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Research Article

Beneficial effects of *Morus alba* and *Azadirachta indica* leaf extract based combination cream in scalding type burn injury in rats

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ABSTRACT

Background: The present study was designed to evaluate the potential of a combination cream of aqueous extracts of leaves of *Morus alba* (MA) and *Azadirachta indica* (AI) in scalding type burn wound injury in rats.
Methods: Plant material was successively extracted and aqueous extracts were selected. Three extract based cream formulations *viz.* 20% w/w (MA), 20% w/w (AI), and combination cream containing 10%+10% w/w (MA+AI) were prepared. Cream base and standard anti-burn cream containing silver sulfadiazine were also used for comparison. Scalding type burn was given by pouring water at 90°C on a shaved dorsal area of 20 mm^2. Deep second-degree burn injury was produced which was evaluated for next 21 days for a percentage of wound contraction and period of epithelialization. On 21st day, animals were sacrificed and histopathological slides were prepared using hematoxylin-eosin staining. Burned tissue was also screened for levels of oxidative stress using thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) estimation.
Results: There was a significant increase in the percentage of wound contraction and significant decrease in period of epithelialization in MA, AI, and MA+AI combination cream treated group as compared with control group. However, most significant results were obtained with MA+AI combination cream. Histologically, MA+AI cream treatment resulted in almost complete re-epithelialization and restructuring of the wound tissue. There was a significant rise in TBARS and decrease in GSH levels in burn injury group which was reversed to a major extent by the application of combination cream.
Conclusions: The results indicate toward the possible role of free radical scavenging potential of extracts in the Burn wound tissue healing.

Keywords: *Morus alba*, *Azadirachta indica*, Scalding, Cream, Burn injury, Free radicals

INTRODUCTION

Burns are a major health problem worldwide, with high mortality and morbidity in addition to causing changes in the quality of life of burn patients. Thermal burn injury is a major cause of death and disability, with a high cost in health care. Burn injury represents a specific type of injury that can be caused by multiple factors such as heat, freezing, electricity, chemicals, radiations, etc. Burn is a wound in which there is coagulative necrosis of tissue. Scalds from hot liquids and steam, building fires, and flammable liquids and gases are the most common causes of burns. Healing of burn wounds still remains a challenge to modern medicine, though many antiseptics have been discovered. Burn management requires significant duration of hospital stay, expensive medication, multiple operative procedures, and prolonged period of rehabilitation. This makes burn care an expensive proposition and every effort should be made to provide a shorter in-patient care for the burn patients.[1] Variability in the range and extent of burn injury is its hallmark and depends

upon the type of tissue affected, the severity of burn and the resultant complications involved.[2]

Burn severity may be characterized by two main factors *viz.* type and extent of skin layers involved (which itself is a function of temperature and exposure time to burn stimulus) and burn body surface area (BSA). In case, more than 20% of BSA is involved, it is usually called burn disease.[3]

One of the major mediators of the necrotic cell injury process is oxidative free radical-induced cell injury. Large amount of reactive oxygen species (ROS) are produced in the region of burn injury and these free radicals have been shown in many studies to be implicated in the tissue necrosis process which is a hallmark of burn injury.

The ROS, released during burn disease, attack the unsaturated fatty acid rich cell membrane. Lipid peroxidation is one of the most dangerous pathological reactions during burn injury.[4,5] Lipid peroxidation triggers changes in the cell membrane, therefore markedly damages the function of cell membrane related proteins.[6] There is a close correlation between the severality of lipid peroxidation, burn-related organ failure, and burn shock. Burn injury causes intravascular neutrophil granulocyte activation, which leads to increased ROS production. ROS oxidize the phospholipid membranes of cells. Lipid peroxidation end-products manifest in the burn affected tissues, edema, and lymphocytosis; this shows the role of this oxidative pathomechanism.

