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Effect of a methanol extract of *Allium cepa* Linn. on incisional wound healing in alloxan-induced diabetic mice

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ABSTRACT

Aim: The objective of the present study was to evaluate the wound healing effect of a methanol extract of *Allium cepa* Linn. *in vivo*. **Materials and Methods:** The healing activity was studied using incision wound on diabetes suppressed wound healing rodent model. **Results:** Data recorded exhibited a significant improvement of the blood glucose level and scare breaking strength in diabetic mice at 300 and 400 mg/kg onion extract. Histological examination of the general morphology of wounds of extract treated group has shown a normal histological pattern of healthy skin at the same doses. **Conclusion:** This study illustrated an excellent potential of the onion consumption on dermal wound healing in diabetic patients, with a tentative mechanism of action related to tensile strength improvement and anti-hyperglycemic effect.

KEY WORDS: *Allium cepa*, diabetes, tensile strength

INTRODUCTION

Wound healing occurs whenever there is a loss of continuity in any body tissue, as a result of trauma, infection or pathological process [1]. In general, wounds cure in an orderly and timely repair process, which is characterized by dynamic, interactive events described in 3 phases: Inflammation, proliferation, and remodeling [2]. In order to assess the healing effects of natural products, *in vivo* and *in vitro* assay models may be employed, so that new therapies that target various aspects of wound repair are emerging in recent years. Among them, plant extracts and therapeutic food have been shown to be beneficial for treatment of wounds [3,4].

Allium cepa (AC) is commonly named onion is either biannual or perennial plant of the Liliaceae family. Previous reports indicate that consumption of dietary onion could contribute to a reduction diabetes mellitus complications. Accordingly, onion could help lower blood glucose levels in diabetes rats [5,6] and improve wound healing in normal rat [7]. However, existing literature does not report a wound healing effect of onion on diabetic wounds. The present study was therefore designed to

investigate whether an onion extract could improve incisional wound healing in diabetic mice celiotomy model.

MATERIALS AND METHODS

Plant Material and Extraction

AC bulbs were collected in the far-north region of Cameroon. 2000 g of onion pulp were macerated in 3 *L* methanol during 24 h. The filtered methanol extract was concentrated in a rotary evaporator. The procedure was repeated twice and gave 56.73 g of a green extract (yielded about 2.8%).

Animal Husbandry and Ethical Considerations

All animal procedures were conducted with strict adherence to the NIH Guide for the care and use of Laboratory Animals (NIH Publication #85-23 Rev. 1985). Locally bred male albino-mice weighing 18-25 g, fed 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.

Drugs and Chemicals

Ketamine (ROTEXMEDICA-TRTTAU-Germany), diazepam (RENAUDIN-France), dexamethasome (Guangdong Medecine & Health Products I/E corp), and nylon surgical treat size 1 (Agary Pharmaceutical Ltd) were purchased from a local pharmacy store. All other chemicals were of laboratory grade and used as received Alloxan. Alloxan was obtained from Sigma-Aldrich (St. Louis, USA). D-glucose, NaCl were from Edu-Lab Biology Kit (Bexwell, Norfolk PE38 9GA, UK). Glibenclamide (Daonil) was purchased from a local pharmacy store.

Animal Grouping and Induction of Diabetes

After 16 h of fasting, mice were treated with a single intraperitoneal injection of alloxan monohydrate (120 mg/kg) in freshly prepared saline. After 72 h of alloxan injection, the diabetic mice (blood glucose >150 mg/dL) [8] were divided randomly into six groups as follows: Normal control; diabetic control: AC 300 mg/kg; AC 400 mg/kg and AC 500 mg/kg. Animals of control groups were given only vehicle (distilled water); ethanol extract of AC or glibenclamide were given by gavage to the animals on 9 consecutive days. Blood glucose levels of all experimental mice were measured using reactive strips and a glucometer (One Touch Ultra Easy, SNXHG29A5AR).

Operative Procedure

Each experimental mouse (diabetic) was anesthetized with ketamine and diazepam. A 3-cm incision was 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. On 10th day post wounding, 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 tensile strength (TS). Fasting blood glucose level and body weight were monitored before (day 0) and after the beginning of the various treatments (day 10).

Determination of Wound TS

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 the wound. The amount of water required to break the wound was noted and expressed as TS of the wound in grams [9]. The TS was calculated according to the following equation:

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

For preliminary screening, an activity >25% is considered significantly important and the sample is described as having positive wound-healing activity. The percentage of activity was calculated according to the following formula:

$$\text{Activity}(\%) = \frac{\text{TSc- TSt}}{\text{TSc}} \times 100$$

TSt = Average of the force necessary to open the wound of a treated mouse.

