Comparative Efficacy of Ampucare and Silversulfadiazine against Burn Wound Rat

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Abstract: The present study was undertaken to investigate the comparative efficacy of ampucare and silversulfadiazine drug on biochemical parameters in burn wound rat model. Burn injury was made on the ventral shaven area of the rats under anesthesia and the animals were treated topically twice daily with ampucare and silversulfadiazine drug for 14 days. Our results showed that in both treated groups, there was statistically significant (p<0.001) decrease in xanthine oxidase, nitric oxide and malonaldialdehyde levels along with significant (p< 0.001) increase in the ascorbic acid, protein and reduced glutathione level after 14 days of treatment when compared with infected group. But these parameters showed better response in ampucare treated group in comparison to silversulfadiazine treated group after 14 days treatment. Therefore, these findings concluded that ampucare has antioxidant property that reduces oxidative stress, improves enzyme activity and increases wound healing effect than silversulfadiazine drug after burn injury.

Key words: wound healing, xanthine oxidase, nitric oxide, ampucare, silversulfadiazine

INTRODUCTION

Burn injury is accompanied by complex pathophysiological alterations that exert deleterious effect on various organ system[1]. Thermal injury initiates systemic inflammatory response, generation limitations of water vapour transport of oxygen radicals leading to peroxidation of biomolecules. It produces performed systematic changes such as anemia, renal failure, and metabolic changes etc. Various biological and metabolic alterations follow burn injury including degradation of adenosine triphosphate and significant reduction in polyunsaturated fatty acids in red cell membrane[2,3]. It is related to alterations in proteins structure and moist environment depends on the quality of grossly visible change in the skin. The skin is one of the largest organs in humans and accounts for 15% of body weight and 10–25% of whole-body protein turnover[4]. Tissue repair and wound closure may last for weeks after burn injury, and closure usually requires extensive surgery and skin grafting[5]. Various complications that are exacerbated or favored by nutritional deficiencies can occur, such as infections, delayed wound healing, and muscle wasting[6], infections remain a leading cause of death after major burns[7,8]. Healing of burn wound has still remained challenge to modern medicine though many drug are discovered. A wounds is described as a' break in continuity of tissue, from violence or trauma' and is regarded as helped if there is restoration of the wound and inflammed tissue to normal conditions. Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma- derived protein all coordinated by an array of cytokines and growth factors[10]. There are four distinct stages involved in wound healing namely inflammatory stage, debridement stage, proliferation stage and maturation/remodeling stage[11]. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodeled to form scar[12]. Certain factors such as bacterial infection, nutritional deficiency, drugs, sterility, movement of wound, edges, site of wound and wasting diseases distorts wound healing process during burn injury[13].

Ampucare is a oil based drug formulation which is used topically for the treatment of burn wounds. It is a polyherbal drug with anti-inflammatory and antimicrobial product, which improves blood flow. It has an immunomodulatory action along with tissue regeneration properties. Wound healing is an important biological process involving tissue repair and regeneration. The active components of ampucare are...
Azadirachta indica and Curcuma longa involved in wound healing[14]. The present study was evaluate the comparative efficacy of ampucare drug and its comparison with SSD (Silversulfadiazine drug) on oxidant enzyme as well as biochemical parameters in burn wound rat model.

MATERIAL AND METHODS

Materials: 5,5 diathiobis (2 nitro benzoic acid ) (DTNB; 55495) Thiobarbaturic acid (TBA; 30231), 2,4 Dinitrophenely hydrazine (52144), Bovine Serum albumin (BSA; 27423) reduced glutathione (GSH; 13679) and other biochemicals were procured from Himedia laboratories Ltd (Mumbai, India) and Sigma, St. Louis, MO, U.S.A. Ltd. Ketamine hydrochloride was purchased from Samarth Life Science Pvt. Ltd. Mumbai All other chemicals and reagents used were of analytical grade.

Drugs: Ampucare is polyherbal drug. It contains Azadirachta indica, Berberis aristata, Curcuma longa, Glycyrrhiza glabra, Jasminum officinale, Pongamia pinnata, Rubia cordifolia, Terminalia chebula, Trichosanthes dioica, Symposoc racemosa, Ichnocarpus frutescens, Capsicum abbreviata, Nymphaea lotus etc. ingredients. These pure ingredients were purchased from authorized dealer and use as such. Ampucare drug was obtained from Venus Remedies Ltd, Baddi, H.P. and silversulfadiazine drug (SSD) was purchased from a pharmacy (Chandigarh) for comparative efficacy of respective drugs on burn rat wound.

