

Effect of aqueous extract of *Tetrapleura tetraptera* on excision wounds in albino rats

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Abstract

In this study, *Tetrapleura tetraptera* was ascertained of its effect on wound healing. The Lethal dose (LD₅₀) of *Tetrapleura tetraptera* was determined and the research on its wound healing effect was carried out. The standard method of Nofal was adopted for the determination of the (LD₅₀). Wound healing effect was done by excising wounds on anaesthetized rats and then the percentage wound closure (epithelialization) was determined from the treatment with different concentrations of the extract, negative control as well as the positive control. The (LD₅₀) of *Tetrapleura tetraptera* was 10,000mg/kg body weight. The least concentration (200mg/ml) gave hundred percent (100%) epithelialization at the end of the experiment; 2000mg/ml concentration of the extract delayed the wound healing effect of the plant. Conclusively, *Tetrapleura tetraptera* at 200mg/ml has a potent value of wound healing effect while 2000mg/ml of *Tetrapleura tetraptera* is not efficacious in wound healing. Thus, *Tetrapleura tetraptera* could be administered at 200mg/ml for the treatment of wounds.

Keywords: Aqueous extracts, Wound healing, Neobacin powder ® *Tetrapleura tetraptera*

INTRODUCTION

Plants have been an important source of medicine for thousands of years. Even today, the world health organization estimates that up to 80% of people still rely on traditional remedies such as herbs for their medicines (World Health Organization, 1997). WHO (World Health Organization, 2001) defines medicinal plants as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Tetrapleura tetraptera also called Taub and commonly known as Aridan (fruit) in South Western Nigeria is a medicinal plant of the Mimosaceae family. The plant is locally known as Uyayak in Ibibio; Edeminang in Efik; Osakirisa or Oshosho in Igbo; Dawo in Hausa and Aidan in Yoruba (all in Nigeria) (Ojewole and Adewunmi, 2004); it is called Prekese in Twi language of Ghana (Herbert et al., 1977). It is generally found in the lowland forest of tropical Africa. The fruits consist of a freshy pulp with

small, brownish-black seeds. Its fruit is used for the management of convulsions, leprosy, inflammation and rheumatism (Okwu, 2003, Ojewole and Adewunmi, 2004) unquiqually reported on the chemical evaluation, nutritional and flavouring properties of *Tetrapleura tetraptera* in which the spice contain crude protein, (7.44% - 17.50%), crude lipid (4.98% - 20.36%) and food energy (234.42 – 379.48g/cal), he also reported that the spice is a source of minerals such as calcium, phosphorous, potassium, zinc and iron, while the phytochemical screening revealed the presence of tannins, phenolic compounds, saponins, alkaloids, steroids and flavonoids which could be assumed to be responsible for its varied biological and pharmacological properties.

Wound is defined as the disruption of the cellular and anatomic continuity of a tissue (Bennet, 1988). Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. It mainly depends on the repairing ability of the tissue type, extent of

damage and general state of the health of the tissue (Clark, 1996).

Wound healing represents a major health problem both in terms of morbidity and mortality, thus this work was aimed at determining the dose response wound healing effect of the aqueous fruit extract fraction of *Tetrapleura tetraptera* plant in rats.

MATERIALS AND METHODS

Collection and identification of plant material

The plant material was collected at Ibiaku Itam in Itu Local Government Area of Akwa Ibom State in October, 2012. It was identified and authenticated by Dr. (Mrs.) Eshiet, a Botanist in the Department of Botany and Ecological studies, University of Uyo, Nigeria.

Extraction of plant material

The dried fruits was washed and grated into coarse particles. The grated particles (300 g) was macerated cold with 1800ml of distilled water for seventy two hours (72 hours) at room temperature, filtered and the filtrate was concentrated to dryness at 40°C and stored in the refrigerator. The yield of the dried extract was 75.03 g.

Animals used for the work

Young adult Swiss Albino mice (20-25g) and Albino Wistar rats of (147-295g) of both sexes obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy of the University of Uyo, Nigeria were used. They were kept in clean cages (wooden bottom and wire mesh top), maintained under standard laboratory conditions (Temperature 25± 5°C, Relative humidity 50-60%, and a 12/12h light/dark cycle) and were allowed free access to standard diet (Guinea Feed Nigeria Ltd) and water *ad libitum*. The principles of laboratory animal care were followed while the Faculty's ethical committee approved the use of the animals and the study design.