TSc = Average of the force necessary to open the wound of an untreated mouse (control).

Histopathological Studies

Skin specimens were immediately fixed in 10% (v/v) neutral formalin until the tissues hardened. Each specimen was embedded in a paraffin block and thin sections (5 μm) were prepared and stained with hematoxylin and eosin (H and E) (for general morphological observations). Slides were examined qualitatively under a light microscope, for collagen formation, fibroblast proliferation, angiogenesis, and epithelization.

Statistical nalysis

All data were expressed as mean ± standard deviation. Statistical analyses were evaluated by one-way ANOVA followed by Hochberg test using SPSS 16.0 software. $P < 0.05$ were regarded as significant.

RESULTS

After the injection of alloxan, a significant increase in blood glucose levels ($P < 0.01$) was recorded compared to normal

control group at days 0-10, which was not accompanied by an important variation in body weight. The mean values of blood glucose levels over the experimental period and the statistical comparisons of the groups are shown in Figure 1. Blood glucose levels in methanol extract of AC treatment groups were down-regulated as compared to the diabetic control group; the difference was statistically significant ($P < 0.05$) except for the dose of 500 mg/kg of the onion extract. The tensiometric analysis shown in Figure 2 revealed that strength for the midline incision was significantly higher (TS >25%) in methanol extract of AC treated groups compared to diabetic control group ($P < 0.001$).

Histological sections of scared tissue from the various groups of experimental animals of the study are illustrated in Figure 3. H and E is commonly used for general morphology stains collagen fibers pale pink, cytoplasm purple, nuclei blue, and red blood cells cherry red. Accordingly, pink color was abundant in glibenclamide treated group and onion extract treated groups at 300 and 400 mg/kg. Other details are described below.

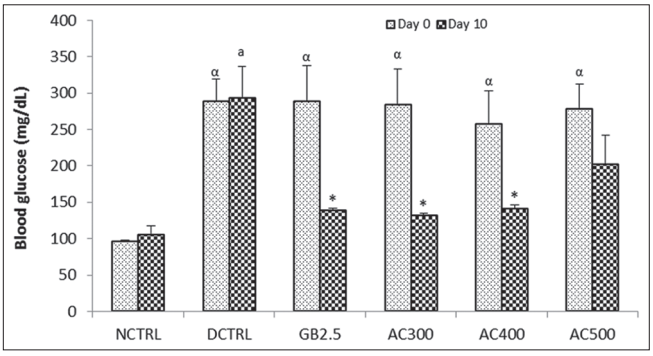

Figure 1: Effect a methanol extract of *Allium cepa* on blood glucose in alloxan-induced diabetes mice. The values are the mean ± standard error of the mean of 5 mice/group. αP < 0.001 compared to NCTRL at day 0; ᵃP < 0,001 compared to NCTRL at day 10. *P < 0.05 compared to DCTRL at day 10. NCTRL: Normal control; DCTRL: Diabetic control; GB2.5: Glibenclamide 2.5 mg/kg; AC300: *Allium cepa* 300 mg/kg; AC 400: *Allium cepa* 400 mg/kg; AC500: *Allium cepa* 500 mg/kg

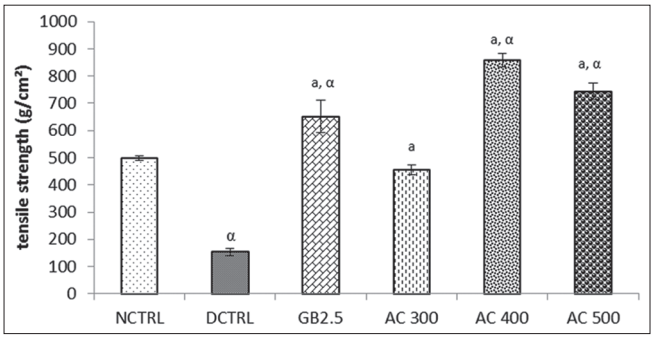

Figure 2: Effect a methanol extract of *Allium cepa* on wound tensile strength in alloxan-induced diabetes mice. The values are the mean ± standard error of the mean of 5 mice/group. αP < 0,05 compared to NCTRL; ᵃP < 0,001 compared to DCTRL. NCTRL: Normal control; DCTRL: Diabetic control; GB2.5: Glibenclamide 2.5 mg/kg; AC300: *Allium cepa* 300 mg/kg; AC400: *Allium cepa* 400 mg/kg; AC500: *Allium cepa* 500 mg/kg

Normal control animals (a) showed abrasive epidermis with hemorrhagic suffusion (Hm) of the dermis within which arise skin appendages. Diabetic control mice (b) showed atrophic epidermis, dermis slightly disorganized, infiltration of inflammatory elements (In), and absence of skin appendages. Glibenclamide treated mouse (c) showed skin lesion associated with abrasive epidermis and rare cutaneous appendages within the dermis. Onion administration at 300 mg/kg lead to the regeneration of the epidermis, hyperplasia of the dermis, which remains well oriented, and the development of skin appendages (arrow indicates blood vessel) (d). At 400 mg/kg the onion extract induced epidermis hyperplasia, skin appendages (arrow indicates hair follicle) (e). Given at 500 mg/kg, the onion extract caused necrosis of the above structures (f).