Experimental Animals: Total Eighteen male wistar rats (250-270 gm) were selected for the present study. They were housed in well-ventilated cages and fed with normal mouse chow and water ad libitum. The experimental room was air conditioned with temperature 23 ± 2°C, humidity 65 ± 5%, and with artificial fluorescent light (10 and 14 hours of light and dark, respectively). Experiment was done after approval from the institutional animal ethical committee.

Burn model: Skin burns were made as described by Mohammed et al.[15]. All experimental animals were anesthetized by kitamine hydrochloride (100mg/kg body weight) and burn wounds were created on the shaved ventral area of rats by pouring hot molten wax at 80 °C. The wax was poured on the shaven area of the animal through a cylinder of 300mm² circulating opening. The second degree of burn injury involving approximately 30-35% of the total body surface area. Wax was allowed to remain on the skin till it get solidified. It was removed very carefully after solidification and each animal was kept in other cages.

Experimental Protocol: Total eighteen wistar rats were divided into three groups of six rats each as follow:

Group I : Burn wound without treatment
Group II: Burn wound treated with ampucare
Group III Burn wound treated with silver sulfadiazine drug

Treatment with these drug was continued up to 14 consecutive days. Blood samples were collected on 14th day. Blood sample was diluted 10 times in chilled distilled water for the estimation of oxidant and antioxidant enzyme levels in burn wound rat model. All enzyme parameters were measured at 0-4 °C.

Xanthine oxidase enzyme activity was carried out according to the method of Roussos[16]. One unit of activity has been defined as change in absorbance at 290 nm within 1 minute by 1 ml of blood sample. Protein was assayed by the method of Lowery et al [17] using bovine serum albumin as standard. Malonaldehyde measurement has been utilized as a maker of oxidative stress by the method of Ohkawa et al.,[18] using thio barbituric acid reagent. 1,1,3,3 tetraethoxy propene (TEP) was used as standard. Reduced glutathione was measured in the blood sample by the method of Hissin and Hilf [19]. Ascorbic level was estimated in the blood sample by the method of Roe et al.[20] Nitric oxide concentrations was determined in the blood sample by the method of Griess reagent [21].

Histopathological Analysis: After 14 days treatment, animals were anesthetized and burn skin tissues were collected in to 10% formalin solution for histological examination. The samples were washed out with distilled water and cut in 5 µm sections and stained with hematoxylin and eosin (H&E), and examined by light microscopy.

Statistical Analysis: The results are expressed in mean ± SD. Statistical evaluation of the data was performed by one way- ANOVA followed by student Newman-Keuls using INSTAT 3.0 software package. The statistical difference was analyzed between without treated vs with treated drug groups. p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results: No mortality was seen in the animals during study.

Histological evaluation, after 14 days treatment, it was found that burn healing was found to be higher in
ampucare treated group than silversulfadiazine treated group. The burned skin (control without treated group) showed denuded epidermis, diffuse infiltration of lymphocytes cells and polymorphs. Epidermis causes the separation of epithelial cell in control without treated group. Epidermis was found clear in ampucare treated group than silversulfadiazine. The keratin layer was observed thickened in silversulfadiazine treated group. see Figure 1.

In the present study, wound diameter was found to be statistically significant decreased in ampucare (p<0.001) as well as silversulfadiazine treated group (p<0.001) as compared to control without treated group after 14 days treatment. This enzyme activity was found to be lowered (p<0.001) in ampucare treated group in comparison to silversulfadiazine (SSD) treated group after 14 days treatment. see Figure 2.

Xanthine oxidase enzyme activity was significantly (p<0.001) decreased in ampucare as well as in silversulfadiazine treated group as compared to control without treated group after 14 days treatment. This enzyme activity was found to be lowered (p<0.001) in ampucare treated group in comparison to silversulfadiazine treated group after treatment of 14 days. see Figure 3.

Protein level was significant evaluated (p<0.001) in ampucare treated group as well as silver sulfadiazine treated group as compared with control without treated group after 14 days treatment. The level of protein was found to be statistically significant (p<0.001) increased in ampucare treated group when compared to silver sulfadiazine treated group after 14 days treatment. see Figure 4.

