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WOUND HEALING ACTIVITY OF URSOLIC ACID STEAROYL GLUCOSIDE (UASG) ISOLATED FROM *LANTANA CAMARA* L.

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ABSTRACT: *Lantana camara* L. is regarded as a notorious weed and has found several practices in folk medicine in many parts of the world. Leaves of *Lantana camara* L. is traditionally used for cuts, swellings, and burns for their healing property. The leaves are found to contain various chemical constituents such as monoterpenes, sesquiterpenes, triterpenes, and glycosides. In the present study, Ursolic acid stearoyl glucoside (UASG) isolated from *Lantana camara* L. (100 mg/kg/day) was evaluated for its wound healing activity in albino rats using excision and incision wound models. UASG treated animals exhibited significant ($P<0.01$ and $P<0.001$) reduction in wound area when compared to controls. UASG treated wounds are found to epithelize faster as compared to controls. Significant ($p<0.01$) increase in granuloma breaking strength was observed when compared with the control group. Histological section of the granuloma tissue of control animal showing lesser collagen formation indicated incomplete healing of the wound compared to UASG treated animals where complete epithelialization and increased collagen deposition occurred. The UASG showed significant wound healing activity, and the effect was comparatively evaluated with the standard skin ointment Framycetin Sulphate.

INTRODUCTION: Enormous number of plants are generally used by tribal in many countries for the treatment of wounds and burns. The presence of various health-promoting substances in plants given an idea to scientists to examine these traditional plants for potential wound healing properties [1]. The medicinal use of these plants is due to the presence of bioactive phytochemical constituents that produce definite physiological actions on the living being.

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These phytoconstituents include various classes of secondary metabolite like alkaloids, terpenoids, saponins, essential oils, flavonoids, tannins, and phenolic compounds [2].

Herbal medicines used for wound activity produce disinfection, debridement, and provides a moist environment that encourages natural wound healing processes [3]. Wound healing is a complex process characterized by homeostasis, re-epithelisation, and formation of granulation tissue and restructuring of the extracellular matrix [4]. Although the healing process takes place by itself, there are many potential factors that interfere with wound healing. Wound infection resulting from impaired immunity and exposure to pathogens or poor hygiene is one of the most commonly encountered and clinically important impediments to effective wound healing.

The injured skin remains vulnerable to invasive microbial infections of all kinds, with the subsequent development of wound sepsis that delays complete epithelial repairs [5]. An injury becomes infected because the wound area is an ideal medium for the multiplication of the infecting organism.

Topical antimicrobial therapy is one of the most important methods of wound care. Some herbal extracts and fractions can effectively arrest bleeding from fresh wounds, inhibit microbial growth, and accelerate wound healing [6]. An increased process of wound healing could be a function of either the individual or the synergistic effects of many bioactive molecules. Optimal management of wound infections is essential to promote a good healing response in the shortest time possible with minimal pain, discomfort, and scarring to the patient and must occur in a physiological environment, conducive to tissue repair and regeneration [7].

Skin fibroblast proliferation is important for tissue repair as fibroblasts are involved in the migration, proliferation, contraction, and collagen production [8]. The different phases of the wound healing processes overlap and ideally a plant-based remedy should affect at least two different processes before it can be said to have some scientific support for its traditional use [9].

Ursolic acid is one of the best known bioactive pentacyclic triterpenoids. It is widely found in more than 120 plant species; most of them used as medicinal plants in traditional medicine and also exist in food products [10]. Ursolic acid is of great interest to scientists because of its several biological activities. These include anti-inflammatory [11], antiulcer [12], cytotoxic [13], antiproliferative, larvicidal activity [14], and antidiabetic effect [15].

Given its easy availability in many plants and strong traditional evidence of wound healing property of the plant, we have evaluated wound healing activity of ursolic acid stearoyl glucoside isolated from *Lantana camara* L.

Ursolic acid stearoyl glucoside (UASG), pentacyclic triterpenoid isolated from *Lantana camara* L. (family: Verbenaceae) [15]. The traditional uses of *Lantana camara* L. mainly refer for the treatment of asthma, ulcers, measles, chickenpox, eczema, tumors, cancers, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria, ataxy of abdominal viscera [16], memory weakness, enhance intellect and cognition [17].

