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Acute Toxicity Study and Ascertaination of Wound Healing Effect of the Acetone Fraction of *Tetrapleura tetraptera* Fruit in Excision Wound Model

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Authors' contributions

This work was carried out in collaboration among all authors. Author EGS designed the study, wrote the protocol and wrote the first draft of the manuscript. Author ADO managed the animals and collected all data. Author UIE did the literature search and also wrote part of the manuscript. Author NEE performed the statistical analysis. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The study was aimed at determining the lethal dose of the fruit of *Tetrapleura tetraptera* and ascertains the wound healing effect of its acetone fraction.
Place and Duration of Study: The department of clinical pharmacy, faculty of pharmacy, University of Uyo, Nigeria, between July and September, 2014.
Methodology: Miller and Tainter method was adopted for acute toxicity testing of the plant's fruit while the Excision wound model were used to evaluate the wound healing activity with the progressive wound closure rate calculated via the wound width and number of days for complete epithelisation was determined.

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Results: The acute toxicity of the aqueous fraction of *Tetrapleura tetraptera* dry fruit was calculated to be 10,000 mg/kg body weight = 10.0 g/ kg body weight. Topical administration of the Acetone fraction with 0.4 g/ml and 0.6 g/ml doses produced significant increases (*P*=0.05) in wound width by 0.29±0.07 cm and 0.47±0.09 cm respectively in comparison with the 0.10±0.03 cm of rats treated with 0.2 g of Neobacin powder on the first 12 days after excision. On day 15, 0.4 g/ml had a non-significant increase of 0.06±0.04 cm when compared to the 0.2 g Neobacin powder with 0.02±0.02 wound width.

Conclusion: The acetone fraction of the dry fruit of *Tetrapleura tetraptera* was not effective on wound healing thus further work should be done using other extracting solvents like Aqueous, Methanol etc. to ascertain the wound healing effect of the dry fruit of *Tetrapleura tetraptera* claimed by the traditional medicine.

Keywords: Acetone fraction; lethal dose; Tetrapleura tetraptera; excision wound; wound healing; epithelisation.

1. INTRODUCTION

There is an increasing interest in finding herbal extracts with wound healing efficacy although the use of such extracts for treating cuts and wounds is a common practice in traditional medicine. Wound infection resulting from the impaired immunity and exposure or poor hygiene is one of the most commonly encountered and clinically important impediments to wound healing. The injured skin remains vulnerable to invasive microbial infections of all kinds subsequent development of wound sepsis until complete epithelial repairs has occurred [1]. 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 [2].

Tetrapluera tetraptera is a species of flowering plant in the pea family native to Western Africa. The tree has many uses and its sweet fragrance is highly valued, its fruit and bark is used to spice dishes and for medicinal purposes. The fruit is used to prepare soup for mothers from the first day of birth to prevent postpartum contraction [3]. The plant has many traditional uses mainly in the management of convulsion, leprosy, inflammation and rheumatic pains, schistosomiasis, asthma and hypertension [4]. The major constituents are tannins, flavonoids and saponins [5].

Tannins act as free radical scavengers, triterpenoids, and flavonoids promote wound healing due to their astringent and antimicrobial property, and saponins due to their antioxidant and antimicrobial activity, which appear to be responsible for wound contraction and elevated rate of epithelialisation. Flavonoids also possess potent antioxidant and free radical-scavenging effect, enhancing the level of antioxidant enzymes in granuloma tissue [6]. Sterols and polyphenols are also responsible for wound healing due to free radical-scavenging and antioxidant activity, which are known to reduce lipid peroxidation, thereby reducing cell necrosis and improving vascularity [7]. Several types of injuries like burn, wounds, and skin ulcers usually generate superoxides and lipid peroxidation through the activation of neutrophils. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage, and by promoting the DNA synthesis. Thus, an intervention into any one of these phases by drugs could eventually lead to either promotion or depression of the collagenation phase of healing [8].

A wound is a type of injury in which the skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or over healing. Therefore the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences [9].

