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A Comparative Study of Plant Extracts on the Healing of Excised Wound: Tensile Strength and Histological Studies

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Abstract:
In this study, we analyzed the wound healing effect of Chromolaena odorataand Calotropis giganteain animal models by monitoring the wound contraction of full thickness excision wounds. The animals used for the purpose were Albino rats. The leaves of Chromolaena odoratawere collected freshly and crushed well in the mortar and pestle without adding any solvent. The crude extract was collected and applied topically over the wound. The latex from Calotropis gigantea was collected from the leaf nodes and applied directly over the wound. The plant materials were applied over the wound on the housed animals on alternate days and tissue samples were collected from them on every fourth day. The tissue samples collected were used for histological studies and biochemical estimations. From the results obtained it was concluded that there were no traces of any serious side effects in the rats under study, except that the group ofanimals treated with the latex of Calotropis gigantea has shown moderate inflammation, ulceration and necrosis with little enhanced edema. But the rate of wound contraction was more when compared to the control group of animals and also Chromolaena odorata. It also exhibited increased tensile strength in the newly formed granulation tissues. Chromolaena odorataalso did not show any undesirableeffects. Unlike Calotropis gigantea, the group of animals treated with the leafjuice from Chromolaena odorata displayed very less edema, inflammation, necrosis and ulceration in the granulation tissues.

1. Introduction

The tensile strength of a material is the maximum amount of tensile stress that can be subjected to the material before its failure. The definition of failure can vary according to material type and design methodology. This is an important concept in engineering, especially in the fields of material science, mechanical engineering and structural engineering. Histology is the study of the microscopic anatomy of cells and tissues of plants and animals. It is performed by examining a thin slice (section) of tissue under a light microscope or electron microscope. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histology is an essential tool of biology and medicine. There are three definitions of tensile strength:

Yield Strength: The stress at which the material changes from elastic deformation to plastic deformation, causing it to deform permanently.

Ultimate Strength: The maximum stress a material can withstand.

Breaking Strength: The stress co ordinate on the stress – strain curve at the point of purple.

1.1. Procedure

The animals were divided into four groups as follows:

Group I: 5 rats
No drug
Group II: 5 rats
Drug used: Betadiene
Group III: 5 rats
Plant used: Chromolaena odorata
Group IV: 5 rats
Plant used: Calotropis gigantea

1.2. Tensile Strength

Dumbbell shaped test pieces of required shape and size are cut from the area of the wound. The test specimens were conditioned and the width of each test piece was measured to the nearest 0.1mm at three places on the grain side and three places on the flesh side.

Thickness gauge having a flat circular pressure foot of diameter 10 + 1mm and a flat circular anvil of diameter greater than or equal to 10 + 1 mm exerting a pressure of 49 + 5KPa between the pressure foot and anvil, was used. The gauge is fitted with a circular dial indicator showing up to 10mm with an accuracy of 0.01mm steel scale graduated in 0.5mm or a vernier was used to calculate the width of the specimen. The test was placed on a flat surface and was flattened without stretching.
The area of cross section of the test specimen was calculated by multiplying its width with its thickness. The jaws of the tensile were set 20mm apart for the sample. The test specimen was clamped in the jaws and the machine was run at the rate of 100 + 2 mm/min until the specimen tore apart. The highest load reached was recorded while the sample was subjected to breaking. The distance between the jaws when the rupture of the test specimen occurred, was noted. The test for the other specimen was carried out similarly.

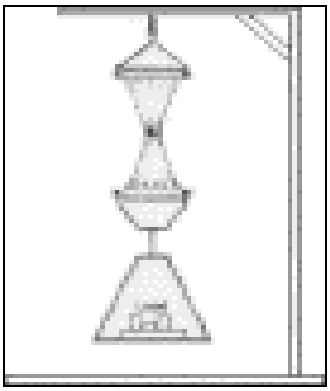

Figure 1: Tensile Strength

Tensile Strength, $kgcm^2$= Breaking Load in kg/Area of Cross Section in cm^2
Area of Cross Section, cm^2= Width (cm)×Thickness(cm)
% Elongation at Breaking Point=(b−a)/a×100
Where, a = Initial distance between the jaws, in mm
b = Final distance between the jaws, in mm

1.3. Histology

1.3.1. Fixation
Fixatives are used to preserve the tissue, the structures of the cell, and the cell organelles found in individual cells (e.g. nucleus, rough endoplasmic reticulum and mitochondria). The tissues are mechanically and biochemically stabilized in a fixative. The most common fixative is neutral buffered Formalin (10% Formaldehyde in Phosphate buffered Saline (PBS)). It is important to consider that a fixative should not be too toxic to its handler and it should also not damage the tissue being preserved.

