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Evaluation of burn wound healing potential of aqueous extract of *Morus alba* based cream in rats

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Abstract

The present protocol was designed to compare the effects of *Morus alba* (MA) aqueous extract based cream on healing of burn wounds in rats. Fifty adult, male Wistar-albino rats were divided into five groups. The first group served as a sham control group. The animals in this group underwent shaving on the back without any burn injury. Burn was generated on the backs of all the rats in second, third, fourth and fifth group. The animals of second group served as burn injury control group and did not receive any treatment. The burned areas of the rats in the third, fourth and fifth groups were covered daily with a cream base, SSD cream and MA creasm (20% MA aqueous extract based cream) respectively. The percentage of wound contraction in a period of 11 days was observed, followed by observation in the period of epithelialization of the wound till the 21st day after injury. On the 21st day, the rats were sacrificed and the burn wound skin tissue samples were collected for histopathological examinations. Morphological and histo-pathological evaluations showed burn healing to be better in the MA and SSD groups with respect to the burn injury control group. Furthermore, it is interesting to note that the MA cream treated group had a significantly better rate of burn wound healing than the SSD group. In conclusion, application of MA cream (20% MA aqueous extract based cream) proved to be highly effective in healing burn related skin wounds in the rat model.

Keywords: *Morus alba*, Silver sulfadiazine, Burn wound, Antioxidant, Rat.

Introduction

A burn is defined as damage to the skin caused by excessive heat or caustic chemicals. The most common burn injuries result from exposure to heat and chemicals. Burn injury is characterized as a kind of inflammation beneath the stratum corneum of the skin, which can progress either to spontaneous healing or can deteriorate to further necrosis, depending on the approach to treatment. Different types of burn injuries are epidemiologically prevalent in different parts of the world yet thermal burns and related injuries remain the most commonly reported cause of burn related deaths and disabilities.[1] Treatment strategies for most of the time rely on topical application of the medicament with an aim to enhance wound healing, minimize inflammatory response and most importantly prevent opportunistic infections that are commonly associated with severe wound injuries.[2] Herbs and spices with antimicrobial activity have been widely used both traditionally and commercially to increase the shelf life and safety of foods.[2, 3] The essential oils produced by different plant genera are in many cases biologically active, endowed with antimicrobic, allelopathic, antioxidant and bio-regulatory properties. The antimicrobial abilities of plant flavanoids have shown to be a particularly interesting field for applications within the food and cosmetic industries.[4]

The genus Morus from the Moraceae family consists of 10–16 species of deciduous trees that are distributed worldwide.[5] Different parts of the Morus plants such as leaves, fruit, branches, bark, root, and shoot have been used as food and herbal medicine in China for over 1900 years.[6] In Taiwan, *M. alba*, commonly known as white mulberry, is possibly the Morus species most frequently used in traditional Chinese medicine. The antioxidant potential of the extracts obtained from mulberry leaves and fruits has been investigated by several authors. Previous studies demonstrated that the *M. alba* leaves extract contained numerous substances possessing antioxidant.[7] Although, it is sometimes used interchangeably with *M. atropurpurea* and *M. bombycis*. As all three species have similar morphological characteristic

and habitat preferences they are frequently misidentified. *M. alba*, the most extensively studied species, has been reported to have anti-hyperlipidemic[8], anti-hypertensive[9, 10], anti-hyperglycemic[6, 11], Hypoglycemic[12], antimicrobial[13, 14] anti-allergic[15], anti-inflammatory[16, 17], hepatoprotective[18, 19], neuroprotective[20], immuno-modulatory[21], and anti-venom activities[22].