1.1 Neobacin^{R}

Neobacin^{R} powder has each gram containing 5 mg neomycin as the sulphate and 250 IU of

bacitracin as zinc bacitracin. The drug is used in surgery and dermatology as prophylactic and therapeutically in wound infections, burns, corrosions, excoriations and gangrene. It is also use in the treatment of impetigo, folliculitis, eczema, seborrheic dermatitis and in dusting wounds after ear and nose surgery [10].

The traditional medicine practitioners and rural dwellers have claimed that *Tetrapluera tetraptera* taub are used to treat wounds, it has to be studied to ascertain the authenticity of this claim scientifically with different fractions of the fruit's plant. Recently, the acqueous fraction of *Tetrapluera tetraptera* fruit was studied by [11] and it proves the authenticity of this claim scientifically. The aim of this study is to investigate if the acetone fraction of the plant has wound healing effect.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant

The plant material was collected at Ibiaku Itam in Itu Local Government area of Akwa-Ibom state, Nigeria in October, 2012. The plant was identified by Dr (Mrs) Eshiet, a botanist of the department of Botany and Ecological studies, Faculty of Science of the University of Uyo, Nigeria and deposited at the Pharmacognosy and Natural Medicine department´s herbarium, Faculty of Pharmacy, University of Uyo, Nigeria with a voucher number –UUH No:42 (F) University of Uyo, Nigeria.

2.2 Extraction of Plant Material

The dried fruit of *Tetrapleura tetraptera* was washed, air-dried and ground into powder. 300 g of the powdered fruit was macerated cold with 1.8L of Acetone for seventy –two hours (72 hours) at room temperature, the extract was filtered and the filtrate was concentrated in vacuum at 40ºC to remove any dissolved, suspended or adhered particles and stored in the refrigerator. The yield of the dried extract fraction was 54.50 g.

2.3 Animals

Swiss albino mice (20-25 g) and albino wistar rats (180-270 g) of both sexes obtained from the animal house of the department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria were used. They were kept in clean cages and maintained under standard laboratory conditions (Temperature; 23±1ºC, Relative humidity; 50-60%, and a 12/12h light/dark cycle) and were allowed free access to standard diet (Bendel feed and flour mill Ltd, Benin, Nigeria) and water *ad libitum*. All authors hereby declare that the "Principles of Laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed while the Faculty of Pharmacy, University of Uyo, Nigeria's ethical committee examined and approved the use of the animals and all the experiments.

2.4 Acute Toxicity Studies

The method of Miller and Tainter [12], was adopted for acute toxicity testing. Swiss albino mice were starved of food but allowed access to water ad libitum 24 hours before the experiment. The acetone fraction was administered intraperitoneally in a dose range of 7000-12500 mg/kg and the animals were randomly selected into groups of six each. The mice were observed for physical signs of toxicity and death, the numbers of deaths per group within 24 hours were recorded and the median lethal dose (LD_{50}) calculated as the square root of the geometrical mean of the maximum dose that did not kill any animal and the minimum dose that killed all.

2.5 Experimental Design of Wound Healing Effect

Excision wound model were used to evaluate the wound healing activity of *Tetrapleura tetraptera* in rats thus: The dorsal skins of the rats were shaved, and the rats were divided into four groups of five (5) animals each. Under Xylocaine 5% anaesthesia, the animals were depilated on the Para vertebral area prior to wound creation and area of 10 mm x 10 mm skin in its full thickness was excised; Modified [13]. Animals of group one were topically treated with the distilled water (Negative control-NC), group two were topically treated with 0.2 g Neobacin powder as the positive control (PC), group three were topically treated with 0.4 g/ml of the acetone fruit fraction of *Tetrapleura tetraptera*, and group 4 were topically treated with 0.6 g/ml of the fraction. The acetone fruit fraction of *Tetrapleura tetraptera* was dissolved in distilled water (as vehicle). Wound healing property was checked by measurement of wound area and Period of epithilization.