Medicinal substances of natural origin possessing free radical scavenging actions such as curcumin[7] have been reported to produce highly beneficial effects in burn injury treatment. Various plant and their phytoconstituents have been utilized both traditionally and recently for affording the curative effect on burn injury wounds of various types. The antioxidant properties of various plants have been explored for the healing of burn wound/injury such as *Crocus sativus,*[8] orange peels,[9] etc. Utilizing antioxidant therapeutic strategies depending on new mechanisms involved in the pathogenesis of burns-related "oxidative stress" may be considered a promising step in burns management. Various antioxidants are used to antagonize ROS: vitamin E, the lipid soluble chain-breaking antioxidant;[10] vitamin C, which in addition to its antioxidant effect serves to recycle vitamin E;[11] zinc sulfate, which has acute and chronic antioxidant effects;[12] allopurinol, the well-known xanthine oxidase inhibitor;[13] melatonin, the pineal gland product that has powerful antioxidant effects through its direct scavenging effect and stimulation of antioxidant enzymes;[14] and finally N-acetylcysteine, which historically was used as a mucolytic agent - this agent is rapidly metabolized to cysteine, which is a direct precursor in the synthesis of intracellular glutathione (GSH), the natural antioxidant.[15] Interference by such antioxidants in the treatment of burns has been proposed to have an improving effect on burn outcomes.

Despite advances in burn care techniques, there is still a tendency toward therapeutic failure in patients who sustain burns, especially in a large percentage of the total BSA (TBSA); the modification of medical treatment protocols and the search for new mechanisms involved in the pathogenesis of burns may be helpful in the successful treatment of burn patients.[16] Burns are a common traumatic injury that results both in local tissue damage and in a systemic mediator-induced response; there is evidence of both local and systemic oxidant changes manifested by increased free radical activity and lipid peroxidation. At the same time, burn injury causes a remarkable decrease in total antioxidant status and a reduction in antioxidant scavenging capacity when compared with control.[17] The presence of free radicals in concentrations that overwhelm natural radical blocking or scavenging mechanisms results in oxidative stress, and targeting of this condition with antioxidants may be considered a promising step for improving the outcome of burns.

The genus Morus from the Moraceae family consists of 10-16 species of deciduous trees that are distributed worldwide.[18] 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.[19] In Taiwan, *Morus alba* (MA), 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 have been investigated by several authors. Previous studies demonstrated that the MA leaves extract contained numerous substances possessing antioxidant.[20]

Biologically active principles isolated from different parts of the plant include: azadirachtin, meliacin, gedunin, nimbidin, nimbolides, salanin, nimbin, valassin, meliacin forms the bitter principles of neem oil, and the seed also contain tignic acid responsible for the distinctive odor of the oil.[21] Neem kernels contain 30-50% of oil mainly used by the soap, pesticide, and pharmaceutical industries and contain many active ingredients which are together called triterpene or limnoids.[22] The four best limonoids compounds are: azadirachtin, salanin, meliantriol, and nimbin. Limonoids contain insecticidal and pesticidal activity.[23]

Azadirachta indica (AI) has been evaluated for its various properties such as antibacterial and anti-diabetic,[24] antioxidant,[25] larvicidal,[26] anti HIV/AIDS,[27] skin disorders,[28] anti-ulcer,[29] anti-malarial,[30] anti-tumor,[31] antifertility,[32] anti-dental caries,[33] anti-hypertensive, and anti-hypercholesterolemic activity.[34]

The thermal model that we prefer is scalding. In our opinion, this model offers conditions of the facility, reliability, and control that make it superior to all others. We produce the thermal lesion on the back of the animal as follows: after anesthetization of the animal an electric shaver is used to expose a cutaneous surface on the back, which