1.3.2. Processing
The most common technique is wax processing. The samples are immersed in multiple baths of progressively more concentrated ethanol to dehydrate the tissue, followed by a clearing agent such as Xylene or Histoclear, and finally, hot molten Paraffin wax (impregnation). During this 12 – 16 hour process, Paraffin wax replaces Xylene.

1.3.3. Embedding
Soft, moist tissues are turned into hard paraffin blocks, which are then placed in a mould containing more molten wax (embedding) and allowed to cool and harden. Embedding can also be accomplished using frozen, non – fixed tissue in a freezing medium. This freezing medium is liquid at room temperature but when cooled, will solidify. Non – fixed tissue allows for procedures such as in situ hybridizations for specific mRNAs that would have been destroyed during the fixing process. It also allows for very short turnaround where it is needed, as with an ongoing surgery.

1.3.4. Sectioning
The tissue is then sectioned into very thin (2 – 8 µm) sections using a microtome. These slices, usually thinner than an average cell, are then placed on a glass slide for staining. Frozen tissue embedded in a freezing medium is cut on a cooled machine called a cryostat.

1.3.5. Staining
Routine Staining: This is done to give contrast to the tissue being examined, as without staining, it is very difficult to see differences in cell morphology. Hematoxylin and Eosin (H & E) are the most commonly used stains in histopathology. Hematoxylin colours nuclei blue, Eosin colours the tissue under a microscope, the sections are stained with one or more pigments.

Special Staining: There are hundreds of other techniques that have been used to selectively stain cells and cellular components. Other compounds used to colour tissue sections include Safranine, Oil Red, Congo Red, Fast Green FCF, Silver salts and numerous natural and artificial dyes that were originated from the development of dyes for the textile industry.

2. Result and Discussion

2.1. Tensile Strength Studies

TABLE 1 TENSILE STRENGTH IN HEALED TISSUE OF THE WOUND TREATED WITH *Chromolaena odorata* And *Calotropis gigantean*

SAMPLE	MAX LOAD (N)	DISPLACEMENTAT MAX LOAD (mm)	TENSILE STENGTH (MPa)	MAX. % STRAIN (%)
CONTROL	9.88	5.08	2.15	25.41
STANDARD CONTROL	19.11	7.26	4.56	38.12
Chromolaena odorata	6.89	10.79	3.37	53.99
Calotropis gigantea	28.35	9.28	5.06	46.40

Table 1

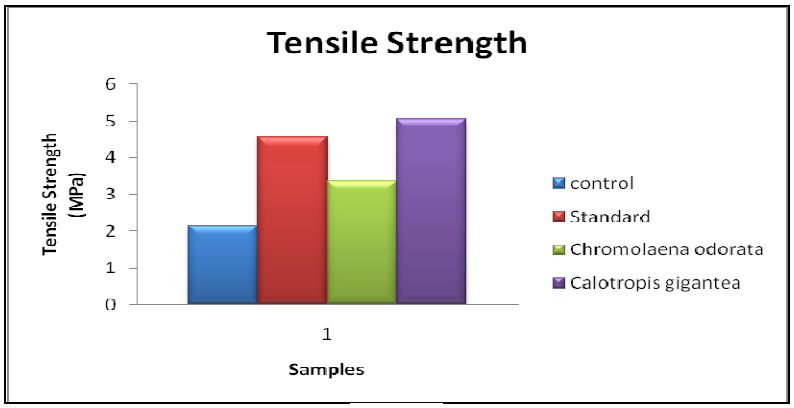

Figure 2

GRAPH1– Tensile Strength In Healed Tissue Of The Wound Treated With *Chromolaena Odorata* And *Calotropis Gigantea*

Tensile strength is the maximum for the material under testing can withstand per unit area. From the data obtained (graph), it was observed *Chromolaena odorataand Calotropis gigantea* has higher tensile strength when compared with control. When we consider the maximum percentage strain it clearly reveals that *Chromolaena odorata* has higher (53.99%) strain compared to *Calotropis gigantea* which has lower strain (46.40%). Overall results prove that all healed tissues treated with *Chromolaena odorataand Calotropis gigantea* have tensile strength higher than the control and standard (Betadiene).

