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Histopathological Observations of The Wound Healing Properties of Plant Exudates of *Jatropha curcas* Linn.

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ABSTRACT
This study is aimed at evaluating the wound healing properties of latex exudates of Jatropha curcas Linn based on histopathological observations. The plant is widely known as a source of medicinals for treatment of a variety of ailments. A total of 15 albino wister rats of both sexes weighing between 200 and 260gms were used for this study and were divided into three groups; negative control, positive control (Betadine (R)) and exudates of Jatropha curcas. All rats were incised at the flanks on both sides. After treatment, smear biopsies were taken at the hours of 12, 24, 48, 72, 96,144 and 168 for histopathological evaluations. The histopathological parameters used were number of neutrophils, microphages, lymphocytes and fibroblasts. The results showed that there was a significant increase in the numbers of inflammatory cells and fibroblasts suggestive of its ability in acceleration of wound healings.
Key words: Jatropha curcas, inflammatory cells, wound healing.

Wound healing is the process of repair following injury to the skin and other soft tissues. It is fundamentally a connective tissue response. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodeled to form a scar.

The human body can sustain a variety of injuries, including surgical, penetrating, burn and blunt traumas. All of these set into motion an orderly sequence of events that are involved in the healing response, characterized by the movement of specialized cells into the wound site. Platelets and inflammatory cells are the first cells to arrive at the site of injury and they provide key functions and "signals" needed for the influx of connective tissue cells and a new blood supply. These chemical signals are known as cytokines or growth factors (Lawrence and Diegelmann, 1994).

Wound healing is influenced by many factors including the kind of medicine or drug used and herbal medicine is one of these kinds (Rose 1969). The use of medicine/ drug is to accelerate the wound healing process and to prevent infection (Prockop and Kivirikko 1995). Parameters used to identify wound healing processes are inflammatory cells and connective tissues (Lazarus et al 1994). *Jatropha curcas* belongs to the family of euphorbiaceae. Its common names are Black vomit nut, Barbados nut and Curcas bean. The plant, originating in Central America, has been spread to other tropical and subtropical countries as well and it is mainly grown in Asia and in Africa. Various parts of this plant have proven to be very useful. Although toxic, *Jatropha* is a known purging nut for its use as purgative/laxative, and is widely known as a source of medicinals for treatment of a variety of ailments. A range of healing properties have been ascribed to leaf preparations for both topical application and ingestion. Duke (1983) provides an extensive list of its various uses in folk medicine. The oil is extensively used for making soap in some countries. The bark of *Jatropha curcas* yields a dark blue dye which is used for colouring cloth, fishing nets and lines. The roots are reported to be used as an antidote for snake-bites. The leaves are used for fumigating houses against bed-bugs. The non-edible vegetable oil of *Jatropha curcas* has characteristics comparable to diesel and the latex and has also been used for promoting wound healing (Nath and Dutta 1999). The latex of *Jatropha* contains an alkaloid known as "jatrophine" which is believed to have anti-cancerous properties and proteolytic enzyme. Its extraction and some of the physico-chemical properties have been reported (Nath and Dutta 1988).

This present study was taken up to screen the wound healing properties of *Jastropha curcas* latex.

MATERIALS AND METHODS

Jatropha curcas latex preparation.

Jatropha curcas plant was confirmed and authenticated with its leaves by the Plant Sciences Department, University of Port Harcourt. The

exudates or latex was collected directly from the stem by cutting the stem aseptically with a knife.

Laboratory Animals:

A total of 15 albino wister rats of both sexes weighing between 200 and 260gms were used for this study. Rats were kept in the individual cage with the optimum environment and temperature (29-310C). Rats were fed with a commercial feed and drinking water was given ad libitum. A period of one week was allowed for acclimatization.

Treatment of the Rats:

Rats were distributed into 3 groups and both flanks of the rats were used;

Group A: negative control on the right and positive control (Betadine (R)) on the left.

Group B: negative control on the right and treatment (*Jatropha curcas*) on the left.