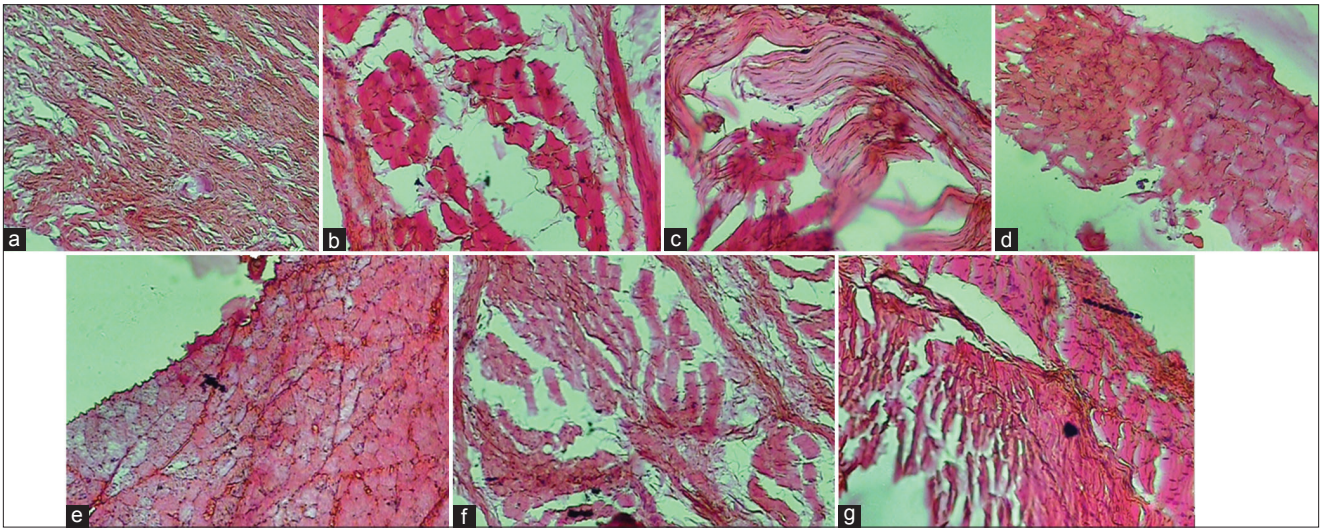

Figure 1: Representative histological slides of skin wound tissue of various groups on 21st day after burn injury. (a) Sham control group slide with no histological changes, (b) control group slide is characterized by incomplete formation of epithelial layer, inflammatory tissue damage and edema, (c) cream base treated group showing significant inflammatory damage and tissue edema, (d) silver sulfadiazine cream (standard) treated group characterized by significant tissue repair and restructuring with little edematous fluid, (e) *Morus alba+Azadirachta indica* (MA+AI) combination extract based cream treated group showing significant healing of the burned tissue the cellular structure of the dermal layers have been restored. Signs of necrosis have been limited to the most extent and the tissue is devoid of any perivascular infiltration into the tissue spaces, (f) MA extract based cream treated group showing some re-structuring and re-organizing of the burn wound tissue. The process of tissue necrosis appears halted and tissue spaces less widened, (g) AI extract based cream treated group showing partial reformation of the epithelial layer with signs of tissue se-structuring and healing. Moreover, there is little edematous swelling of the tissue. However, the results are not as marked as that of MA+AI combination extract based cream treated group.

constitutes 30% of TBSA; the rat is then placed on its back in a moldable metal wire cage (Figure 1). This device is a modification of that described by Walker and Mason.[35] The method has been slightly modified to get consistent results. Once the animal is securely immobilized in the metal cage, the shaved dorsal area is submerged for 15 sec in water at 90°C. This model inflicts a deep dermal burn in the entire cutaneous area exposed. Histologically, skin burned in this way shows the classic signs of Epidermal necrosis, a diffuse perivascular infiltrate, and an important level of collagen degeneration at the papillary dermis.[36] Our laboratory has recently documented the potential of MA (Mulberry) leaf extract based cream in burn injury in experimental animals. However, the benefit could possibly be enhanced by addition of an anti-inflammatory as well as antiseptic component such as neem leaf extract.

However, as per our knowledge, there is no documented research conducted to evaluate the potential of combination of mulberry and neem leaves extract in scalding burn injury-induced pathological changes in experimental animals.

Therefore, the present study has been designed to evaluate the potential of aqueous extract of MA (Mulberry) and AI (Neem) leaves based cream preparation on scalding type burn injury in rats.

METHODS

Preparation of MA and AI extract

The leaves of MA and AI were collected from the Botanical garden of Khalsa College, Amritsar, Punjab, India. The plant material was authenticated by Dr. Ahuja, Professor, Postgraduate, Department of Botany, Khalsa College Amritsar, India. Leaves of both plant species 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. Solvent evaporation from the extracts was performed under reduced pressure using rotary evaporator. Obtained extracts were further dried in hot air oven at 50°C to get dry powder. The aqueous extracts of both plant species were selected in the present protocol for further evaluation.

Preparation and formulation of MA extract cream

Two grams of liquid white paraffin, 7.5 g of stearyl alcohol, 3 g of solid white paraffin, and propylparaben (0.015 g) were mixed and heated to a boiling point as aqueous phase. 20 g of dried extract powder mixed in 70 ml deionized water were added to the mixture of 7 g of propylene glycol, 3 g of sodium lauryl sulfate, and 0.025 g methylparaben. The

mixture was heated as the 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 in an easily squeezable tube. Cream contained 20% of dried extract.