2.2. Histology

2.2.1. 4th day

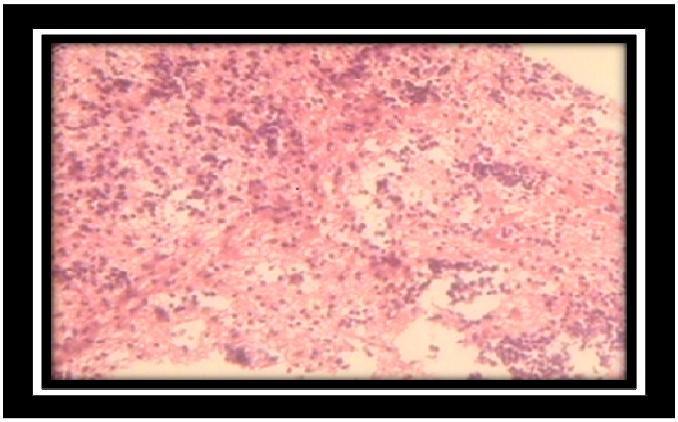

Figure 3.A – Control

MICROSCOPIC EXAMINATION: Ulceration is mild, Exudate is mild, Necrosis is mild, PMN is moderate, Edema is mild.

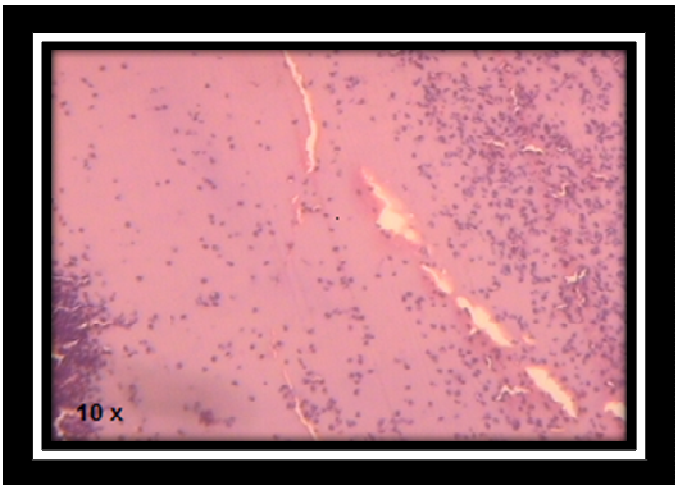

Figure 3.B - Standard
MICROSCOPIC EXAMINATION: Ulceration is mild, Necrosis is mild, PMN is mild, Edema is mild, Exudate is moderate.

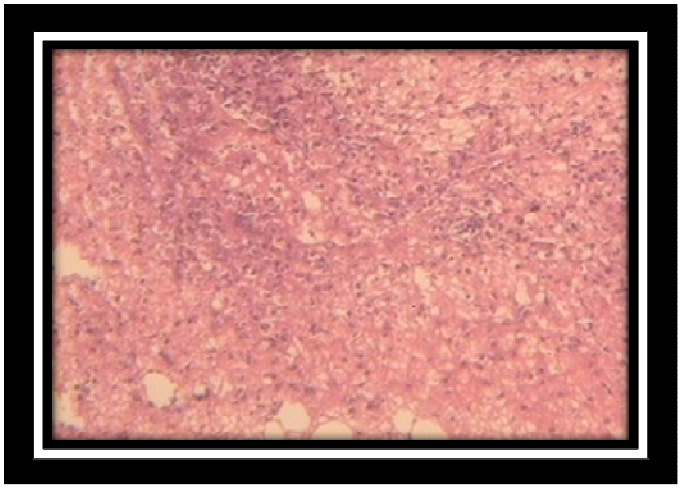

Figure 3.C - Sample 1
MICROSCOPIC EXAMINATION: Ulceration is mild, Necrosis is mild, No epithelialization, Congestion is mild, PMN is mild, Edema is mild.