2.6 Measurement of Wound Area

The progressive wound closure rate was accessed by tracing the wound on day 3, 6, 9, 12 and 15 post wounding using a transparent paper and a permanent marker. The wound areas recorded were measured using a millimetre scale graph paper. Number of days required for filling of scar without any residual raw wound was taken as end point of complete epithelisation. The wound healing effect was determined by the reduction of injured area in relation to initial day and calculated using the formula:

$$\text{Wound width} = \frac{W_{exp.} \times 10}{W_m}$$

Where,

W_m = Initial wound area on day one.
W_{exp} = Wound area on day of measurement.

2.7 Statistical Analysis

Results were expressed as multiple comparison of mean ±SEM significance was determined using one way analysis of variance (ANOVA) followed by Duncan test. A probability level of less than 5 per cent (P=.05) was considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Acute toxicity studies

Acute toxicity studies of the crude extract of *Tetrapleura tetraptera* dry fruit was calculated to be 10000 mg/kg which is 10.0 g/kg as seen on Fig. 1 and Table 1.

Specifically derived by the method of Miller and Tainter [12] in which 0.25 was added to zero mortality and subtracted from 100% mortality.

3.1.2 Wound healing effect

Wound healing effect of the acetone fraction of *Tetrapleura tetraptera* fruit extract showed no significant difference (P = 0.05) between groups 1, 2, 3, and 4 on days 3, 6 and 9 as shown on Table 2. Acetone fruit extract of *Tetrapleura tetraptera* in groups1, 3 and 4 showed a

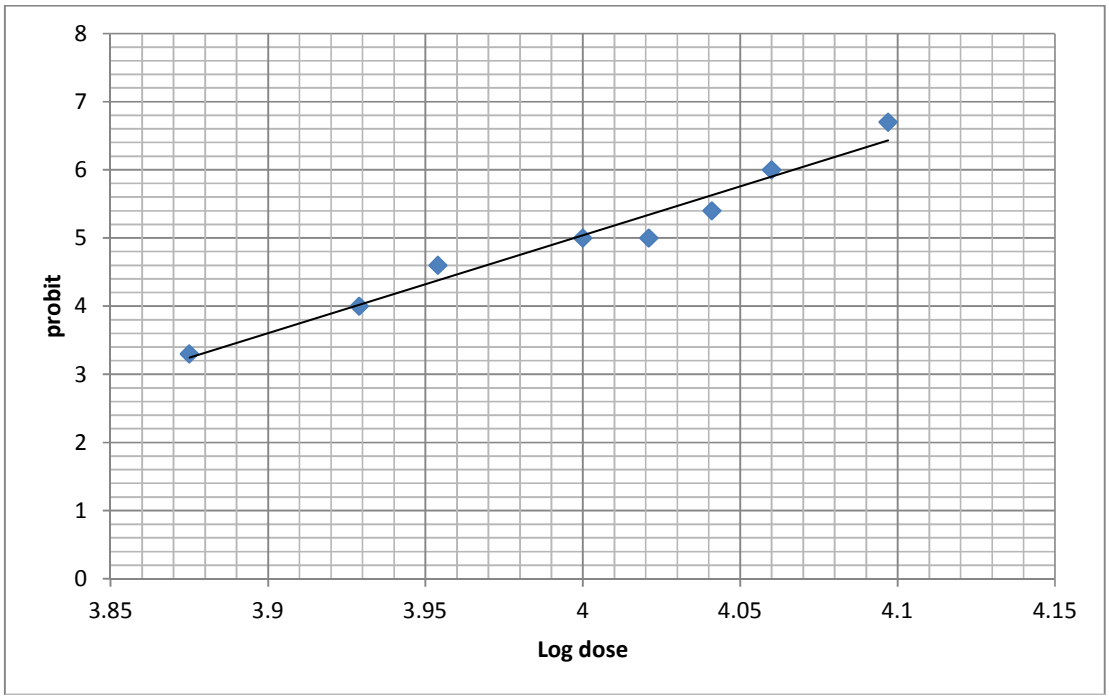

Fig. 1. A Graph of Probit versus Log dose indicating a graph of intraperitoneal acute toxicity study of *Tetrapleura tetraptera* fruit crude extract in mice. Dotted lines indicate an LD_{50} of approximately 10000 mg/kg = 10.0 g/kg. (Antilog of 4.00 = 10000 mg/kg)

