WOUND HEALING POTENTIAL OF THE AQUEOUS EXTRACT OF *Acacia hockii* De WILD ON WOUND EXCISED ALBINO RATS

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Abstract: The Fali tribe of Mubi, Adamawa State, uses the pulverized bark of *Acacia Hockii* De Wild (Kuffran) for treatment of wounds and traditional embalmment. This experiment was aimed at evaluating the wound healing potential of the aqueous extract of *A. hockii De* Wild bark on albino rats. A total of twelve rats were used for the experiment and the animals were divided into four groups (Gp) of three rats each. Gp 1-wounded-untreated; Gp 2- wounded, treated with petroleum jelly; Gp 3- wounded, treated with standard drug and Gp 4 - wounded, treated with *A. hockii* extract. Wound excision was done following standard procedures, while treatment was performed by topical application for 9 days, and healing was measured (cm) at intervals of 3 days. Results obtained, showed significant ($p<0.05$) increase in the percentage wound contractions in all the experimental groups, though the highest (11.00 ± 0.02%) was in the group 4 treated with *A. hockii* extract when compared to control group 1 (1.0 ± 0.65%), group 2 (5.33 ± 0.65%) and group 3 (6.83 ± 0.65%) which was observed between day 0 and day 3. The rate of wound contractions increased significantly ($p < 0.0\ 5$) by the 9th day when compared to the 3rd and the 6th day, respectively. Higher contractions were observed mostly in groups 4 and 3 when compared to the other groups (control and group 2). This research therefore provides scientific justification to the use of *Acacia hockii* De Wild bark powder in wound treatment and possibly having some antimicrobial activities as well.

Keywords: *Acacia hockii*, contraction, evaluation, excision, wound healing

Introduction

Wound is a disruption of the normal anatomical structure and function which may be caused by chemical, physical, microbial, thermal or immunological damage to the tissue (Ikobi *et al.*, 2012). Impaired skin integrity leads to development that could involve different tissue, from the epidermis to deeper layers, such as muscles (de Bruin *et al*., 2011) immediately after injury, the blood clot forms a scab that protect the injured area (Garcia-Gubern *et al*., 2010). The consequent release of chemical mediators induces an acute inflammatory response characterized by the presence of neutrophils, followed by microphages and lymphocytes (Kondo and Ishida, 2010).

Wounds heal by primary intention or secondary intention depending upon whether the wound may be closed with sutures or left to repair, whereby damaged tissue is restored by the formation of connective tissue and re-growth of epithelium (Cooper, 2005). Repairing the damaged tissues restore the function required for robust and effective mechanism to protect it from trauma. The wound healing process is divided into four (4) phases: coagulation (haemostasis), inflammation and proliferation (granulation), and remodeling (maturation) (Eming *et al*., 2014). The successful management of wounds will decrease the number of complications and permit speedy return to normal function (Liptak, 1997). Majority of the populace in developing countries use traditional medicine as a prime healthcare to address their healthcare needs and concerns (Robinson and Zhang, 2011). Numerous medicinal plants were scientifically proven to be used for the treatment of wounds and other ailments. The healing actions of many traditional medicines are conferred by natural ingredients produced within the plant. *Acacia hockii De – Wildis locally called "kuffran"* in *Fali language and wawakajemagori* in *Hausa language.* The plant's bark is used by the Fali tribe of Adamawa state to treat deep cuts to the skin, ulcers of the foot or open body or wounds that are presumed stubborn to other treatment and for traditional embalmment during burial ceremonies in ancient times. It is known that foot ulcers and other wounds are healed by the use of the plant's powder. The traditional medical practitioners in the area uses the plant bark alone and sometimes may also mix it with other medicinal plant for treatment which affects the skin or ectopic problems (Soromessa *et al*., 2004).

Acacia hockii is a multi-stemmed shrub 2–4 m tall, or a small tree to 6–7 m tall with an open crown occasionally 9 m wide, with 12 m also recorded in exceptional individuals. Bark is red-brown to greenish or greenish-brown, rarely pale-yellow, peeling off in papery layers, brown when not burned or grey brown and in Plates where burned, yellow underneath. Young twigs and branchlets puberulous to densely puberulous, rarely glabrous, with sessile glands, reddish, sometimes numerous, usually elongate and slender. Leaves have 2-11 pairs of pinnae; each with 9-29 pairs of leaflets, 2.0-6.5 mm long and 0.5-1.2 mm wide, usually densely ciliolate but sometimes glabrous, obtuse to acute but not spinulose-mucronate at the apex, with lateral nerves invisible beneath. Leaves often have a gland on the petiole and between the top 1(3) pairs of pinnae. Flowers are bright yellow or orange, in axillary, pedunculate heads 5-12 mm in diameter. The pods are reddish brown, narrow, straight or crescent shaped, puberulous and (4)5-14 cm long and 0.3-0.8 cm broad. Seeds are olive-brown, 5-7 x 3-4 mm, smooth, elliptic and compressed (Gurib-Fakim, 2006).