Photochemical studies have identified alkaloids, flavonoids, flavones, flavanones, stilbenes, benzophenones, coumarin derivatives and terpenoids in Morus species.[10, 23] These compounds are likely responsible for the bioactivities of the Morus plants. Among them, the stilbenes, oxyresveratrol and resveratrol, were reported present in the Morus plants and demonstrated antioxidant activity.[24] Oxyresveratrol inhibited nitrogen oxide (NO) production, inducible NO synthase (iNOS) expression, prostaglandin E2 (PGE2) production, and activation of nuclear factor kappa-light-chain enhancer of activated B cells (NFκB) in macrophages; and consistently reduced edema induced by carrageenan in a mouse model.[25]

The plant is a very good source of ascorbic acid, of which over 90% is present in a reduced form, and also contains carotene, Vitamin B1, folic acid, folinic acid, isoquercetin, quercetin, tannins, flavonoids and saponins, which act as a good source of natural antioxidants . *Morus alba* leaves contain rutin, quercetin and apigenin as bioactive constituents. [9] The one of major constituents of *Morus alba* is 1-deoxynojirimycin. [26] *Morus alba* leaf extract has been found to produce nitric acid, prostaglandin E2 and cytokines in macrophages. Further, a polysaccharide isolated from *Morus alba* root bark. [27] Many flavones were isolated from the root bark as active principles. [28] Many biochemical compounds such as Moranoline, Albafuran, Albanol, Morusin, Kuwanol, Calystegin and Hydroxymoricin are isolated from mulberry plants which play an important role in the pharmaceutical industry. [29]

Animal models of thermal trauma implicate oxygen radicals as a causative agent in local wound response, development of burn shock and distant organ injury. It has been proposed that the source of reactive oxygen metabolites could be neutrophils sequestered in systemic organs as a result of the systemic inflammatory reaction to a local burn insult.[3] However to the best of our knowledge, no experimental study has yet documented the effect of orange peel on thermal burn injury. Therefore, the present study was designed to evaluate the burn wound healing property of aqueous extract based cream of *Morus alba* (Moraceae) in experimentally induced burn injury in rat.

Burn injury is characterized as a kind of inflammation beneath the stratum corneum of the skin, which can progress either to spontaneous healing or can deteriorate to further necrosis, depending on the approach to treatment.

Materials and Methods

Preparation of *Morus alba* extract

The leaves of *Morus alba* were collected from the botanical garden of Khalsa College, Amritsar, Punjab, India. The plant material was authenticated by Dr. P.K. Ahuja, Professor, postgraduate, Department of Botany, Khalsa College Amritsar, India. Leaves were dried in shade at room temperature. Powdered leaves (500 g) were extracted successively with Petroleum Ether, Chloroform, Methanol and Water in increasing order of polarity of solvents. The solvents were evaporated from the extracts under reduced pressure using Rota evaporator. The extracts were further dried at an oven temperature of 50ºC to get dry powder. The Aqueous extract obtained was selected in the present protocol for further evaluation.

Preparation and formulation of *Morus alba* extract cream

Two grams of liquid white paraffin, 7.5 grams of stearyl alcohol, 3 grams of solid white paraffin, and propyl paraben (0.015 g) were mixed and heated to a boiling point as aqueous phase. Twenty grams of dried extract powder mixed in 70 ml deionized water were added to the mixture of 7 grams of propylene glycol, 3 grams of sodium lauryl sulfate and 0.025 grams methyl paraben. The mixture was heated as organic phase. Then, two separate phases were mixed continuously while being treated to a constantly decreasing temperature. Thus, the uniform cream was produced after cooling. The cream was filled with easily squeezable tube. Cream contained 20% of dried extract. Our experimental research and formulations were carried out under sterile conditions. The final cream formulation was tested for sterility and for any probable contaminating microbes.

Animals

Wistar albino Rats of either sex weighing 175±25 g were employed in the present study. Animals were fed with standard laboratory feed (Kisan Feeds Ltd., Chandigarh, India) and water *ad libitum*. They were housed in the departmental animal house and were exposed to natural cycles of light and dark. The experimental protocol was approved by the Institutional Animal Ethics Committee and the care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Registration No. 1753/PO/a/14/CPCSEA).