The plant has reported as anticonvulsant [18], anticancer [19], antiulcer [20], antioxidant [21], anti-diabetic [22], antifungal, antibacterial [23], antifeedant, larval mortality/repellency [24], activities.

Hence, in the present study, UASG isolated from *Lantana camara* L. was evaluated for its wound healing activity in albino rats using excision and incision wound models.

MATERIALS AND METHODS: All the studies conducted were approved by the Institutional Animal Ethical Committee Siddhartha Institute of Pharmacy, Dehradun, India (1435/PO/a/11/ CPCSEA).

Plant Material: *Lantana camara* L. leaves were collected from Hamdard University and identified by Dr. S. B. Singh, Scientist, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/ consult/-20-09-10/1322/125) was deposited in the herbarium of NISCAIR, India.

Extraction and Isolation of UASG: Dried powder of *Lantana camara L.* leaves (4 kg) was extracted with methanol (12 L) at 50 ºC for 1 day. The extract was concentrated to dryness under reduced pressure to obtain slurry (605 g). The slurry was dissolved in a minimum amount of methanol and was adsorbed on silica gel (60-120 mesh). The slurry was subjected to a silica gel column using $CHCl_3$/MeOH gradient system (49:1; 2.0 L for gradient system); leads to elution of colorless crystals of USAG (yield 11.2 g, 0.28 %). The same experiment was repeated to collect more amount of UASG required for wound healing activity. It was found to be 100% pure by HPTLC by using solvent system $CHCl_3$/ MeOH (99:1).

Structure of compound was identified by comparison of their spectroscopic data from the reported literature [15]. The structure of the USAG is depicted in **Fig. 1**.

Animals: Wistar albino rats (150-200 g) were obtained from Animal House, Siddhartha Institute of Pharmacy, Dehradun and kept at 25 ± 1 °C, 55 ± 5% humidity along with 12 h light/dark cycle. The animals were given a standard pellet diet (Lipton rat feed, Ltd, Pune) and water *ad libitum* throughout the experimental period. The experiment was approved by the 'Institutional Animal Ethics Committee.'

Chemicals: Framycetin Sulphate cream 1% w/w (Ranbaxy, India), diethyl ether, ethanol, sterilized cotton were purchased from Chopra Pharmaceuticals, New Delhi. All other chemicals used were of analytical grade. Ursolic acid stearoyl glucoside was suspended in Tween 80 in saline and used.

Wound Healing Activity: Excision and incision wound models were used to evaluate the wound-healing activity of USAG. Studies conducted were approved by the Institutional Animal Ethical Committee (1435/PO/a/11/CPCSEA) of Siddhartha Institute of Pharmacy, Dehradun, India.

Wistar albino rats of either sex weighing between 150-200gms were divided into six groups of six animals each.

Group I: Received no treatment and served as control.

Group II: Received application standard Framycetin Sulphate cream 1% w/w.

Group III: Received application of UASG (100 mg/kg/day.).

Excision Wound Model: Excision wounds were used for the study of the rate of contraction of wound and epithelization. Animals were anesthetized with slight vapor inhalation of di-ethyl ether, and the right side of each rat was shaved. Excision wounds sized 300 $mm^{2,}$ and 2 mm depth were made by cutting out a layer of skin from the shaven area. The entire wound was left open.

The treatment was done topically in all the cases. The UASG was applied at a dose of 100 mg/kg/day for 15 days. Wound areas were measured on days 1, 3, 6, 9, 12, and 15 for all groups, using a transparency sheet and a permanent marker [25].

Incision Wound Model: The incision wound model was studied. Under light ether anesthesia, the animal was secured to operation table in its natural position. One paravertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. UASG was applied at a dose of 100 mg/kg/day for 10 days. The sutures were removed after 8 days, on the tenth day the tensile strength was measured by continuous constant water supply technique [26].

Histopathological Examination: For histological studies, granulation tissues were fixed in 10% neutral formalin solution for 24 h and dehydrated with a sequence of ethanol-xylene series of solutions [19, 20]. The materials were in filtered and embedded with paraffin (40-60 ºC). Microtome sections were taken at 10 µm thickness. The sections were processed in alcohol-xylene series and stained with hematoxylin-eosin dye. The histological changes were observed under a microscope.

Statistical Analysis: The means of wound area measurement and wound breaking strength between groups at different time intervals were compared using one-way ANOVA, followed by Tukey's tests.