Table 1. Acute toxicity determination of the crude extract of *Tetrapleura tetraptera* dry fruit

Group	Dose	Log dose	24h mortality	% Mortality	Probit	Probit (Approx)
1.	7000	3.845	0/6[a]	04.7	3.2721	3.3
2.	7500	3.875	0/6[a]	04.7	3.2721	3.3
3.	8000	3.903	0/6[a]	04.7	3.2721	3.3
4.	8500	3.929	1/6	16.6	4.0299	4.0
5.	9000	3.954	2/6	33.3	4.5684	4.6
6.	9500	3.978	2/6	33.3	4.5684	4.6
7.	10000	4.000	3/6	50.0	5.0000	5.0
8.	10500	4.021	3/6	50.0	5.0000	5.0
9.	11000	4.041	4/6	66.6	5.4289	5.4
10.	11500	4.060	5/6	83.3	5.9661	6.0
11.	12000	4.079	6/6*	95.8	6.7279	6.7
12.	12500	4.097	6/6*	95.8	6.7279	6.7

*Key: [a] = 0% Mortality and * = 100 % mortality*

significant decrease in wound healing in comparison with group 2 (Neobacin Powder 0.2g) on day 12. Day 15 also indicated a significant decrease in wound healing in group 1 and 4 when compared to group 2 while group 3 showed a non-significant decrease compared to group 2 (Table 2 and Figs. 2-5).

3.2 Discussion

The mean lethal dose (LD50) was calculated to be 10000 mg/kg = 10.0 g/kg. The physical signs of toxicity included excitation, paw-licking, decreased motor activity. Others were increased respiratory rate, convulsion and death. According to the OCED protocol [14,15]. Acetone fraction of *Tetrapleura tetraptera* can be classified as non toxic since the limited dose of an acute toxicity is generally considered to be 5.0 g/kg bw [1,16].

There is an increasing interest in finding herbal extracts with wound healing efficacy although the use of such extracts for treating cuts and wounds is a common practice in traditional medicine [1]. Wound healing effect of the acetone fraction of *Tetrapleura tetraptera* fruit extract was not enhanced up to the 12th day as it showed no significant difference between the negative control rats and the fraction treated groups, this was in contrast to the report [17,18] that reported on the aqueous extracts possession of anti-inflammatory and hypoglycaemic properties. This could be due to the portraying sign of toxicity to wound area and thus resulting in expansion rather than reducing the width of the wound.

Acetone fruit fraction of *Tetrapleura tetraptera* in groups 1, 3 and 4 showed a significant decrease in wound healing in comparison with the rats in group 2 treated with Neobacin Powder on day 12. Day 15 also indicated a significant decrease in wound healing in groups 1 and 4 when compared to group 2. This finding contradicts Badu and Effiong's [18,11] reports on aqueous fruit fraction of the plant. The reason could be traced to the poor extractive property and toxic nature of acetone or may be due to the fact that the necessary phytochemical components or nutrients of the plant responsible for management and treatment of wounds are more soluble in polar solvents (eg; Water) rather than non-polar solvents like acetone, hence not suitable for wound healing.

The effect of acetone fraction of the plant confirmed that the plant extract has dose limitation effect, thus at high doses, the extract may have induced severe tissue damage and even lead to neoplastic transformation, which further impede the healing process by causing damage to cellular membranes, DNA, proteins, and lipids as well as reported by [19]. Also, oxidative stress plays an important role in impaired wound healing. This oxidative stress may cause damage to the growing tissue at the repair site towards cellular proliferation, granular tissue formation and epithelisation [20], thus the negative result of the acetone fraction of *Tetrapleura tetraptera* fruit extract.