Group C: positive control (Betadine (R)) on the right and treatment (*Jatropha curcas*) on the left.

Each group has a total of 5 rats. The hairs of the anaesthetised animals were shaved at both flanks and an incision of about 1.5 x 0.5cm produced extending to the muscle with the aid of a surgical blade. According to the groups, rats were treated topically. Rats were euthanized using chloroform and smear biopsies were taken from wound edges at different time points; 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 144 hours, and 168 hours post surgery. Tissues were prepared and the histopathological changes were studied.

Data analysis

Data of inflammatory cells and connective tissues were statistically evaluated by one-way ANOVA. The values of $p< 0.05$ were considered as statistically significant

RESULTS AND DISCUSION

The first line of leucocyte response to acute inflammation is the appearance of neutrophiles which play an important role in phagocytosis (Lever and Gundula 1968)It does this in order to clean up the wound from contaminate microbes through their phagocytic activies (Kalangi 2004) and thereafter, a decrease in the number and activities of neutrophiles in line with the cleanliness of the wound.

In this study, there was a significant increase in the number of neutrophiles at 12hrs and 48hr in comparison to both negative and positive control groups. While the negative control achieved a peak at 24hr, the *Jatropha* and Betadine treated peaked at 48hrs as shown in Table 1. The higher number of neutrophiles seen in *Jatropha* and Betadine treated indicated that the phagocytic and clean up activities occured earlier in these groups.

Macrophages which are also inflammatory cells, have the ability to digest microbes. They release vasoactive mediators, growth factors, enzymes such proteases (Kalangi 2004). The macrophage, referred to as the orchestrator, is essential for wound healing. Numerous enzymes and cytokines are secreted by the macrophage. These include collagenases, which debride the wound; interleukins and tumor necrosis factor (TNF), which stimulate fibroblasts (produce collagen) and promote angiogenesis and

Table 1. Number of neutrophils

Time (hours)	Betadine	Jatropha	Negative control
12	110.08 ± 08.37^{A}	113.30 ± 09.20^{A}	100.50 ± 06.28^{C}
24	180.30 ± 32.15^{A}	190.00 ± 14.10^{A}	182.05 ± 04.34^{A}
48	210.54 ± 16.20^{A}	240.04 ± 29.03^{B}	134.45 ± 33.16^{C}
72	170.13 ± 14.07^{A}	134.14 ± 32.40^{B}	135.20 ± 08.41^{B}
96	94.15 ± 07.20^{A}	103.62 ± 10.42^{A}	98.14 ± 13.08^{A}
144	84.16 ± 21.00^{A}	52.93 ± 07.19^{B}	40.90 ± 05.37^{C}
168	53.49 ± 30.10^{A}	40.25 ± 11.20^{A}	38.72 ± 19.42^{A}

The same alphabet (superscript) indicates no significant difference ($p >0.05$

transforming growth factor (TGF), which stimulates keratinocytes (Cohen et al, 1992). The significant higher number of macrophages in *Jatropha* treated group as shown in Table 2 below, would have produced a lot of growth factors thereby stimulating the growth of new cells (cell proliferation), faster formation of granulation tissues and thereby affecting the acceleration of wound healing process.

In this study it was revealed that the number of lymphocytes at 96hrs, 144hrs and 168hrs is significantly higher in *Jatropha* treated group than others with a peak value at 168hrs as shown in Table 3. It showed that *Jatropha* could have stimulated the presence of macrophages that triggered lymphocytes to proliferate to produce antibodies. These Lymphocytes are natural killers which could destroy alien substances or produce specific antibody. When specific lymphocytes are activated by antigens they proliferate and produce antibodies.

It was also revealed in this our study that *Jatropha curcas* exudates treated rats showed a significant presence of more fibroblast at hours between 24-168 as shown in Table 4. It is evidenced that fibroblast form the most important cellular elements after the inflammatory phase of wound healing until probably healing is complete (Wokalek and Ruh 1999). The fibroblast is the connective tissue cell responsible for collagen deposition that is needed to repair the tissue injury (Rose 1969).The main function of fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix.