Acacia gum has a naturally sticky texture and materials with this property are often used to reduce irritation and inflammation. The gum has been shown to be effective in easing stomach or throat discomfort (Ogaji *et al*., 2012). Though the plant powder is also used for traditional embalmment during ancient practices like burials, corpses were said to be well preserved for the period intended.

Materials and Methods

Plant material

The plant material used in the study was *Acacia hockii De Wild* bark. It was obtained from Vimtim village, Mubi in Adamawa State and identified by Dr. Akasa T.M. of the Department of Botany, Adamawa State University, Mubi Adamawa, Nigeria.

Animals

Albino rats of either sex weighing 150 – 200 g were purchased from Veterinary Research Institute, Vom, Jos,

Plateau State, Nigeria. The rats were kept in the animal house of the Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria. The experimental animals were kept in standard cages and allowed to acclimatize under standard condition of ambient temperature of 20–25˚C with 12 h day light cycles. The rats were fed with standard pelletized feed and water *ad libitum*.

Instruments/equipment

The instruments used for the present study include; Razor blade, Meter rule, marker/Pen, mechanical blender, weighing balance, rotary evaporator, Autoclave, Oven, Magnetic stirrer.

Chemicals/reagents

All chemicals and reagents in this study were of analytical grades and products of Sigma Aldrich Ltd. (USA). They include; Methanol, Normal Saline, extract ointment, n-Hexane, Whatman No. 1 filter paper, Silver sulfadiazine(standard), Diethyl ether, Methylated Spirit, vacuum and pressure pump.

Preparation of plant material

The medicinal plant samples were chopped into small pieces and air dried at room temperature until dry. The dried samples were grounded into fine powder using a steel blender.

Preparation of the plant extract

Two hundred (200) grams of powdered plant material was kept in 500 ml conical flask and 100 ml of water was added. The mouth of the conical flask was covered with a cork and aluminum foil and kept in a reciprocating shaker for 24 h for continuous agitation at 150 rpm in order to ensure proper mixing and to dissolve in the solvent. The solution was filtered using muslin cloth followed by Whatman No. 1 filter paper. The solvent from the extract was removed by using rotary vacuum evaporator RE52 at the temperature of 50°C. Finally, the solid material was collected, stored at room temperature and subsequently used for the experiment.

Experimental design

Total of twelve (12) rats were used for the experiment. The animals were divided into four groups of three rats per group.

Group 1-untreated but wounded.

Group 2-wounded and treated with petroleum jelly.

Group 3-wounded and treated with standard drug (Dermazine).

Group 4- wounded and treated with extract powder.

Wound excision

Experimental procedure was approved by Nasarawa State University Keffi Animal Ethics Committee (NSUKAEC) and animals received tender care as contained in the guide lines of WMA Statement on Animal Use in Biomedical Research, which was reaffirmed by the 203rd WMA Council Session, Buenos Aires, Argentina, April 2016

The excision wound model was done using the method described by (Rodriguez-Merchan, 2012). The animals were anesthetized with anesthetic ether and shaved at the predetermined site before wounding. A circular wound was inflicted by cutting away approximately 2 cm of diameter of the predetermined area on the anterior-dorsal side of each rat using sterile surgical blade and then wiped with sterilized cotton wool to control bleeding. The wounded animals were then placed in separate cages to avoid any disturbance. The cage bedding was changed daily. After skin excision, the wound was left open to the environment. A standard treatment was used, 1% silver sulfadiazine (Dermazine) on infected wounds. Dermazine groups were topically treated once a day with 0.1 g of each ointment. Wound contraction were measured with calipers and calculated as percentage reduction in wound area.

Treatment of surgical wound

Animals in all groups were treated daily from the immediate postincision period to the day of euthanasia. Before daily treatment, the wounds were cleaned with distilled water. Then treated with 0.1 g petroleum jelly, silver sulfadiazine (dermazine) the standard, 0.1 g of *Acacia* powder (plant extract) using applicator stick which were discarded after each application. The treatment was done topically for 9 days. Wound areas were measured on day 3, 6 and day 9 (at three days intervals).

Results and Discussion

Effect of A. hockii extract on wound contraction rate of albino rats

As shown in Table 1, on day 0, all the rats in all groups were excised with the same size of wounds (2 cm), and hence all groups assumed 0 percent contraction on that day. Percentage wound contractions were observed three days later (day 3). The 4th group had the highest percentage contraction (11.00±0.02), though the differences in contractions among the groups were not statistically significant ($p > 0.05$). Wound contractions were further observed to increase on day 6 and these were significantly ($p < 0.05$) higher when compared to day 3 and 0 with group 4 having the highest percentage wound contraction. The rate of wound contractions increased significantly ($p < 0.0\ 5$) by the 9th day when compared to the 3rd and the 6th day, respectively. Higher contractions were observed mostly in groups 4 and 3 when compared to the other groups (control and group 2). The contraction could be attributed to *A. hockii* medicinal properties to the presences of phytochemicals, such as alkaloids glycosides, and flavonoids as reported by Subhan *et al*. (2018).