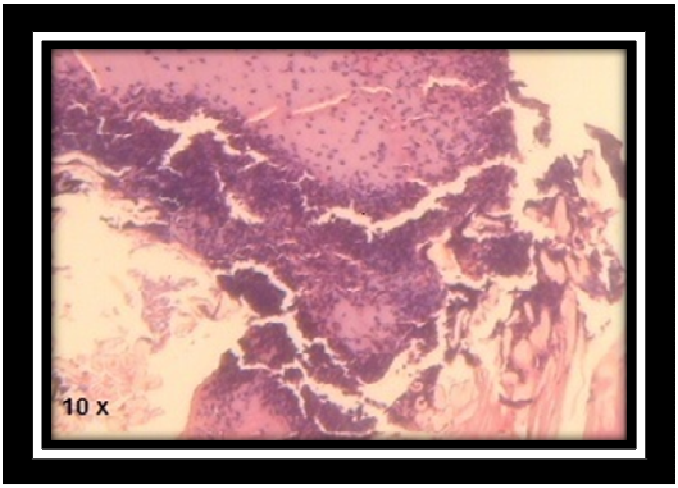

Figure 3.D - Sample 2
MICROSCOPIC EXAMINATION: Ulceration is moderate, Necrosis is moderate, PMN is moderate, Edema is moderate, Exudate is moderate.

2.2.2. 8th day

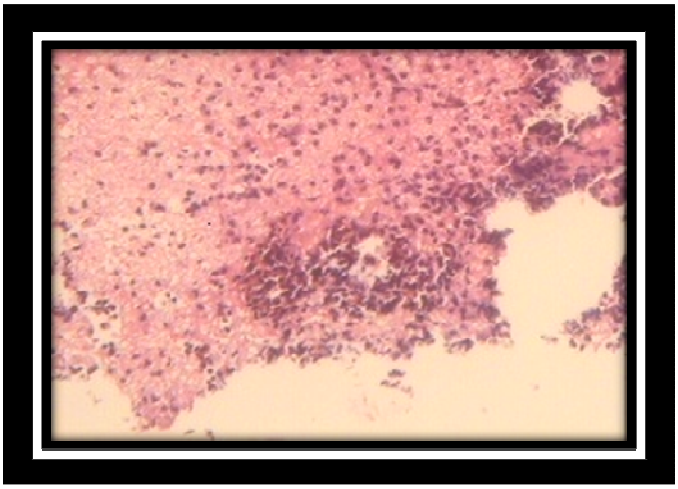

Figure 4.A - Control

MICROSCOPIC EXAMINATION: Necrosis is mild, PMN is moderate, Edema is moderate, Exudate is mild.

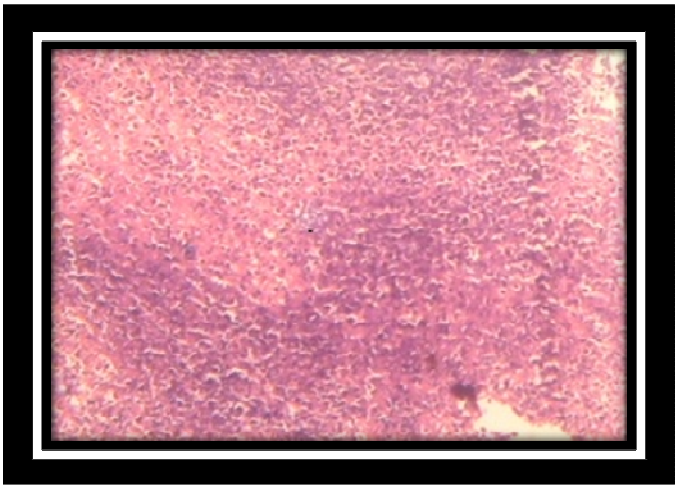

Figure 4.B - Standard

MICROSCOPIC EXAMINATION: Ulceration is mild, Necrosis is mild, PMN is mild, Edema is mild.

