E-ISSN: 2378-654X

Recent Advances in Biology and Medicine

Original Research Article

Antidiabetic and Wound Healing Effects of Smeathxanthone A

HATASO, USA

Antidiabetic and Wound Healing Effects of Smeathxanthone A

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Received: Jan 19, 2016; Accepted: Mar 8, 2016; Published: Mar 23, 2016

Abstract
The purpose of this study was to investigate whether smeathxanthone A isolated from G*arcinia smeathmanii* improves incisional wound healing in diabetic mice. Male albino alloxan-induced diabetic mice ($n = 20$) were divided into five groups: normal control, diabetic control, 2.5 mg/kg glibenclamide given orally, 0.05 and 0.1 mg/kg smeathxanthone A given subcutaneously. Animals were euthanized on postoperative day 10 after wounding; body weight, blood glucose, breaking strength, and histologic examination were reviewed. Smeathxanthone A significantly increased skin tensile strength (24% higher than diabetic control group when given at 0.1 mg/kg), stimulated hair growth, and reduced signs of inflammation in the scar sections. Smeathxanthone A also reduced blood glucose levels in diabetic mice (45% higher than diabetic control group when given at 0.1 mg/kg). The present study demonstrates that administration of smeathxanthone A after laparotomy expedites wound healing in mice. We suggest that it could confer benefits to tissue healing by significantly enhancing tissue collagen deposition and controlling blood glucose levels.

Keywords: Smeathxanthone A; Wound; Tensile strength; Antihyperglycemia.

1. INTRODUCTION

Wound healing is a natural and spontaneous phenomenon that takes place in three orderly and timely interactive phases: inflammation, proliferation, and remodeling [1]. Normal wound healing cascade begins immediately following injury. Tissue damage and the activation of clotting factors during the vascular phase stimulate the release of inflammatory mediators, such as prostaglandins and histamine, from cells such as mast cells. The transition from the inflammatory to the proliferative phase, the stage characterized by the filling of the wound with new connective tissues, is orchestrated by macrophages. A decrease in wound size is achieved by a combination of the physiological processes of granulation, contraction, and epithelialization. Reepithelialization phase rebuilds the structure while the remodeling phase involves the final form [2]. Surgery in diabetic patients is associated with slow wound healing process and hence requiring longer hospital stay, higher health care resource utilization, and greater perioperative mortality than nondiabetic subjects [3]. The exact pathogenesis of the poor wound healing process in diabetic patients is not clearly understood, but evidence from studies involving both human and animal models reveal increased rate of infections and several abnormalities in the various phases of wound healing process [3]. With the worldwide diabetes incidence now considered to be increasing in an epidemic proportion [4], there is a growing need to search for novel drugs to combat diabetes and the associated disorders, such as wound complications.

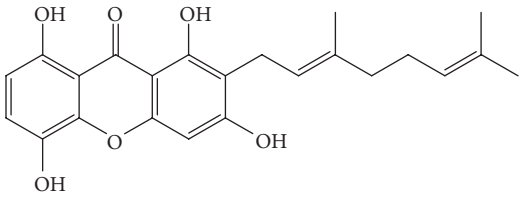
Over 278 natural xanthones belonging to the plant families of Gentianaceae, Guttiferae, Moraceae, Clusiaceae, and Polygalaceae are known to occur [5]. Most xanthones are polyphenols and hence regarded as powerful antioxidants that can offer beneficial health effect either by direct scavenging of reactive oxygen species or by acting as chain-breaking peroxyl radical scavengers. In addition to possessing antioxidant effects, xanthones have also been reported to be hepatoprotective, mutagenic, immunomodulatory, anticomplement, cardioprotective, antitumoral, antidiabetic, anti-inflammatory, antiulcer, and analgesic agents [5-8]. Smeathxanthone A (Figure 1) is a unique xanthone that combines a polyphenolic skeleton with four free hydroxyl groups and a terpenoid geranyl structural moiety [9]. Although the compound has previously been isolated in our laboratories from *Garcinia smeathmanii* [7], it has never been investigated for its potential antidiabetic properties. In the present communication, the blood glucose lowering and wound healing effects of smeathxanthone A in diabetic mice are reported.