Ascorbic acid level was significantly (p<0.001; p<0.01) increased in ampucare treated group as well as in silver sulfadiazine treated group when compared with control without treated group after 14 days treatment. When ampucare treated group was compared with silversulfadiazine treated group, the level was statistically significantly (p<0.05) increased in ampucare treated group after 14 days treatment. (see Figure 5.)

Reduced glutathione level was significantly (p<0.001) increased in ampucare treated group as well as in silver sulfadiazine treated group when compared to control without treated group after 14 days treatment. When ampucare treated group was compared with silversulfadiazine treated group, the level of reduced glutathione was significantly (p<0.001) increased in ampucare treated group. see Figure 6.

Nitric oxide and MDA levels were statistically significantly (p<0.001; p<0.05; p<0.001) decreased in ampucare as well as in silversulfadiazine treated group as compared with control without treated group after 14 days treatment. When ampucare treated group was compared with silversulfadiazine treated group, the NO and MDA levels were statistically significantly lowered in ampucare treated group after 14 days treatment. see Figure 7 and 8.

Discussion: Wound healing is a major issue in burns, and delayed healing with graft failure is a serious problem. Burns involving >20% of the body surface result in extensive inflammatory, endocrine, metabolic, and immune changes[22]. Infection is also another major complication during burn injury[23]. Healing of burn tissue is a complex process, that involves in re-epithelisation, granulation, tissue formation and remodeling of extracellular matrix. Burn injury initiates inflammatory response, generation of oxygen free radicals leading to peroxidation of biomolecules. Various biological and metabolic changes occur during burn injury including degradation of adenosine triphosphate (ATP) and significant reduction in polyunsaturated fatty acids in red cell membrane[24].

Oxygen-derived free radicals play a significant role in the pathophysiology of thermal injury. There are several report suggested that the involvement of superoxide radical in the pathogenesis of burn injury[24]. Free radicals as well as histamine and prostaglandins released from the burn wound cause lipid peroxidation in the skin tissue. These radicals appear to be involved in the appearance of lipid peroxidation products extractable from burned skin and distant organs[25-27]. Lipid peroxidation is a most spectacular manifestation of cell damage which causes the transformation of membrane polyunsaturated fatty acids to hydroperoxides and degradation to low molecular species, respectively. In burn injury condition, the adenosine tri phosphate (ATP) levels gradually fall, and increased adenosine mono phosphate (AMP) is converted to hypoxanthine and xanthine. Simultaneously xanthine dehydrogenase is converted by selective proteolysis into xanthine oxidase. Xanthine oxidase (XO) is important source of free radical generating enzyme which acts on xanthine and hypoxanthine to produce uric acid and oxygen free radicals. During burn injury, the ratio of free radical generating enzyme and antioxidant defense mechanism may altered which may lead to several pathophysiological state. Because xanthine oxidase has been reported to be an important source of oxidants involved in cell and tissue damage in situation of ischemia followed by reperfusion. Till et al.[28] has been reported that xanthine oxidase may also play a role in thermal injury of skin. Burn injury also leads to increase oxidative stress in the cells as seen by decreased endogenous non enzymatic and enzymatic antioxidant activity. Adequate antioxidant defense is also required for the normal wound healing process.
Fig. 1: Histopathological section of skin after 14 days treatment. Slide A Shows control thermally burned animal without treatment and slide B and C shows thermally burned animal treated with ampucare and silversulfadiazine (H E) 10X E; epidermis, I; inflammation

Fig. 2: Values are presented in mean ± SD. C= Control without treated group, A = ampucare treated group, SSD= silversulfadiazine treated group. P***< 0.001 highly significant; P** < 0.01 significant; P* < 0.05 significant; P > 0.05 insignificant (ns).
Fig. 3: Values are presented in mean ± SD. C= Control without treated group, A = ampuca treated group, SSD= silversulfadiazine treated group. P*** < 0.001 highly significant; P** < 0.01 significant; P* < 0.05 significant; P > 0.05 insignificant (ns)