Table 1: Effect of *A. hockii* extract on percentage wound contraction of albino rats

	Period			
	Day 0 (%)	Day 3 (%)	Day 6 (%)	Day 9 (%)
Group 1	100±0.00^{ab}	1.0±0.65^{cb}	18.83±0.65^{de}	20.67±0.65^{ge}
Group 2	200 ± 00^{ab}	5.33±0.65^{cb}	19.16±0.65^{de}	24.83±0.65^{ge}
Group 3	300 ± 00^{ab}	6.83±0.65^{cb}	27.83±0.65^{fe}	35.00±0.65^{he}
Group 4	400 ± 00^{ab}	11.00±0.02^{cb}	29.66±0.01^{fe}	40.6±0.04^{he}

Results are expressed in Means ± SD (n = 3); Mean values with different letters down the groups and across the periods are considered to be significant at $p < 0.05$

Group 1 = Control, Group 2 =Petroleum jelly, Group 3 =Standard, Group 4 =Plant extract

Fig. 1: Wound healing on day 3

Fig. 2: Wound healing on day 6

Fig. 3: Wound healing on day 9

The images below depict the visible contractions of the wounds excised on albino rats as observed on days 3rd, 6th and 9th day, respectively.

The Fali people of Vimtim, Mubi North Local Government Area of Adamawa state very much believe in the use of *A. hockii* (kuffran) as a wound healer and an essential material for traditional embalmment during traditional burial ceremonies. Hence, this experiment was designed to evaluate effect of the medicinal plant extract on wound healing. It is known that foot ulcers and other bodily wounds are treated with the use of the plant (bark) powder, sometimes in addition with some oils but mostly alone. It was observed that Acacia species has been used in medicines and woodwork for centuries (Wickens, 1995) and in locally for hides and skin treatment (leather processing). It has a long history in civilizations as ancient as the Egyptians and the aboriginal tribes of Australia. These kingdoms and tribes used acacia in surprisingly diverse ways, from making desserts to treating hemorrhoids (Soromessa *et al*., 2004).

Topical application of *A. hockii* may have exploited unknown angiogenic and mitogenic potential confined in the phytochemical constituent of the bark of the plant. In excision wound model, aqueous extract of *A. hockii* showed faster wound contraction on the 9th day, compared with reference standard and control groups in percentage contraction 40.6±0.04, 35.00±0.65, 24.83± 0.65 and 20.67±0.65, respectively. This is in agreement with the studies of Suriyamoorthy *et al*. (2014). Various inflammatory cells, fibroblasts and keratinocytes may have been acted upon by organic constituents of the plant extract which may have resulted to the acceleration of the wound contraction rate of *A. hockii* (Sembian *et al*., 2012). In the fibroblasts the gap junctional intercellular communication may be increased to induce a more rapid maturation of granulation tissues (Moyer *et al*., 2002).

It is obvious that, the phyto-compounds present in *A. hockii* extract which may be similar to other species of Acacia may have played significant antimicrobial activity in the healing process of the wounds (Tchatchedre *et al.*, 2019; Okoro *et al.*, 2012). Lagnika *et al*. (2016) reported that the presence of alkaloids, lignan, flavonoid, saponin, tannin, triterpene in the aqueous extracts of *A. hockii*, could be responsible for the medicinal properties of plant extract. Secondary metabolites that serve as the defensive agents (wood preservatives) and those phytocompounds which are produced specifically when the plant is under stressed condition provide invaluable resources. These secondary metabolites have been observed in previous studies to be sources of novel compounds which historically served as templates for the development of many important classes of drugs (Gurib-Fakim, 2006).

Therefore, the wound healing action observed with *A. hockii extract, can be attributed to the* activity of the phyto-compounds working synergistically present in the plant, by promoting clot formation and possible antimicrobial activity. This experiment justifies the use of *A. hockii* bark extract by Fali people of Adamawa State, as a wound healer.

Conclusion

The results of this study showed that, the aqueous extract of *A. hockii* bark demonstrated significant wound healing effect on albino rats and it is obvious that this effect is accompanied with antimicrobial activity. Further studies are however necessary to fractionate and test the various fractions and subsequent sub-fractions on same experiments with anti-microbial properties *in vitro*, as steps towards understanding its *modus operandi* and antimicrobial properties. These will serve as guide towards the purification and isolation of pure anti-biotics from the plant extract.

Recommendation

Further research should be conducted on diabetic foot ulcer models to ascertain the medicinal plant efficacy as it is one of the claims by the traditional medical practitioners.

Conflict of Interests

Authors have declared that no conflict interests exist.

Ethical Consent

The Institutional Animal Ethics Committee, Nasarawa State University, Keffi, Nigeria, approved the study before the experiment and certified all experimental protocols. The certification number is NSUKAEC021

Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work.

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