Experimental Protocol

Animals were divided into 5 groups, each consisting of ten animals. The duration of the experimental protocol was 21 days. The groups assigned were as follows:

- Sham control Group
- Burn injury Group
- Cream base treated Group
- Silversulfathiazine (Standard) treated Group
- Burn injury + MA Extract Based Cream treated Group

Experimental Procedure

Group 1 *Sham control group*: Animals in this group were just made to undergo the shaving procedure on the ear and then were kept undisturbed for the whole study protocol.

Group 2 *Acute burn injury group*: Animals were given scalding burn injury, according to a pre-devised method. [30] Animals were restrained in the rat holder and 5cm of the area on the back of the rats were carefully shaved to expose the skin. Boiling water was poured for 15 seconds on the exposed skin to induce full thickness burn. After the burn injury was

induced, 0.8 mL of normal saline was given intraperitonially to the animals to prevent spinal shock.

Group 3 *Cream Base treated group*: Animals were given scalding burn injury as discussed in Group 1. After the administration of saline solution, cream base (without any medicament) was applied on the affected area so as to cover the whole area.

Group 4 *Silversulfathiazine (Standard Drug) cream treated Group:* Animals were given scalding burn injury as discussed in Group 1. After the administration of saline solution, Silversulfathiazine cream (Silverex®) was applied on the affected area so as to cover the whole area.

Group 5 *MA Extract based cream treated group*: Animals were given scalding burn injury as discussed in Group 1. After the administration of saline solution, aqueous extract of *Morus alba* based cream was applied on the affected area so as to cover the whole area.

Pharmacological Evaluation

The parameters observed in the study were as follows:

Epithelialization period: It was monitored by noting the number of days required for the eschar to fall off from the burn wound surface *without leaving a raw wound behind.*

Wound Contraction: It was noted by following the progressive changes in wound area planimetrically, excluding the day of the wounding the size of the wounds was traced on a transparent paper every two days, throughout the monitoring period. The tracing was then transferred to $1mm^2$ graph sheet, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound 500 mm^2 as 100%, by using the following equation:

Percentage of wound contraction = [(Initial wound size – specific day wound size) / initial wound size] X 100.

Histopathology of the skin tissue: On the 21st day, the animals were sacrificed after being anesthesized, burned skin tissue samples were collected after sacrificing the rats for histopathological examination purposes. These tissue samples were fixed at 10% neutral buffered formalin solution, embedded in paraffin wax, cut into 5 µm-thick sections and stained with hematoxylin-eosin and Masson's trichrome stain for examination by light microscopy. The slides were examined under

Statistical analysis

The results were analyzed using one-way Anova followed by Tukey's *post-hoc* analysis with $p \leq 0.001$ considered significant for all values.

Results

The percentage of wound contraction was significantly higher in the *Morus alba* Leaf extract based cream treated group as compared to that of burn injury group (Table 1). Furthermore, it was interesting to note that on the 11th day of study, the percentage of wound contraction in the MA cream treated group was significantly higher than that of the standard drug Silver sulfadiazine. The mean period of epithelialization was found to decrease significantly in the *Morus alba* leaf extract based cream treated group as compared to that of burn injury group as well as Silver sulfadiazine (Standard Drug) group (Table 2).

The Histo-pathological changes in the burned tissue were also significantly less evident in the extract based cream treated group as compared to control group as is evident from a the formation of an incomplete epithelial layer formation even on the 21st day after injury, neutrophillic infiltration and perivascular inflammatory changes in the control group as compared to the MA extract based cream and SSD cream treated group (Figure 1). No pathological changes were seen in the sham control group.

A significant increase in the percentage of wound contraction in the extract treated group also indicated the rapidity with which the *Morus alba* Leaf extract based cream led to burn injury healing.