Preparation and formulation of AI extract cream

The procedure adopted was similar as described for the MA based cream. However, in this case, 20 g of dried aqueous extract of AI was added in the formulation. The prepared cream contained 20% w/v 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.

Preparation and formulation of MA and AI extract combination based cream

The procedure adopted was similar as described for the MA based cream. However, in this case, 10 g of dried aqueous extract of AI leaves and 10 g of dried aqueous extract of MA leaves were added in the formulation. The prepared cream contained 10% w/v of dried extract of each plant material. 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 on 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 Institutional Animal Ethics Committee and 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 7 groups each consisting of 10 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
- Silver sulfadiazine (SSD) (standard) treated group
- Burn injury+MA extract based cream treated group
- Burn injury+AI extract based cream treated group
- Burn injury+MA and AI extract combination based cream treated group.

Experimental procedure

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

Group 2 - Scalding type burn injury group: animals were given scalding burn injury according to a pre-devised method (Bhatia et al., 2011). Animals were restrained in the rat holder and 20 mm^2 of the area on the back of the rats were carefully shaved to expose the skin. Boiling water was poured for 15 sec on the exposed skin to induce full thickness burn. After the burn injury was induced, 0.8 ml of normal saline was given intraperitoneally to the animals to prevent spinal shock.

Group 3 - Cream base treated group: animals were given scalding burn injury as discussed in Group 2. 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 - SSD (standard drug) cream treated group: animals were given scalding burn injury as discussed in Group 2. After the administration of saline solution, SSD cream (Silverex®) was applied on the affected area, so as to cover the whole area.

Group 5 - MA+AI combination extract based cream treated group: animals were given scalding burn injury as discussed in Group 2. After the administration of saline solution, a combination cream of aqueous extract of MA and Aqueous extract of AI was applied on the affected area, so as to cover the whole area.

Group 6 - MA extract based cream treated group: animals were given scalding burn injury as discussed in Group 2. After the administration of saline solution, aqueous extract of MA-based cream was applied on the affected area, so as to cover the whole area.

Group 7 - Neem extract based cream treated group: animals were given scalding burn injury as discussed in Group 2. After the administration of saline solution, aqueous extract of AI (neem) 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 2 days, throughout the monitoring period. The tracing was then transferred to 1 mm^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 20 mm^2 as 100%, by using the following equation:

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

Histopathology of the skin tissue

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

Biochemical estimation of the wound tissue

On 21st day, the animals were sacrificed after being anesthetized and approximately 2 cm^2 of the burned skin tissue samples was dissected out for biochemical estimations. Biochemical estimations involved tissue thiobarbituric acid reactive substances (TBARS) and tissue reduced GSH concentrations respectively.

Tissue TBARS estimation

The concentration of TBARS in the skin tissue was estimated by the method of Niehaus and Samuelsson.[37] In brief, 0.1 ml of tissue homogenate (supernatant: Tris-HCl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA- trichloroacetic acid (TCA)-HCl reagent (0.37% w/v thiobarbituric acid, 0.25 N HCl and 15% w/v TCA), placed in water bath for 15 mins, cooled, and centrifuged at 1000 rpm at room temperature for 10 mins. The absorbance of the clear supernatant was measured against reference blank at 535 nm. The values were expressed as mmol/100 g of tissues.

Tissue reduced GSH estimation

The amount of reduced GSH was estimated in the burn wound tissue by the method of Ellman.[38] Briefly, 1 g of skin tissue was homogenized with Tris - HCl buffer, pH 7.5. The homogenate was centrifuged at 4000 rpm for 5 mins. About 1 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5'- dithiobis nitrobenzoic acid in 100 ml of sodium citrate) and 3 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

Statistical analysis

The results were analyzed using one-way Analysis of Variance followed by Tukey's *post-hoc* analysis with $p \leq 0.05$ considered significant for all values.

RESULTS

In the present study, the percentage of wound contraction was significantly high in the MA and AI leaf extracts combination based cream treated group as compared to that of burn injury group, as well as MA leaf aqueous extract based cream treated group and AI leaf aqueous extract based cream treated group (Table 1). Furthermore, it was interesting to note that on the 11th day of study, the percentage of wound contraction in the MA+AI combination cream treated group was significantly high than that of the standard drug SSD. Significant increase in the percentage of wound contraction in the extract treated group also indicated the rapidity with which the MA+AI combination cream led to burn injury healing.

