wound measurement was done from day 4th and then on day 8th, 12th and 16th and when necessary thereafter.

Period of epithelisation was observed in days.

Statistical analysis was carried out by independent t test to compare the wound healing i.e average wound measurements and average time of epithelisation between the control and test group. Probability of $p<0.05$ was considered to be significant. All the analysis was conducted using SPSS ver 13.0.

RESULTS

Group A showed an average wound size of 348±13.0 on day 4, 278±10.5 on day 8, 119±9.8 on day 12, 86±9.2 on day 16 and 100% wound healing was observed day 20 onwards. Group B showed an average wound measurement of 296±10.0 on day 4, 192±10.0 on day 8, 86±9.6 on day 12 and complete wound healing was observed day 16 onwards in the test group. Independent t test results showed a statistically significant difference in the average wound measurements between both the groups at day 4, 8, 12, 16. (table 1)

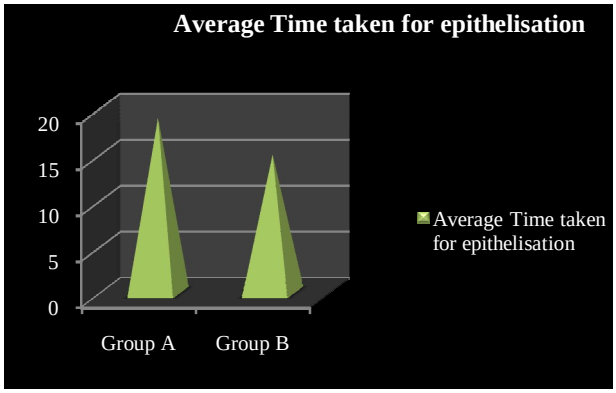
Table 1 Comparison of average wound measurement between the control and test group

Groups	Wound area mm^2			
	Day 4	Day 8	Day 12	Day 16
Group A (control group)	348±13.0	278±10.5	119±9.8	86±9.2
Group B (test group)	296±10.0	192±10.0	86±9.6	Complete wound healing (0.0±0.0)
P value	< 0.001	<0.002	<0.002	< 001

* p value <0.05 is considered significant

The average time taken for epithelisation of the wound was found to be on an average of 19 ± 1.0 for group A and 16± 0.0 for group B. Independent t test shows a significant difference between the average time taken for epithelisation of the wound between group A and Group B. (Graph 1)

Independent t test



Graph 1 Average time taken for epithelisation of wound

DISCUSSION

The results of this study shows that beberine has better potential to heal the wound created in experimental animals as compared to control group.

The wound healing effect of berberine can be explained on the basis of its various pharmacological actions like antimicrobial property, action on blood vessels and platelets, antinflammatory and antioxidant actions.

Antimicrobial action of berberine

Berberine was found to have antimicrobial property against bacteria, fungi, viruses and helminths. The possible mechanisms for these antimicrobial actions are

1. Suppression of cell adhesion and migration (e.g Streptococci, E.coli).
2. Inhibition of microbial enzymes responsible for antimicrobial drug inactivation.
3. Production of compounds like methoxy hydno carpine which inhibit the bacterial efflux mechanism responsible for antimicrobial resistance.
4. Generation of inhibitor compounds which suppress bacterial multidrug resistance pumps resulting into increased accumulation of berberine in the microbial cells.
5. By inhibiting FtSz protein regulated cell division by binding to C terminal inter domain cleft of FtSz projecting 9 methoxy group towards the outside of cavity.[3]

Berberine and blood flow

Berberine has an action on endothelium and vascular smooth muscles.[8] It was found to dilate the blood vessels. This may be due to ACE enzyme inhibition and stimulation of NO-cGMP axis.[9] Alpha 1 adrenoreceptor blockade,[10-12] enhanced sensitivity to acetylcholine [12] activation of K+ channels and hyperpolarisation of cell membrane, inhibition of intracellular Ca2+ release and blocking of L type calcium channels observed in experimental studies are the other mechanisms for its vasorelaxant effect resulting in to increased blood flow to the affected region.[13]

Expression of endothelial nitiric oxide synthase (eNOS) mRNA is increased and that of inducible nitric oxide synthase (iNOS) mRNA is decreased by berberine[14-16] Berberine improved vascular endothelial function through rise in nitric oxide levels.[17] Evidence from both human and animal studies

highlighted the role of nitric oxide in wound repair which has been attributed to its potential of angiogenesis and inhibition of inflammation.[18] Induction of iNOS enhances the oxidative stress, increases the levels of peroxinitrite and protein tyrosine nitration and hydroxyl radical production resulting in to tissue damage.[19,20]

AMPK activation by berberine inhibits platelet derived growth factor (PDGF) induced vascular smooth muscle cell growth resulting into antiproliferative action on blood vessels.[21]

Antiplatelet action of berebrine

Antiplatelet action of berberine was observed.[22] which was attributed to the inhibition of thromboxane synthesis in response to collagen, adenosine diphosphate and arachidonic acid.[22,23] inhibition of Ca 2+ influx in platelets and action on platelet adrenoreceptors.[24] Berberine also accelerated thrombolysis.[25,26] Thus berberine maintains the blood supply to the site of injury and promotes the healing of wound.

Effect of berberine on inflammation

Berberine administered orally for few weeks reduced the levels of 8 iso prostane in response to pro inflammatory lipopolysachharides [LPS] and also inhibited LPS induced rise in TNF alpha, and IL-1a [27]. Through ERK ½ signalling pathway, berberine inhibits cyclooxygenase 2 [COX- II] and also via JNK pathway in human peripheral blood monocytes when the drug is given in high doses.[28]

Nuclear factor kappa B [NFk-B] activation which modulates the transcription of genes codifying for COX II , marix metalloprotease C-9, cyclin D1 and survivin gets inhibited by the berberine and is responsible for its anti inflammatory effect.[29] In normal human keratocytes berberine exert antinflammatory effect via inhibition of matrix metalloprotein 9 and IL- 6.[30]

Berberine was found to down regulate mRNA tumour necrosis factor alpha, IL 6 and C reactive protein and heptoglobulin in 3T3N-1 adipocytes and macrophages.[31,32] There was significant reduction in inflammation and TNF alpha levels in endotoxin induced uvitits and turpentine liniment induced ocular inflammation in rabbit by the topical instillation of aqueous extract of berberine. This proves that berberine has potential anti inflammatory effect when used locally. [33,34] Berberine was found to be selective inhibitor of JAK 3 about 20 times more than JAK 2.[35] JAK 3 /STAT plays an important role in cytokine induced inflammation.[36,37] The mechanism of inhibition appears to be due to blocking of JAK 3 kinase activity at ATP binding site.[35]

Antioxidant effect of berberine

Studies conducted on rodent heptocytes have shown that berberine reduced oxidative stress, accelerated DNA repair and attenuated tissue injury. Berberine treatment opposed to the damaging effect of hydrogen peroxide and also increased cell viability, nitric oxide production and superoxide dismutase activity. It decreased lactate dehydrogenase release and manodialdehyde content.[38]

Thus various actions of berberine like anti-inflammatory, antioxidant, inhibition of iNOS mRNA and stimulation of eNOS mRNA, antiplatelet activity and effect on thrombolysis can contribute to its wound healing property. This suggests that berberine can be the potential drug for enhancing wound healing in normal being and also in diabetic. Due to its antidiabetic potential it can be more promising for diabetic wounds.

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