Fig. 4: Values are presented in mean ± SD. C= Control without treated group, A = ampuca treated group, SSD= silversulfadiazine treated group. P*** < 0.001 highly significant; P** < 0.01 significant; P* < 0.05 significant; P > 0.05 insignificant (ns)
Fig. 5: Values are presented in mean ± SD. C = Control without treated group, A = ampucare treated group, SSD = silversulfadiazine treated group. P*** < 0.001 highly significant; P** < 0.01 significant; P* < 0.05 significant; P > 0.05 insignificant (ns)

Fig. 6: Values are presented in mean ± SD. C = Control without treated group, A = ampucare treated group, SSD = silversulfadiazine treated group. P*** < 0.001 highly significant; P** < 0.01 significant; P* < 0.05 significant; P > 0.05 insignificant (ns)

Effect of ampucare and silversulfadiazine drug on GSH level in burn wound rat
Fig. 7: Values are presented in mean ±SD. C = Control without treated group, A = ampucare treated group, SSD = silversulfadiazine treated group. P*** < 0.001 highly significant; P** < 0.01 significant; P* < 0.05 significant; P > 0.05 insignificant (ns)

Fig. 8: Values are presented in mean ±SD. C = Control without treated group, A = ampucare treated group, SSD = silversulfadiazine treated group. P**** < 0.001 highly significant; P*** < 0.01 significant; P* < 0.05 significant; P > 0.05 insignificant (ns)
Ascorbic acid is an essential cofactor for the synthesis of collagen and other organic components of the intracellular matrix of tissues such as bones, skin, capillary walls, and other connective tissues.

Ascorbic acid deficiency causes abnormal collagen fibers and alterations in the intracellular matrix that manifests as cutaneous lesions, poor adhesion of endothelium cells, and decreased tensile strength of fibrous tissue. Glutathione play a significant role in the detoxification of foreign compounds, hydrogen peroxide and free radical[29]. Reduced glutathione (GSH) enzyme detoxification of foreign compounds, hydrogen peroxide and free radical[29]. Reduced glutathione (GSH) enzyme is free radical scavenging enzyme which quench the superoxide radical and thus prevent the damage of the cell caused by free radical. In the present study, the MDA, NO levels and free radical generating enzyme xanthine oxidase were significantly lowered in ampuccre as well as in silversulfadiazine treated group when compared to control (infected) without treated group after 14 days treatment. Due to enhancement of MDA level and xanthine oxidase enzyme activity, it may be confirmed that free radical mediated damge occur in control without treated group during burn injury. The ascorbic acid, reduced glutathione and protein level was found higher in ampuccre and SSD treated group as compared to control without treated group after 14 day treatment. It means that both drugs had increased cellular proliferation and collagen synthesis at the burn wound site which has been evidenced by increasing protein levels in both treated group. It was also found that the levels of MDA, NO and xanthine oxidase enzyme activity were decreased along with increased in the ascorbic acid, protein and reduced glutathione level in ampuccre treated group in comparison to silversulfadiazine treated group after 14 days treatment.

_Curcuma longa _and _Azadirachta indica _are the active constituents of the ampuccre and these constituents play a significant role in wound healing process. _Curcuma longa _is considered to have natural medicinal properties, including antibacterial, anti-inflammatory, antineoplastic, and analgesic activities because it contains a number of monoterpenoids, sesquerpenoids, and curcuminoids[30-32]. _Azadirachta indica _possesses significant anti-inflammatory and hepatoprotective activity[33,34]. The anti-inflammatory and antioxidant properties of curcumin, an active component of turmeric suggest that curcumin may exert anti-ulcer activity and wound healing property through scavenging reactive oxygen species (ROS) and also regulating MMP activity[35]. Several studies have been reported that curcumin treated wound heal much faster as indicated improved rates of epithelialisation, wound contraction and increased tensile strength[35]. Curcumin treatment caused significant increase in free radical scavenging enzymes along with decrease in the MDA level thus accelerating wound healing process. Ampuccre treatment caused significant increase in antioxidant enzyme activities (ascorbic acid and GSH) along with significant decrease in MDA, NO levels and xanthine oxidase enzyme activity in blood when compared with silversulfadiazine treated group. With these findings, it is concluded that ampuccre possesses free radical scavenging property and plays a significant role in the treatment of burn wounds. It has a better therapeutic and faster burn wound healing effect than silversulfadiazine drug due to the presence of curcumin and gallotannins.

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REFERENCES