Acute toxicity study (LD₅₀)

The method of (Nofal et al., 2009) was adopted for acute toxicity testing. This was carried out in Swiss Albino mice. For this purpose, five groups of six (6) mice each, weighing 20 – 25 g body weight were dosed intraperitoneally in different gradual doses of (8000 – 12000mg/kg body weight). The animals were observed for physical signs of toxicity and death for 24 hours, after which the number of dead mice were counted in each group and percentage mortality calculated.

LD₅₀ of the tested extract was calculated using the method of (Nofal et al., 2009), according to the following formula:

$$LD_{50} = D_m - \frac{\sum (z \times d)}{n}$$

Where:

- D_m = The largest dose which kill all animals
 z = Mean of dead animals between 2 successive groups.
 d = The constant factor between 2 successive doses
 n = Number of animals in each group
 Σ = The sum of (z x d).

Experimental design on wound healing in albino rats

Animals of group one were topically treated with distilled water (Negative control). Group two (2) treated topically with 10000mg/5ml (of extract (LD₅₀) that is 2000mg/ml), Group three (3) received 400mg/ml, Group four (4) received 200mg/ml, and Group five (5) was treated with 200mg of Neobacin powder (positive control) as standard drug. Number of days required for falling of scar without any residual raw wound was taken as end point of complete epithelialization.

Statistical analyses of data

Results were expressed as multiple comparisons of Mean ± SEM. Significance was determined using one way analysis of variance (ANOVA). A probability level of less than 5 percent (P<0.001) was considered significant.

RESULT AND DISCUSSION

Result

Acute toxicity studies (LD₅₀)

Aqueous extract of *Tetrapleura tetraptera* (8000-12000mg/kg) produced signs and symptoms of toxicity ranging from weakness, reduced respiration and locomotor activity to death. Acute toxicity of the aqueous extract of *Tetrapleura tetraptera* dry fruit was calculated to be 10,000mg/kg body weight = 10.0g/ kg body weight.

Effect of aqueous fruit extract of *Tetrapleura tetraptera* on wound healing in albino rats

The wound healing activity of aqueous extract of *Tetrapleura tetraptera* dry fruit was evaluated in this study. On day 3, there was a significant increase (P<0.001) of percentage wound closure in the negative control when compared with the various concentrations of

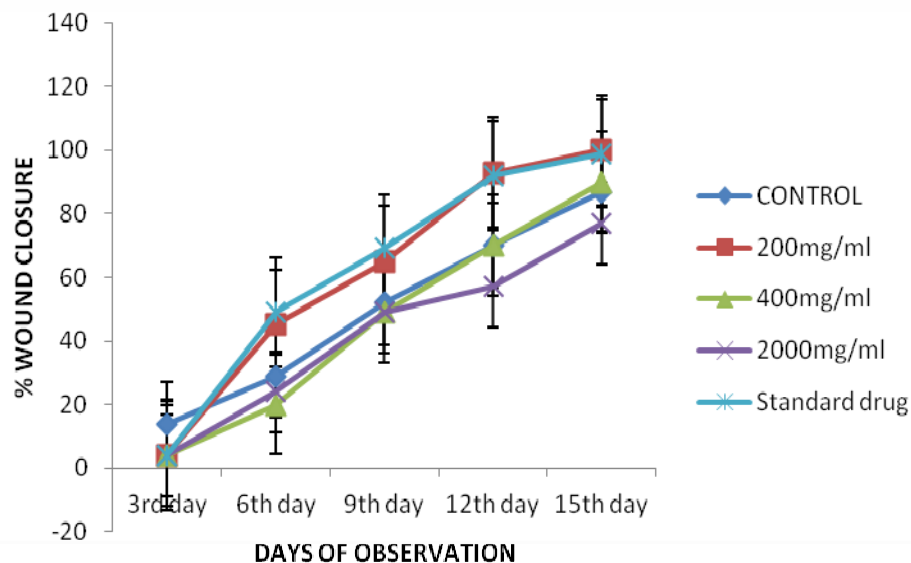


Figure 1. The graph of percentage wound closure versus days of observation.