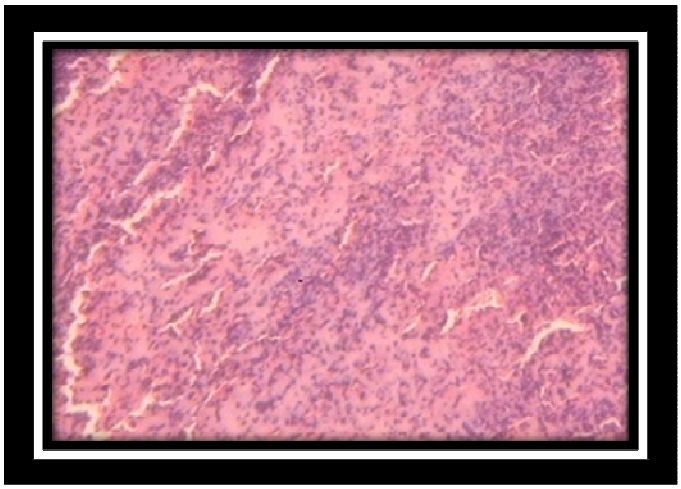

Figure 4.C - Sample 1

MICROSCOPIC EXAMINATION: Ulceration is mild, Necrosis is moderate, Congestion is moderate, PMN is moderate

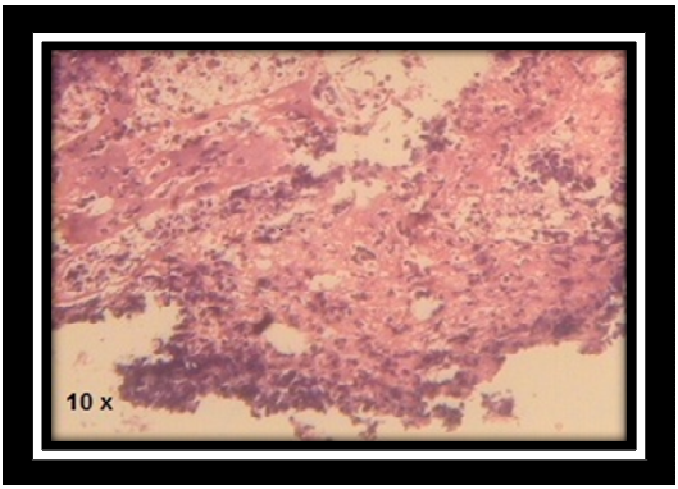

Figure 4.D -Sample 2
MICROSCOPIC EXAMINATION: Necrosis is moderate, PMN extensive, Edema is mild, Exudate is mild.

2.2.3. 12th day

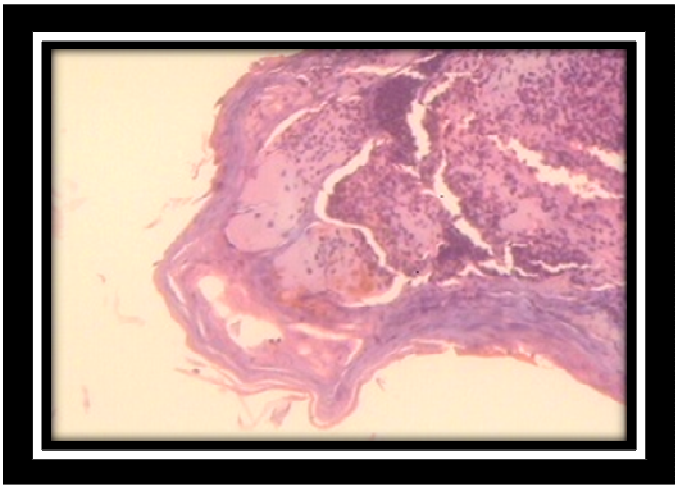

Figure 5.A - Control
MICROSCOPIC EXAMINATION: Epithelization is moderate, PMN is moderate, Exudate is moderate.