RESULTS AND DISCUSSIONS: UASG was administered to the test groups in graded doses ranging up to 100 mg/kg body weight/day, and the rats were observed 10 days for any signs of mortality and behavioral disabilities. Then the dose was increased up to 500 mg/kg and again observed for signs of mortality and behavioral disabilities for another 10 days. Its LD50 value was found to be higher than 500 mg/kg body-weight in rats. $1/10^{th}$ and $1/20^{th}$ of 500 mg/kg of UASG were used for further experimentation.

In studies using the excision wound model, the UASG treated group III showed significantly greater wound healing as compared to standard drug-treated animals **Table 1**. In the incision wound model, a significant increase was observed in the skin tensile strength of UASG treated group on 10^{th} post wounding day **Table 2**.

TABLE 1: EFFECT OF UASG ON EXCISION WOUND [WOUND AREA (mm^2)]

Day	Group I	Group II	Group III
0	268.15±6.19	251.28±5.88	271.25±4.86
3	238.45±8.40[c]	198.16±4.36[c]	214.43±6.17[b]
6	210.18±8.01[b]	152.26±1.99[c]	159.26±2.19[b]
9	181.47±3.17[c]	111.33±1.78[b]	110.51±1.81[c]
12	133.49±2.27[c]	056.54±1.06[b]	043.35±0.92[b]
15	072.36±3.17[b]	017.58±0.13[b]	013.35±0.25[c]

All values are mean ± SEM; n=6. [b] P<0.01, [c] P<0.001, when compared to control

From histopathology, in disease control group **Fig. 2, Group I**, stroma reveals fibrous tissue and non-specific inflammation, broken portion of skin shows abnormal histology, broken appearing epidermis, and dermis, and little inflammatory reaction or granuloma is seen while in standard drug **Fig. 2, Group II** and UASG treated group **Fig. 2, Group III** there is recovery as compared to disease control **Fig. 2**.

TABLE 2: EFFECT OF UASG ON WOUND HEALING IN INCISION WOUND

Groups	Incision wound breaking strength (g)
Group I	279.27±10.17
Group II	467.34±13.19[b]
Group III	471.40±15.45[b]

All values are mean ± SEM; n=6. [b] P<0.01, [c] P<0.001, when compared to control.

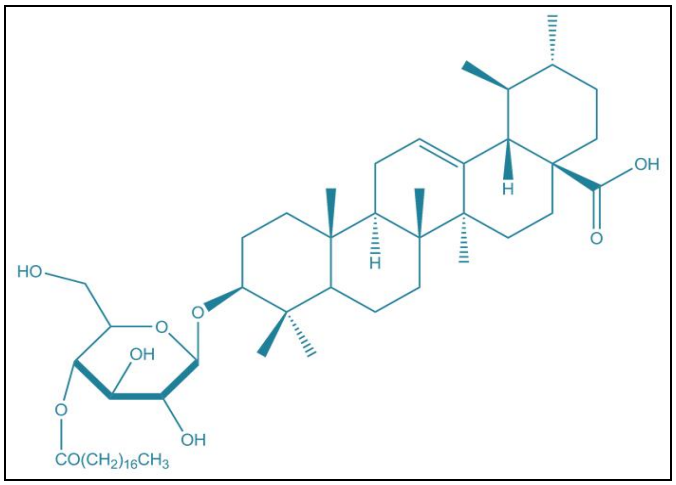


FIG. 1: STRUCTURE OF URSOLIC STEAROYL GLUCOSIDE

Healing is a physiological phenomenon and does not normally need much help, but still, wounds create discomfort, and living being is prone to infection and other associated problems [27]. Synthesis of fibroblasts is one mechanism by which drug might enhance the wound healing process. Wound healing generally requires support at three stages.

Firstly, increasing general resistance and support mechanisms that come from palliative, antioxidant, rejuvenating, adaptogenic, cleansing, detoxifying, buffering, and lubricous activities.

Secondly, by increasing and stimulating the repair and regenerative mechanisms to prolong cell life, cell migration, and cell binding, remove skin blemishes, and improve tensile strength or elasticity of the skin, improve the moisture-holding capacity of the skin.