The mean period of epithelialization was found to decrease significantly in MA+AI combination cream treated group as compared to the MA leaf aqueous extract based cream treated group and AI leaf aqueous extract based cream treated group. As well as SSD (standard drug) treated group (Table 2).

The histopathological changes in the burned tissue were also significantly less evident in the MA+ AI combination cream treated group as compared to control group as is evident from a the formation of an incomplete epithelial layer formation even on 21st day after injury, neutrophilic infiltration and perivascular inflammatory changes in the control group as compared to the MA extract based cream and SSD cream treated group (Figure 1). Furthermore, individual MA extract based cream treated group and AI extract based cream treated group also produced a decrease in histopathological changes. However the results were less prominant as compared to the results with the combination cream group. No pathological changes were seen in sham control group.

In biochemical estimation of oxidative stress parameters, it was observed that burn injury results in a significant increase in the wound tissue TBARS levels. Furthermore, treatment with MA+AI combination cream resulted in a significant decrease in the burn wound tissue TBARS levels as compared to control group (Figure 2). It was interesting to note that individual MA extract based cream treated group and AI extract based cream treated group also produced a decrease in wound tissue TBARS levels as compared to control group. However, the MA and AI combination cream produced more marked decrease in the TBARS levels.

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

Treatment groups	Percentage of wound contraction (mean±SEM)					
	Day 1	Day 3	Day 5	Day 7	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
SSD (standard drug) cream treated group	0	8.6±0.2[a]	21.3±0.4[b]	41.5±0.2[c]	57.3±0.2[d]	65.6±0.2[e]
MA+AI combination extract based cream treated group	0	15.4±0.3[a]	31.4±0.2[b]	49.8±0.4[c]	72.4±0.2[d]	93.3±0.3[e,f]
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]
AI extract based cream treated group	0	8.3±0.3[a]	22.7±0.5[b]	43.2±0.2[c]	61.7±0.2[d]	79.6±0.6[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 SSD treated group on day 11, SEM: Standard error mean, MA: *Morus alba*, AI: *Azadirachta indica*, SSD: Silver sulfadiazine

Table 2: Effect of MA leaf extract based cream on wound contraction in a period of 21 days.

Treatment	Period of epithelialization in days (mean±SEM)
Sham group	-
Burn injury group	20.1±0.4
Cream base treated group	19.6±0.2
SSD cream treated group	16.5±0.4[a]
MA+AI combination extract based cream treated group	11.8±0.3[a,b]
MA extract based cream treated group	12.1±0.3[a,b]
AI extract based cream treated group	14.6±0.2[a,b]

[a]p<0.05 against burn injury group, [b]p<0.05 against SSD treated group, SEM: Standard error mean, MA: *Morus alba*, AI: *Azadirachta indica*, SSD: Silver sulfadiazine

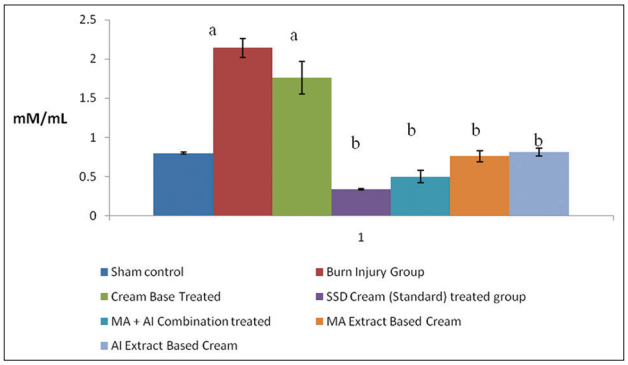

Figure 2: Changes in wound tissue thiobarbituric acid reactive substances (TBARS) after scalding type burn injury. The graph represents tissue levels of TBARS (mm/ml) of sham, burn injury control, burn injury+cream base treated, burn injury+silver sulfadiazine (standard) treated, burn injury+different extract based cream treated groups. Results are represented as the mean±standard error mean with n=06 in each group. [a]p<0.05, as compared to the sham group; [b]p<0.05, as compared to burn injury group.

Moreover, estimation of concentration of levels of reduced GSH revealed that burn injury results in a significant decrease in the wound tissue GSH levels. Furthermore, treatment with MA+AI combination cream resulted in a significant increase in the burn wound tissue GSH levels as compared to control group (Figure 3). Similar to the estimation of TBARS levels, GSH estimation also depicted that individual MA extract based cream treated group and AI extract based cream treated group also produced increase in wound tissue GSH levels as compared to control group. However, the MA and AI combination cream produced a more marked increase in the GSH levels.