Fibroblasts secrete the precursors of all the components of the extracellular matrix, primarily the ground substance and a variety of fibres. The composition of the extracellular matrix determines the physical properties of connective tissues. Fibroblasts grow and form a new, provisional extracellular matrix (ECM) by excreting collagen and fibronectin (Midwood et al 2004). One of fibroblasts' most important duties is the production

Time (hours)	Betadine	Jatropha	Negative control
12	0.00±0.00^{A}	0.00±00^{A}	0.00±00^{A}
24	0.00±0.00^{A}	0.00±00^{A}	0.00±00^{A}
48	1.25±1.22^{A}	1.6±0.84^{A}	0.00±00^{B}
72	1.50±1.30	2.50±0.43^{A}	1.00±1.09^{B}
96	2.00±0.71^{A}	3.40±1.52^{B}	2.10±0.48^{A}
144	2.20±1.70	3.50±1.20^{A}	2.04±1.52^{B}
168	2.40±2.60	4.20±1.46^{A}	2.00±1.58^{B}

The same alphabet (superscript) indicates no significant difference ($p > 0.05$). no significant difference to either of the groups.

Table 3.Number of lymphocytes.

Time (hours)	Betadine	Jatropha	Negative control
12	72.48±9.86^{A}	73.24±17.16^{A}	62.29±14.2^{A}
24	62.90±4.14^{A}	76.32±10.20^{B}	61.14±8.04^{A}
48	65.63±8.20^{A}	60.16±4.35^{B}	48.11±11.60^{C}
72	43.04±8.35^{A}	62.43±7.16^{B}	50.54±9.20^{C}
96	48.75±31.09^{A}	51.08±20.24^{A}	45.30±21.90^{A}
144	47.40±18.14^{A}	50.14±18.50^{A}	40.12±9.62^{A}
168	30.30±10.40^{A}	38.53±30.12^{A}	36.64±20.16^{A}

The same alphabet (superscript) indicates no significant difference ($p > 0.05$).

Table 4. Number of fibroblast.

Time (hours)	Betadine	Jatropha	Negative control
12	10.10±1.84 ^{A}	17.17±14.45 ^{B}	03.12±1.14 ^{C}
24	12.10±2.72 ^{A}	21.34±8.62 ^{A}	06.26±2.11 ^{B}
48	20.12±11.23 ^{A}	23.20±7.70 ^{A}	18.45±6.42 ^{A}
72	21.08±4.30	25.16±3.12 ^{B}	19.18±2.16 ^{A}
96	24.42±3.28	26.54±2.24 ^{A}	20.92±5.13 ^{B}
144	13.20±1.64	16.17±3.72 ^{A}	11.02±1.14 ^{B}
168	07.32±3.10	15.28±2.34 ^{A}	09.08±0.48 ^{B}

The same alphabet (superscript) indicates no significant difference ($p > 0.05$). No significant difference to either of the groups.

of collagen (Wokalek and Ruh 1999). Fibroblasts begin secreting appreciable collagen by the second or third post-wounding day (Romo and Pearson 2005 , and its deposition peaks at one to three weeks (Mercandetti and Cohen 2005). In normal tissues collagen provides strength, integrity and structure. When tissues are disrupted following injury, collagen is needed to repair the defect and restore anatomic structure and function. If too much collagen is deposited in the wound site, normal anatomical structure is lost, function is compromised and fibrosis occurs. Conversely, if an insufficient amount of collagen is deposited, the wound is weak and may dehisce (Lazarus et al 1994). The formation of bundles of collagen fibrils by fibroblasts is fundamental for a wound healing to occur and the rate of wound healing depends on the amount present (Gianluca et al 2004).

The wound healing properties of Jatropha exudates is most probably due the presence of proteoltic enzyme curcain isolated from its latex (Lazarus et al 1994).

CONCLUSION

The results of this present study strongly suggest that application of the latex of *Jatropha curcas* on wound resulted in more profound cellular reaction and most probably a better quality healing.

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