Table 1: Effect of Morus alba Leaf extract based cream on wound contraction in a period of 11 days

Treatment Groups	Percentage of wound contraction (Mean ± S.E)					
	Day 1	**Day 3**	**Day 5**	**Day7**	**Day 9**	**Day 11**
Sham group	-	-	-	-	-	-
Burn Injury group (Control)	0	6.4± 0.4	18.2± 0.3	21.8± 0.4	32.6±0.3	41.4± 0.2
Cream base treated Group	0	6.9± 0.3	19.4±0.4	23.3±0.2	34.7±0.4	43.6±0.3
Silversulfadiazine (Standard drug) cream treated Group	0	8.6± 0.02[a]	21.3±0.4[b]	41.5±0.2[c]	57.3±0.2[d]	65.6±0.2[e]
MA Extract based cream treated group	0	9.8± 0.02[a]	24.7± 0.3 [b]	44.7± 0.3 [c]	64.6± 0.4 [d]	84.6± 0.4[e,f]

(a P< 0.05 against burn injury group on day 3; b P< 0.05 against burn injury group on day 5; c P< 0.05 against burn injury group on day 7; d P< 0.05 against burn injury group on day 9; e P< 0.05 against burn injury group on day 11; f P< 0.05 against silversulfadiazine treated group on day 11)

Table 2: Effect of Morus alba Leaf extract based cream on wound contraction in a period of 21 days.

Treatment	Period of Epithlialization in days (Mean±S.E)
Sham group	-
Burn injury group	20.1±0.4
Cream base Treated Group	19.6 ± 0.2
Silversulfadiazine cream treated group	16.5± 0.4[a]
MA Extract based cream Treated group	12.1±0.3[a, b]

a P< 0.05 against burn injury group; b P< 0.05 against silversulfadiazine treated group