2. MATERIALS AND METHODS

2.1. Animal Husbandry and Ethical Considerations

All animal procedures were conducted with strict adherence to NIH's Guide for the Care and Use of Laboratory Animals (NIH Publication #85-23 Rev. 1985). Male albino mice of 2-3 months of age whose weights oscillated between 25-29 g and that fed

Figure 1: Structure of smeathxanthone A.



on standard chow pellet diet and water given *ad libitum* were used. Animals were caged under laboratory environment with 12-h dark and light cycles.

2.2. Drugs

The isolation of smeathxanthone A from *G. smeathmanii* in our laboratories has been described previously [7]. Alloxan was obtained from Sigma-Aldrich (St. Louis, USA) while D-glucose was from Edu-Lab Biology Kit (Bexwell, Norfolk PE38 9GA, UK). Ketamine (Rotexmedica, Tritau, Germany), diazepam (Renaudin, France), and nylon surgical treat size 1 (Agary Pharmaceutical Ltd) were all purchased from a local pharmacy store. All other chemicals were of laboratory grade and freshly prepared.

2.3. Animal Groups and Induction of Diabetes

After 6 h of fasting, mice were treated with a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) in freshly prepared saline. After 72 h of alloxan injection, the diabetic mice (blood glucose > 150 mg/dL) [10] were divided randomly into four groups as follows: water (0.2 mL s.c.) or diabetic control, glibenclamide (2.5 mg/kg p.o.), smeathxanthone A (0.05 mg/kg s.c.), and smeathxanthone A (0.10 mg/kg s.c.). A fifth group of normal mice was added: normal control group. Blood glucose levels of all experimental mice were measured using reactive strips and a glucometer (One Touch Ultra Easy, SNXHG29A5AR).

2.4. Cicatrizant Activity

Surgical procedures were carried out as previously described [11], 4 days from induction of diabetes, and treatment started immediately. Briefly, animals were anesthetized by an intramuscular injection of ketamine/diazepam (ketamine 25 mg/kg and diazepam 10 mg/kg). A 3-cm incision was then made perpendicular to the axis of symmetry of the animal and the two borders of the wound were stitched together at its center with interrupted sutures at a distance of 1 cm. Treatment started immediately and the experimental agent was tested on a daily basis. On the 10th day of postwounding, blood glucose was measured at the tail vein. Animals were sacrificed by chloroform overdose and wound areas from each animal were dissected carefully. Stripes of equal size (width) from one side were cut and a line was drawn on either side, 3 mm away from the wound, for breaking strength determination. One piece of tissue was fixed in 10% formalin for histopathological examination and the other was used to quantify the wound breaking strength.

2.5. Determination of Wound Tensile Strength

Both ends of each skin stripe were fixed with a pair of steel clip, one clip was allowed hanging on a stand and other clip with a freely suspended polyethylene bag through a string run over the pulley. It was then gradually filled with water from a polyethylene reservoir till the wound stripe was broken at the site of wound. The amount of water required to break the wound was noted and expressed as tensile strength of wound in grams [12]. The tensile strength was calculated according to the following equation:

$$\text{Tensile strength} = \frac{\text{Total breaking load}}{\text{Cross-sectional area}}$$

2.6. Histomorphological Study

Skin specimens were immediately fixed in 10% (v/v) formalin and each specimen was embedded in paraffin. Sections (5 μm) of paraffin block were prepared and stained with hematoxylin and eosin (H&E) [13]. Slides were examined qualitatively under a light microscope, for mononuclear cells, macrophages, fibroblast proliferation, vascularization, congestion, and epithelialization.