Increased cellular proliferation may be due to the mitogenic activity of the positive control, which might have significantly contributed to the healing process of this group. The plant *Portulaca oleracea* containing the tannins possesses wound healing activity [21,19] as do *T. tetraptera*

thereby suggesting the mechanism of action of the extract effect on wound. The less effective nature of the acetone extract could be traced to the poor extraction property of less polar solvent compared to more polar solvent, thus the hampered wound healing effect of the fraction. It was reported that the dry fruit of *Tetrapleura tetraptera* constitutes of Zinc, Calcium, Iron, Magnesium, Pottasium, Vitamin C, and Phosphorus [22] and saponins, tannins, sugar, glysocides, steroids. Triterpenes [23] which are found to be effective in wound healing. This could be attributed to the report that the process of wound healing is promoted by several herbal extracts, which are composed of active agents like triterpenes, alkaloids, flavonoids, tannins, saponins, anthraquinones, and other biomolecules [24,20].

Table 2. Effect of various doses of acetonic extract of *Tetrapleura tetraptera* dry fruit on excision wound healing in rats

Group	Treatment	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
1	Negative control (1ml of distilled water)	1cm	0.88±0.80	0.72±0.09	0.44±0.09	0.28±0.07*	0.12±0.04*
2	Positive control (0.2g of neobacin powder)	1cm	0.96±0.04	0.70±0.05	0.34±0.05	0.10±0.03	0.02±0.02
3	0.4g/ml of *T. tetraptera*	1cm	0.88±0.02	0.78±0.03	0.44±0.05	0.29±0.07*	0.06±0.04
4	0.6g/ml of *T. tetraptera*	1cm	0.89±0.01	0.74±0.02	0.56±0.08	0.47±0.09*	0.14±0.09*

*Values are expressed as Mean ± SEM (n=5), significant at *P=0.05 when compared to positive control*

Group 1: Negative control (treated with distilled water).

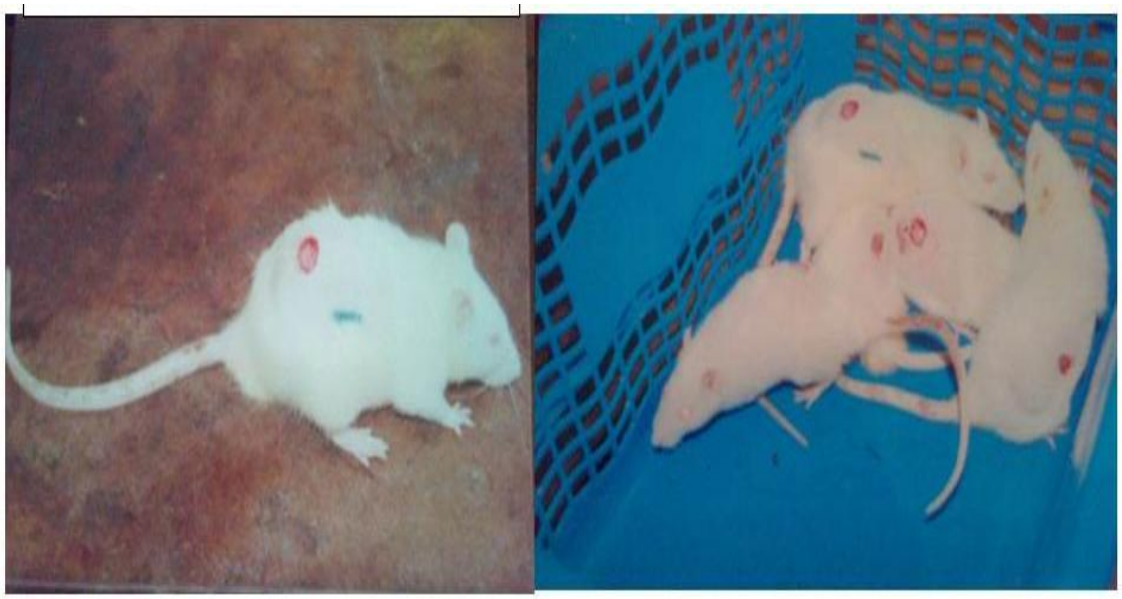

Fig. 2. Width of excision wound on day 1

Group 1: Negative control (treated with distilled water).