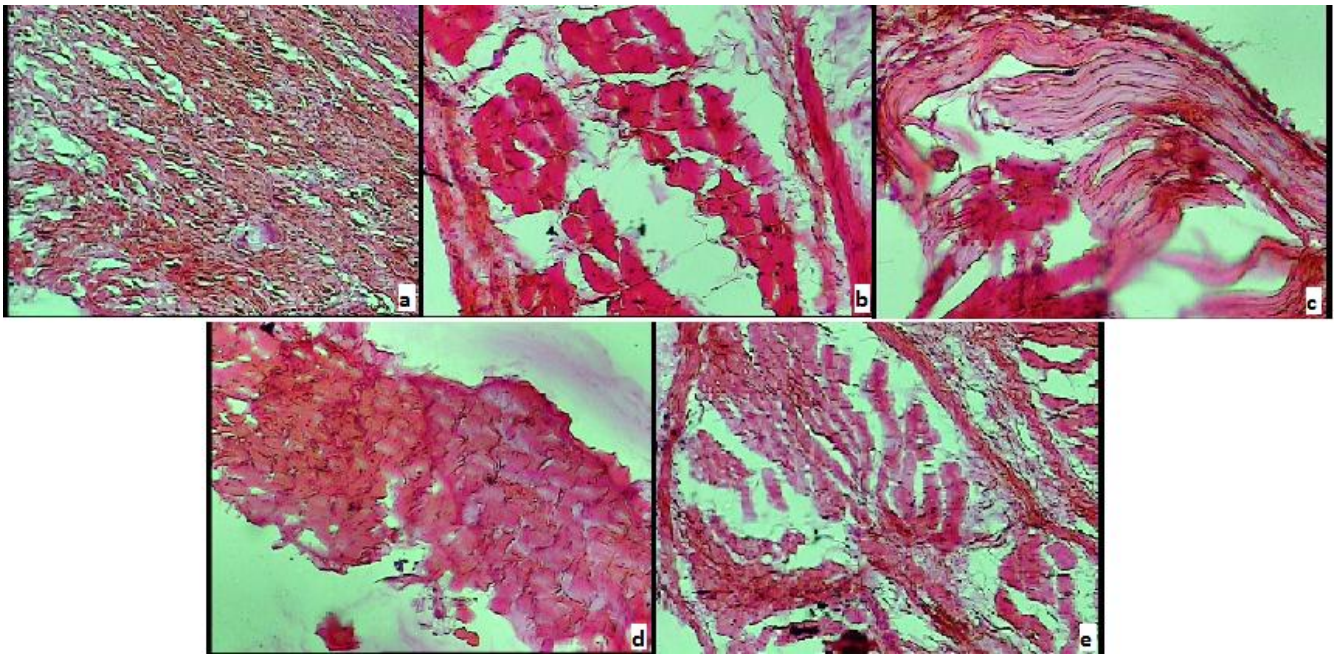

Figure 1: Representative Histological slides of skin wound tissue of various groups on 21st Day after burn injury. **a) Sham control group** slide with no histological changes, **b) Control group** (*Thermal burn*) slide is characterized by incomplete formation of epithelial layer, inflammatory tissue damage and edema, **c) Cream base treated group** showing significant inflammatory damage and tissue edema, **d) SSD cream (Standard) treated group** characterized by significant tissue repair and re-structuring with little edematous fluid, **e) MA extract based cream treated group** showing marked re-structuring and re-organizing of the burn wound tissue. There is complete reformation of the epithelial layer with no edematous swelling of the tissue.

Discussion

Plants of the genus *Morus* are known to be a rich source of flavonoids, including quercetin 3-(-malonylglucoside), rutin, isoquercitin[12], cyanidin 3 rutinoside and cyanidin 3-glucoside.[31, 32] From the present study, it was found that treatment with aqueous extract of leaves of *Morus alba* based cream in thermal burn injury in rats led to faster recovery and less tissue damage as compared to untreated animals.

In the past many studies have been done using natural products for the treatment of burn wounds, but these were mainly aimed at controlling infections associated with burns. However, production of free radicals, such as superoxide and peroxynitrite, in the early phase of an extensive burn exacerbates many aspects of the injury process, such as an increase in microvascular permeability and the production of inflammatory mediators. The aggressive fluid replacement needed to stabilize burn patients in this period appears to exacerbate the lipid peroxidation that leads to the production of free radicals.[33, 34] Free radical release subsequently exacerbates inflammatory cytokine production and the influx of activated neutrophils into the lungs. Therefore, several antioxidant therapies have been tried to improve critical condition in extensive burns Flavonoids exist widely in the plant kingdom. Mulberry leaves have been shown to contain at least four flavonoids, including rutin.[35] Flavonoids have long been recognized to possess anti-inflammatory, antioxidant,

antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities. The antioxidant properties of mulberry leaf extracts were investigated by Arabshahi-Delouee[36] using various experimental methods, including the iron (III) reducing capacity, the total antioxidant capacity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and an *in vitro* inhibition of ferrous sulfate-induced oxidation of lipids. The percentage of superoxide ion scavenged by extracts of mulberry leaves, mulberry tender leaves, mulberry branches and mulberry bark were 46.5, 55, 67.5 and 85.5%, respectively, at a concentration of 5 μg/mL.[37] The antioxidant activities of ethanolic extracts of five mulberry cultivars from Korea were determined using the DPPH radical assay, hemoglobin-induced linoleic acid system, and reducing power. The extracts were found to be strong scavengers of hydroxyl radicals and superoxide anions.[38] Anti-inflammatory activity of certain natural products also plays a vital role in their anti-burn potential. Although the present study did not explore the exact mechanism of the beneficial effect of *the Morus alba leaf extract* based cream on burn injury, it could be attributed to anti-oxidative property of phytoconstituents such as stilbenes, oxyresveratrol and resveratrol present in *the Morus alba leaf extract*. [24]

Conclusions

From the present findings, it can be postulated that MA extract based cream exhibited potent wound healing potential against thermal burn injury and that the anti-burn activity of the MA extract could be attributed to the anti-oxidative potential of its major components such as oxyresveratrol and resveratrol or other flavanoids. However, still further research is warranted to delineate the mechanism of present findings and to evaluate the major phytoconstituents responsible for the burn healing property exhibited by *Morus alba* leaves extract.

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