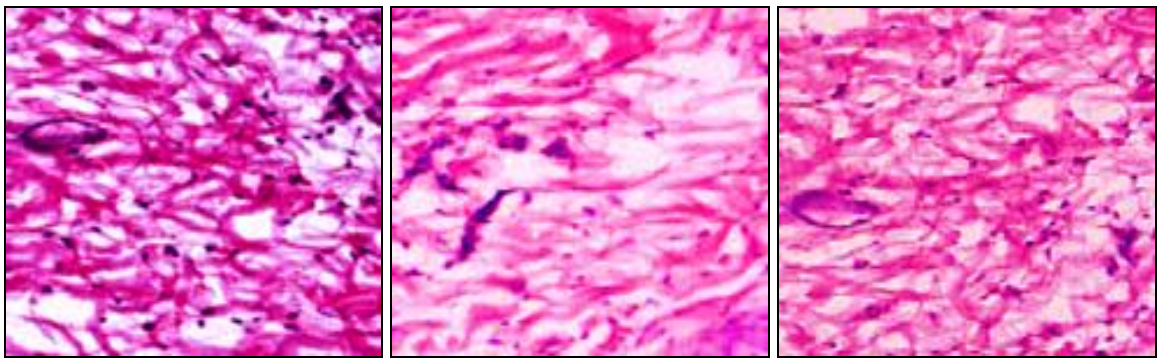
Thirdly, by therapeutic and nutritional activities, which includes anti-inflammatory, antiseptic, and antimicrobial, improving protein and collagen synthesis and increase in the stability of biomembranes. The wound healing process is stimulated by several herbal extracts, which contains active ingredients like triterpenoids, alkaloids, flavonoids, tannins, saponins, anthraquinones, and other biologically active molecules [7]. Terpenoids are known to enhance the wound healing process, mainly due to their astringent and antimicrobial properties, is responsible for wound contraction and an increased rate of epithelialization [28].

Terpenoids or isoprenoids also have antifungal or antimicrobial activity due to possible effects on the non-mevalonate pathway. This pathway is essential in fungi, protozoans, bacteria, and other micro-organisms for the synthesis of cell membrane components, prenylation proteins and as a secondary source of carbon. Inhibition of crucial enzymes of this pathway provides a future use for terpenoid derivatives as potential antimicrobial agents [29].

In the present study, our molecule is triterpenoid, which promotes wound healing as it is established from **Table 1** and **2**.

It is also well established that several local growth factors help in the wound healing process. It is possible that UASG may have a growth factor-like activity or can stimulate the expression of growth factors like the basic fibroblast growth factor (bFGF). Basic FGF has the broadest range of target cells, including all those involved in wound healing *viz.* endothelial cells, fibroblasts, myoblasts, *etc.* [30] Therefore, pro-inflammatory cytokines have been implicated in stimulating the synthesis of platelet-

activating factors by the recruited monocytes, which in turn induce several angiogenic factors and chemokines [31]. More in-depth studies would, therefore, be needed to delineate the likely beneficial properties of this agent in wound healing at the pro-inflammatory cytokine level.



GROUP I **GROUP II** **GROUP III**

FIG. 2: PHOTOGRAPH OF HISTOLOGICAL SPECIMEN OF RATS FROM DIFFERENT GROUPS WITH ORIGINAL MAGNIFICATION OF 45 X. (Group I) HISTOLOGICAL SECTION OF THE GRANULOMA TISSUE OF CONTROL ANIMAL SHOWING LESS EPITHELIAZATION AND LESSER COLLAGEN FORMATION INDICATED INCOMPLETE HEALING OF THE WOUND. (Group II and III) STANDARD SKIN OINTMENT FRAMYCETIN SULPHATE AND USAG APPLIED ANIMAL SHOWING COMPLETE EPITHELIALIZATION

In the present study, incision wounds healing by granulation, collaboration, and tensile strength was measured indirectly to assess the collagen content and maturation. The results indicate that UASG significantly promoted collagen as compared to that of control.

Further, histopathology of skin reveals UASG was effective in wound healing comparable to standard drug. Use of a single model is inadequate, and there is no reference standard which can collectively represent the various components of wound healing as drugs which, influence one phase may not necessarily influence another.

Hence in our study, we have used two models to assess the effect of UASG on various phases of wound healing.

CONCLUSION: The wound healing activity of UASG from *Lantana camara* L. was studied by using excision and incision wound model and the UASG showed the significant wound healing activity as like as standard FSC (Framycetin sulphate cream).

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CONFLICT OF INTEREST: Nil

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