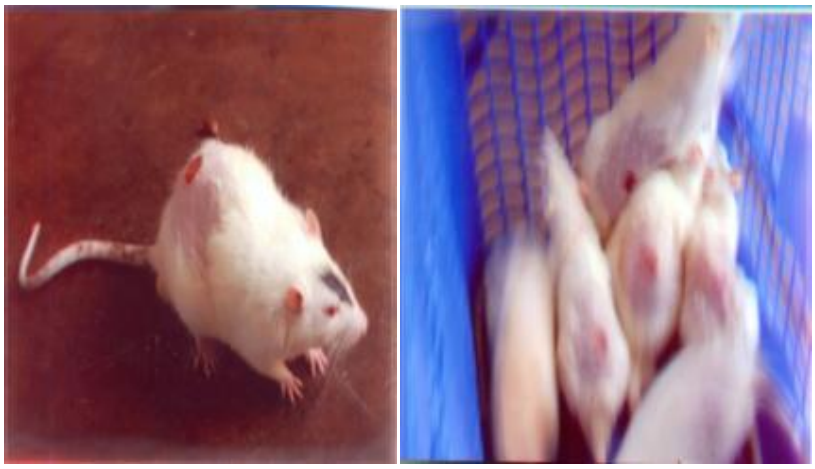

Fig. 2. Width of excision wound on day 15

Group 2: Positive control (treated with Neobacin powder).

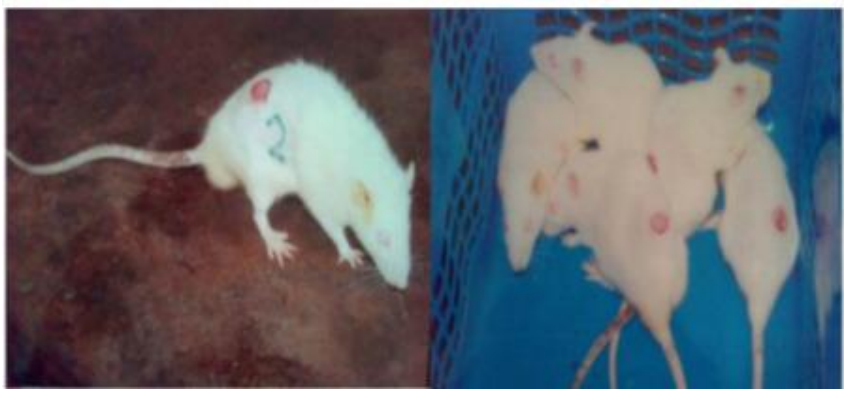

Fig. 3. Width of excision wound on day 1

Group 2: Positive control (treated with Neobacin powder).

Fig. 3. Width of excision wound on day 15

Group 3: Treated with 0.4g/ml of acetone fraction of *Tetrapleura tetraptera* fruit.

Fig. 4. Width of excision wound on day 1

Group 3: Treated with 0.4g/ml of acetone fraction of *Tetrapleura tetraptera* fruit.