DISCUSSION

Normal wound cures in an orderly and timely repair process characterized by interactive events described in 3 phases: Inflammation, proliferation, and remodeling [10]. Re-epithelialization is achieved by migration, proliferation, and differentiation of epidermal keratinocytes [11]. The present study aimed to evaluate an incisional wound healing action of a methanol extract of the onion on diabetic mice. Wounds were created similar to celiotomy that might be experienced by a diabetic patient. Impaired wound healing constitutes a major health problem in patients with diabetes. It is generally accepted that alloxan is diabetogen by exerting a toxic action on pancreatic β cells which is the sum of several oxidative and disturbances in intracellular calcium homeostasis [12]. In wounds that occur in diabetes, a persistent inflammatory phase is commonly witnessed at histopathology, associated with deficient in collagen and a subsequent reduction in linear wounds TS to a degree curvilinearly related to the severity of hyperglycemia [13]. The methanol extract of AC given orally has shown antihyperglycemic effect and improved incision wounds healing in alloxan-induced diabetic mice. Accordingly, the activity described probably comes from the well-known antioxidant potential and the antihyperglycemic effect of the extract of AC that was confirmed in this study.

Factors that modulate wound repair can be evaluated according to their influence on the development of wound strength [14]. The increasing amount of stable collagen and the alignment of its fibers gradually increase the strength of the healing wound [11]. Increased TS in onion treated groups of mice, therefore, indicates an increase in a collagen matrix. Histopathological examination of wound scare provides additional insights into the status of the healing process, particularly in studies of impaired wound healing [14]. Specifically, use of alloxan was associated with poor wound healing, which was related to the atrophy of the epidermis, disorganization of the dermis, as well as infiltration of inflammatory elements and absence of skin appendages. The present work showed interesting healing action of the onion extract that was evidenced by the inhibition of vascularity and inflammation in all treated groups. However, use of the extract at doses over 400 mg/kg

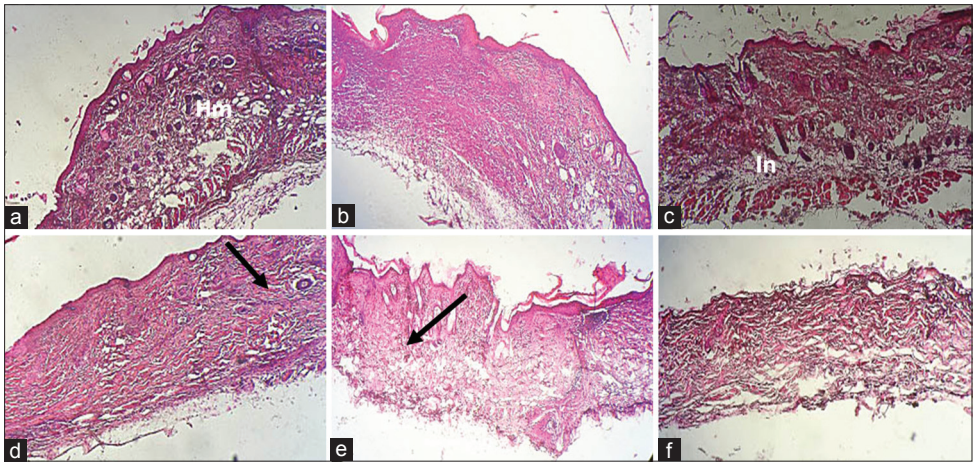

Figure 3: Histological results. (a) Normal control mouse, (b) Diabetic control mouse; (c) Glibenclamide treated mouse; (d) *Allium cepa* 300 mg/kg treated mouse; (e) *Allium cepa* 400 mg/kg treated mouse and (f) *Allium cepa* 500 mg/kg treated mouse (haematoxylin and eosin stain, ×100)

was not suitable since they produced important necrosis of the skin envelops.

CONCLUSION

The study provides evidence that adequate supplementation of onion after surgery would benefit diabetic mice by lowering blood glucose levels and producing high TS at the wound site.

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