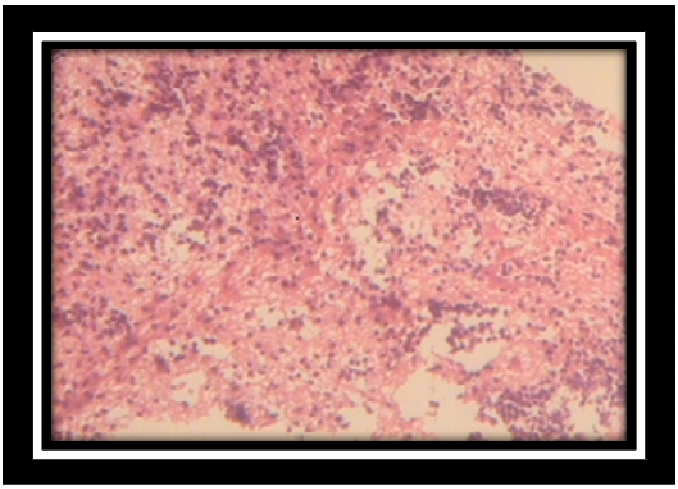

Figure 5.B – Standard
Necrosis is mild, PMN mild, Edema is absent, Exudate is mild.

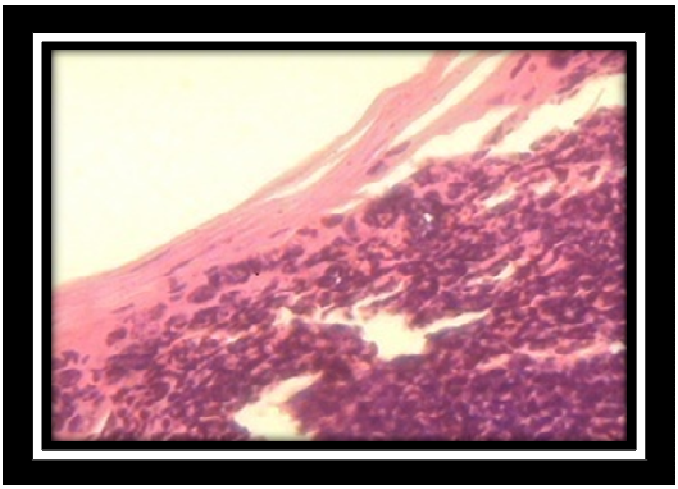

Figure 5.C - Sample 1

MICROSCOPIC EXAMINATION: Epithelialization is mild, Necrosis is mild, Congestion is mild, PMN is moderate, Vascularization is mild.

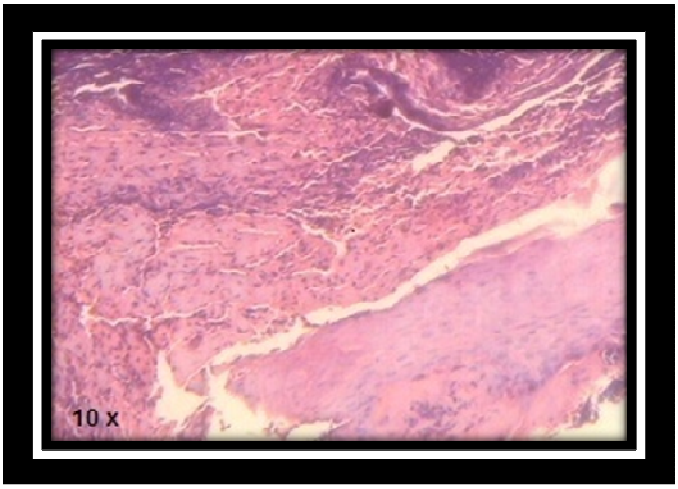

Figure 5.D - Sample 2

MICROSCOPIC EXAMINATION: Epithelialization is mild, Exudate is moderate, Vascularization is moderate, Fibroblasts is mild, PMN is moderate.

3. Conclusion

It is inferred from the study that leaves of Chromolaena odorata and Calotropis gigantea possess good wound healing activity when applied locally. The effectiveness is based on the dosage used topically. Further, isolation of active constituents from the extracts of the leaves may bring about the development of a new wound healing agent. These active constituents seem to be responsible for wound contraction and increased rate of epithelization. These plants also showed better results than the control and standard.

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