2.7. Statistical Analysis

All data were expressed as mean $\pm$ SD. Statistical analyses were evaluated by one-way ANOVA followed by Hochberg test using SPSS 16.0 software. *P* values of less than 0.05 were considered significant.

3. RESULTS

3.1. Effect of Smeathxanthone A on Body Weight and Blood Glucose Level

No significant body weight change was recorded on mice after treatment with smeathxanthone A and glibenclamide (data not shown). As shown in Figure 2, blood glucose level of diabetic control mice remained significantly high when compared with normal control mice 10 days postinduction. Additionally, alloxan administration in mice caused an increase in mean blood glucose level from 104.40 (normal control on day 10) to 200 mg/mL (diabetic control on day 10). Administration of the standard antidiabetic drug, glibenclamide, resulted in the reduction of blood glucose level by 40% ($p < 0.001$) in the normal control group when compared with the diabetic control group. Similarly, smeathxanthone A has potential antidiabetic effect as blood glucose was reduced by 35.50 and 45.10% at the doses of 0.05 and 0.1 mg/kg, respectively.

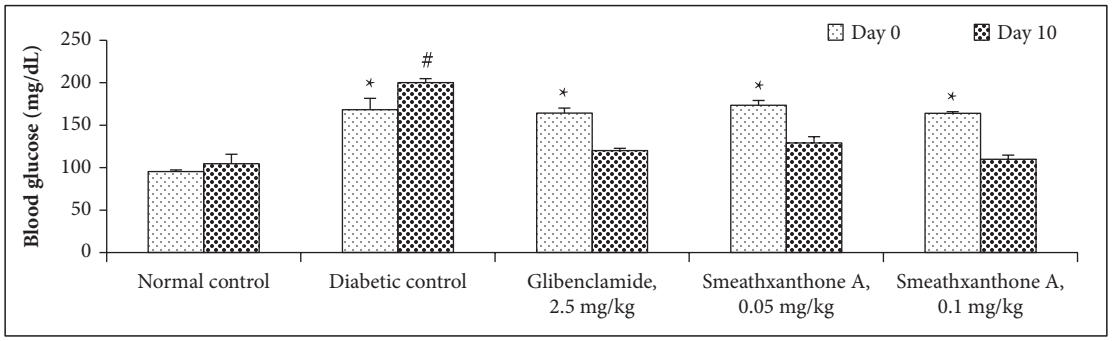
3.2. Effect of Smeathxanthone A on Skin Tensile Strength

In the linear incision wound model, there was a significant ($p < 0.001$) increase in the tensile strength of the smeathxanthone—treated wounds, as compared with those of the various control groups (Figure 3). The tensile strength required to disrupt the wound was found to be 13.34 and 24.14% higher at the doses of 0.05 and 0.1 mg/kg, respectively, than the diabetic control group (486.55 g/cm^2). Inversely, the standard antidiabetic drug, glibenclamide, group needed 391.22 g/cm^2 (19.59% lower than the diabetic control group) to tear out the wound.

3.3. Histomorphological Analysis

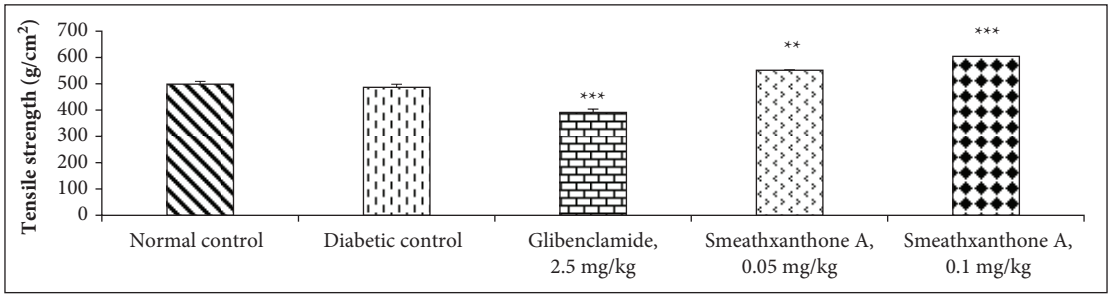
Representative results from the histopathological examination of the various treatment groups are shown in Figure 4. Healing was completed in the wound tissues treated with smeathxanthone A. A thin epidermis with keratinization and mature dermal layers and hair follicles were observed in these groups. In contrast to the positive wound healing effect of smeathxanthone A, and in some extend the normal control wound, a parallel histopathological examination of the reference drug, glibenclamide, demonstrated an incomplete healing (Figure 4).