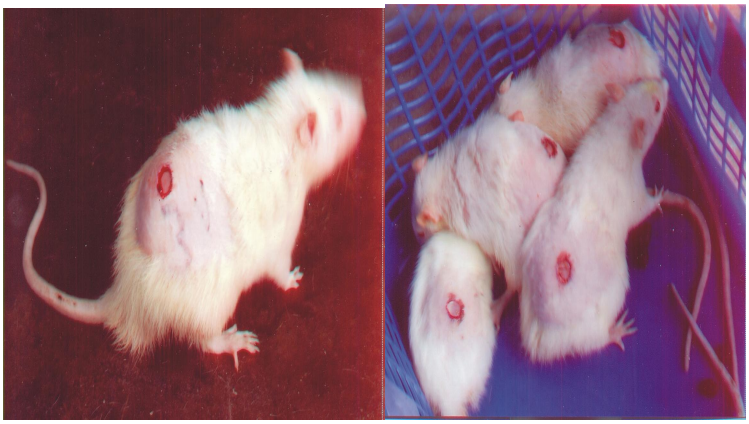

Fig. 4. Width of excision wound on day 15

Group 4: Treated with 0.6g/ml of acetone fraction of *Tetrapleura tetraptera* fruit.

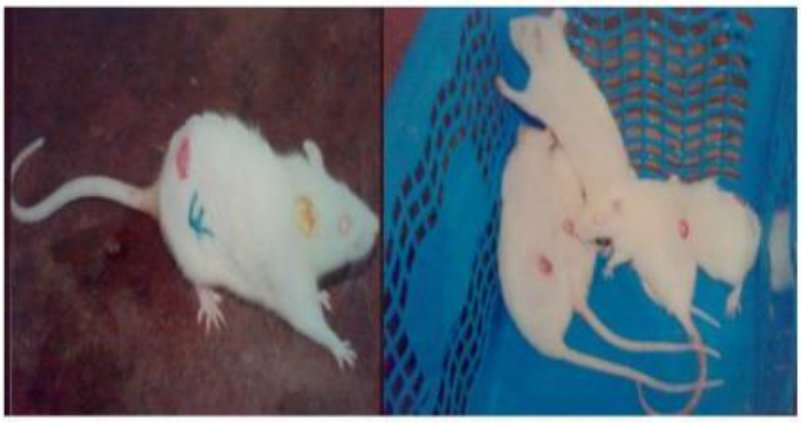

Fig. 5. Width of excision wound on day 1

Group 4: Treated with 0.6g/ml of acetone fraction of *Tetrapleura tetraptera* fruit.

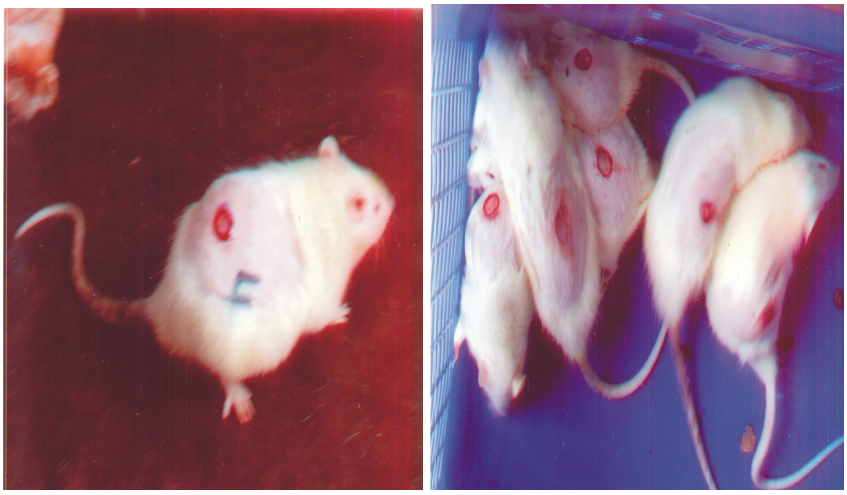

Fig. 5. Width of excision wound on day 15

4. CONCLUSION

In conclusion, the acetone fraction of the dry fruit of *Tetrapleura tetraptera* is not effective on wound healing. The high dose treatment even indicated that the plant has a limit dose treatment for wound healing at which it was toxic, thereby increasing the wound surface area. Thus, it is recommend that further work should be done using other extracting solvents like Water, Methanol etc., to ascertain the wound healing effect of the dry fruit of *Tetrapleura tetraptera*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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