the extract as well as the positive control. A significant decrease ($P < 0.001$) was observed on day 6 comparing groups of rats treated with 400mg/ml and 2000mg/ml with the negative control. Whereas, the groups treated with 200mg/ml and positive control showed a significant increase ($P < 0.001$) when compared with the negative control. Also, there was significant increase of positive control compared with various concentrations of the extract. On day 9, there was a significant increase ($P < 0.001$) in the percentage wound closure of the negative control when compared with the 400mg/ml and 2000mg/ml concentrations of the extract, whereas, there was a significant decrease ($P < 0.001$) in the negative control when comparing to 200mg/ml extract and the positive control. Significant increase ($P < 0.001$) was observed in the percentage wound closure of the negative control in comparison to the 2000mg/ml extract group on day 12. Whereas, there was a significant decrease of the negative control in comparison to the 200mg/ml and the positive control group. The 15th day showed a significant increase in percentage wound closure of 200mg/ml, 400mg/ml and positive control when compared with the negative control, but showed a significant decrease in treatment with 2000mg/ml compared to negative and positive controls (Figure 1).

DISCUSSION

The median lethal dose (LD_{50}) value of 10.0g/kg, indicated that the extract was practically non-toxic; extract can be classified as non-toxic since the limited

dose of an acute toxicity is generally considered to be 5.0 g/kg bw hence a high safety margin and tolerability, this correspond to a report by (Assam et al., 2010).

Area of the wounds decreased with time from day 3 till day 15 in all the groups and increased rate of wound contraction was observed in this study; this was in consonance with the work of Murthy et al., 2004. This might be due to antimicrobial and anti-inflammatory activities of *Tetrapleura tetraptera* (Ekwenye and Okorie, 2010) as plant with anti-inflammatory and antimicrobial activities have been reported to exert wound healing effect (Ojewole and Adewunmi, 2004). A decrease of 20.0 % with 400mg/ml, 24.0% with 2000mg/ml in percentage wound closure was observed on day 6 when compared to the negative control with a 29.0% increase. Whereas, there was an increase of 45.0% with 200mg/ml when compared with the negative control, this was in lieu with the 49.0% increase in positive control. The percentage wound closure of 400mg/ml and 2000mg/ml decreased equally by 49.0% as against 52.0% increase of the negative control whereas there was a 65.0% increase of 200mg/ml in line with the 69.0% increase of the positive control on day 9. On day 12, there was a 57.0% decrease of 2000mg/ml but an increase of 93.3% of the 200mg/ml compared to the negative control in percentage wound closure with 200mg/ml in line with the positive control group's increase of 92.0%. The 15th day showed a 100% increase of 200mg/ml in percentage wound closure which was more than the 99.0% of positive control in comparison to the 87.0% with the negative control. 400mg/ml showed an increase of 90.0% while 2000mg/ml had a decrease of 77.0%

compared with both negative control and positive controls. This result was similar with the report of work by (Badu et al., 2012).

Antioxidant activity is known to account for anti-inflammatory activity of plants. *Tetrapleura tetraptera* has been reported to possess anti-inflammatory (Agarwal et al., 2009), antioxidant activity (Badu et al., 2012) which may have contributed to the wound contraction and increased rate of epithelialisation observed in this study. *Tetrapleura tetraptera* fruits have been reported to contain many chemical compounds such as triterpenoid glycoside (aridanin), flavonoids and other phenolic compounds (Adewunmi and Marquis, 1987; Maillard et al., 1992). These compounds may have accounted for the wound healing effect observed in this study.

CONCLUSION

In summary, the aqueous fruit extract of *Tetrapleura tetraptera* dry fruit produced wound healing effect. The healing effect was dependent on the concentration and was highest with the lowest concentration (200mg/ml) whereas the highest concentration (2000mg/ml) produced delay in the wound healing activity. Thus, topical application of *Tetrapleura tetraptera* has a positive influence on wound healing, and the findings of this study ascertain the usage of the aqueous fruit extract of *Tetrapleura tetraptera* in traditional treatment of wounds.

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