Figure 2: Effect of glibenclamide and smeathxanthone A on blood glucose level of mice. Data represent mean and SD values, *n* = 5.



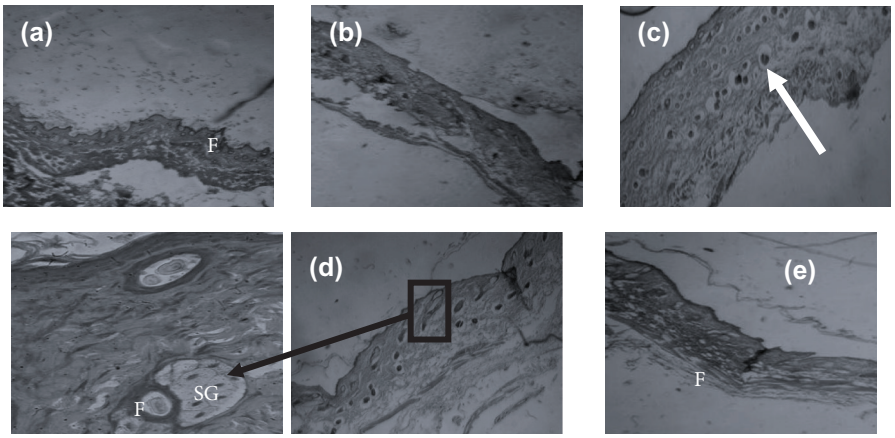
*$P < 0.001$ when compared with normal control group at day 0; #$P < 0.001$ when compared with diabetic control group at day 0 and day 10 posttreatment.

Figure 3: Effect of smeathxanthone A on wound tensile strength of alloxan-induced diabetic mice. Data are mean and SD values, *n* = 5.



**$P < 0.01$, ***$P < 0.001$ when compared with the control groups.

Figure 4: Microscopic view of the portion of skin tissue sections after 10 days of treatment: (a) normal control group, (b) diabetic control group, (c) glibenclamide-treated group, (d) smeathxanthone A 0.05 mg/kg-treated group, and (e) smeathxanthone A 0.1 mg/kg-treated group (H&E stain, ×100). Black arrow indicates high power view (×400) of smeathxanthone A 0.05 mg/kg. Control animals show thin epidermal layer. Granulation tissue of glibenclamide-treated mice contains macrophages (white arrow), poor coloration of the collagen matrix, within the dermis. A thin squamous epidermis is displayed in smeathxanthone A-treated skin. The dermis is well organized, with fibroblasts arranged longitudinally to the incision and no inflammatory pattern. Interestingly, numerous follicle hairs (F) also arise from these specimens and sometimes with sebaceous glands (SG).