DISCUSSION

Plants of the genus Morus are known to be a rich source of flavonoids including quercetin 3-(-malonylglucoside), rutin, isoquercitin,[39] cyanidin-3 rutinoside, and cyaniding-3-glucoside.[40,41] From the present study, it was found that treatment with an aqueous extract of leaves of MA-based cream in thermal burn injury in rats led to faster recovery and less tissue damage as compared to untreated animals.

Mulberry leaves have been shown to contain at least four flavonoids, including rutin.[42] 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 and Urooj[43] using various experimental methods including the iron (III) reducing capacity, the total antioxidant capacity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) 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.[44] The antioxidant activities of

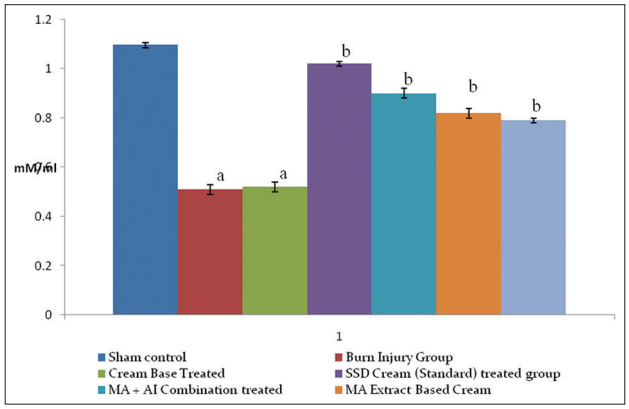

Figure 3: Changes in wound tissue glutathione (GSH) after scalding type burn injury. The graph represents tissue levels of GSH (mm/ml) of sham, burn injury control, burn injury+cream base treated, burn injury+silver sulfadiazine (standard) treated, burn injury+different extract based cream treated groups. Results are represented as the mean±standard error mean with n=06 in each group. [a]p<0.05, as compared to the Sham group; [b]p<0.05, as compared to burn injury group.

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.[45] The anti-inflammatory activity of certain natural products also plays a vital role in their anti-burn potential.

Neem oil contains margosic acid, glycerides of fatty acids, butyric acid, and trace valeric acid. Alcoholic extract of neem is useful in eczema, ringworm, and scabies. Neem leaf extracts and oil from seeds has proven an antimicrobial effect. This keeps any wound or lesion free from secondary infections by microorganisms. Clinical studies have also revealed that neem inhibits inflammation as effectively as cortisone acetate; this effect further accelerates wound healing.[46]

Significant increase in the levels of burn tissue TBARS levels and a significant decrease in the burn wound tissue GSH levels indicate prominent role played by oxidative free radicals in the development and progression of burn injury and associated pathogenesis. Application of MA and AI combination extract based creams as well as individual extract based creams resulted in a significant decrease in the wound tissue TBARS levels and a significant increase in the burn wound tissue GSH levels. The observations highlight the possible role of free radical inhibition by the anti-oxidant phytoconstituents present in the aforementioned extracts in their observed effects.

The beneficial results obtained in the present study by the application of MA and AI leaf aqueous extract based cream could possibly be attributed to the synergism of anti-oxidant effect of phytoconstituents present in MA and the proven potent anti-inflammatory properties of aqueous extract of AI. The results obtained with a combination cream were significantly better than that of individual extract based cream groups. These findings further strengthen the possibility of development and utilization of a multi-component plant-based pharmaceutical formulation for combat of second-degree and third-degree burn injury and associated inflammatory changes. However, further characterization and standardization of the plant material is required. Moreover, the mechanism of action needs to be elucidated in more details in terms of molecular site of action of phytoconstituents.

CONCLUSIONS

From the present findings, it can be postulated that MA and AI combination extract based cream exhibited potent wound healing potential against thermal burn injury (even significantly more than that of individual cream groups) and that the anti-burn activity of the MA and AI combination extract could be attributed to the anti-oxidative potential of its major components such as oxyresveratrol and resveratrol or other flavonoids, as well as the anti-inflammatory nature of various phytoconstituents present in neem. 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 aqueous extracts of MA and AI based cream formulation in scalding type burn injury in rats.

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