4. DISCUSSION

The present study was designed to evaluate the potential antidiabetic effect and wound healing potential of smeathxanthone A using the incision wound model. Alloxan-induced diabetes mellitus was used, as the model represents a pathological biomodel for testing a substance with suspected antidiabetic activities *in vivo* [10]. Wound healing is a natural repair process in response to tissue injury [1]. In normal tissues, epithelialization is ensured by the epidermal predominant cells, keratinocytes, which detach from the basal membrane, migrate toward the wound margin, and proliferate to extend the newly formed epithelial carpet made of several layers of cells in the epidermis. At the end of stage 2, myofibroblasts transformed from fibroblasts contract and try to bring the wound edge together. They subsequently disappear by apoptosis in the dermis [14] to lead to the final stage, which is characterized by remodeling and increased covalent cross-linking of collagen molecules. More changes in collagen organization in the repaired tissue will slowly increase the tensile strength. Smeathxanthone A interfered with the primary phases of wound healing by resorbing inflammatory features as observed in the saline- and glibenclamide-treated scares. The proliferative phase was also improved in smeathxanthone A-treated animals. This was evident by the development of many hair follicles and sebaceous glands (viewed at 400× magnification).

Therapeutic agents that modulate wound repair can also be evaluated based on their influence on the development of wound strength [15]. The increasing amount of stable collagen and the alignment of its fibers are known to gradually increase the strength of the healing wound [16]. Mice treated with smeathxanthone A developed tensile strength suggesting good amount of mature collagen deposition. In humans and in animal models with diabetes, hyperglycemia has been shown to cause multiple defects in wound healing, including reduced collagen synthesis, reduced wound tensile strength, and reduced neovascularization and capillary volume at the site of injury [3]. Consequently, the blood glucose-lowering effect of smeathxanthone A is an additional mechanism that strengthens its beneficial effect on the skin tensile strength. Histopathological examination further provided additional evidence on this healing potential of smeathxanthone A. Fixation of tissues with formalin followed by hematoxylin and eosin provides additional insights into the status of the healing process, according to cytoplasmic, nuclear, and extracellular matrix features [15]. Our data unequivocally showed that smeathxanthone A inhibited the signs of inflammation as evidenced from reduced macrophages and necrosis events in the normal control mice when compared with diabetic control mice. Scar treated with the same molecule also showed many fibroblasts arranged longitudinally to the incision suggesting an important collagen cross-linkage feature [15, 17]. Although the exact mechanism remains to be elucidated, healing effects were reported for other xanthones on inflammatory, subchronic wound conditions and diabetic animals [18, 19]. More recently, xanthones from the roots of *Hypericum oblongifolium* WALL have shown weak to higher antiulcer activity by inhibiting the effects of enzyme urease, depending on the number of hydroxyl groups [20]. Given the polyphenolic structure of smeathxanthone A (Figure 1), it is reasonable to assume that its pharmacological activity may be attributed at least in part to its antioxidant effect. While offering glucose-lowering effect as with the positive control antidiabetic drug, glibenclamide, the additional wound healing benefit of smeathxanthone A gives it a great potential in surgery, particularly in diabetes patients.

5. CONCLUSION

In conclusion, our study shows that smeathxanthone A has glucose-lowering effect and accelerates cutaneous wound healing in alloxan-induced diabetic mice. Our findings also indicated that the smeathxanthone A effect is based on collagen deposition and inhibition of inflammation. Although the therapeutic potential of smeathxanthone A was effectively demonstrated in the present study, the precise underlying molecular mechanisms need to be proven through further researches.

Acknowledgment
AML would like to thank the Alexander von Humboldt Foundation for providing Georg Forster Research Fellowship for Experienced Researchers (ID No. 1137675) at the Bielefeld University, Germany.

Author Contributions
DET is the principal investigator. AML contributed to the compound isolation and identification, whereas NS supervised the work and provided facilities. JAE and BMG conducted pharmacological tests. SH contributed to the design of the study and revised the manuscript. TD contributed to the design of the study and supervised the pharmacological work.

Source of Funding
None.

Conflict of Interest
None.

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Citation: Tsala DE, Lannang AM, Dimo T, Habtemariam S, Ekanga JA, *et al*. Antidiabetic and wound healing effects of smeathxanthone A. Recent Adv Biol Med. 2016; 2:5-10.