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Wound Healing Efficacy Assessment Of Trichosanthes Dioica Fruit Extract In Anaemic Wound Model

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Abstract: Wound healing, as a normal biological process in the human body, It is the process of repair that follows injury to the skin and other soft tissues. Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. In the condition of iron deficiency anemia the deficiency of iron take place since Iron is a vital co-factor for proteins and enzymes involved in energy metabolism, respiration, DNA synthesis, cell cycle arrest and apoptosis. Anaemic condition in animals induced by Haloperidol drug. Methanolic extract of Tricosanthese Doica fruit treated group with showed significant increase in hydroxyproline content compared to control group ($P < 0.01$).Tensile strength for Group treated with methanolic extract of T. Diocia (10%)on 10th day was found to be significant ($P < 0.01$) than control group. The treatments with methanolic extract of T. Diocia (10%) received gel base (10%) showed complete epithilaztion process within 13 days while while it was about 22 days in control rats.

Keywords: Wound healing, Trichosanthes Dioica fruit, anaemic wound model.

Introduction

Wound healing, as a normal biological process in the human body, It is the process of repair that follows injury to the skin and other soft tissues. Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure. Early studies investigated the effect of anemia on wound healing using a variety of experimental methodology to establish anemia or iron deficiency and focused on wound-strength rather than effect on macroscopic healing or re-epithelialization [(1-4)]

Trichosanthes dioica plant is a perennial, dioecious, commonly known as Pointed gourd or parval. It grows as a creepercultivated mainly as a vegetable, the fruit is, being consumed as a vegetablein Indian dietto belong to family Cucurbitaceae. Trichosanthes Dioica or parval Vineare thin in size having dark green cordate, rigid, leaves. The fruits of Trichosanthes dioica are green with white stripes. Fruits are oblong in shape and Size can vary. Juice of leaves of T. Dioica is used as tonic, febrifuge, and in subacute cases of enlargement of liver and spleen, in Charaka Samhita, leaves and fruits is used for treating alcoholism and

jaundice. Leaves are used in edema and alopecia. It is also used as antipyretic, diuretic, cardiotonic, and laxative.[5]

Pointed gourd (parval) iseasily digestible and rich source of protein and vitamin. It has pharmacological activities like lowering of blood sugar and serum triglycerides, cardiotonic and anthelmentic properties. The fruits are easily digestible and diuretic in nature, antiulcerous effects. The leaves of the plant have glycemic property. The juice of the leaf is useful to patches of alopecia areata. The seeds are reported to have anti-hyperglycemic properties. [6-10] Tricosanthese Doica is also reported having wound healing activity. In present work was undertaken to study the wound healing potential of Tricosanthese Doica in anaemic animal using excision and incision wound healing model.

Materials and methods

Plant material: Trichosanthes dioica fully grown fruits were purchased from local market of Bhopal. It was authenticated by Botanist Safia College, Bhopal, M.P. Thefruits of Trichosanthes dioicawere powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies and stored in air tight bottles.

Extraction of plant drug: The completely dried powdered fruits of Trichosanthes Dioica (200g) was defatted with non polar solvent petroleum ether were packed in soxhlet apparatus and extracted with petroleum ether till completion of extraction. The exhausted powdered fruits extracted with methanol till completion of extraction. The obtained extract was concentrated under reduced pressure using rotary evaporator to get methanol extract of Trichosanthes Dioica. The methanol exhausted fruit powder macerated with water, filtered and concentrated to get aqueous extract of Trichosanthes Dioica.

Preliminary phytochemical screening: Methanolic and aqueous extract of Trichosanthes Dioica was subjected to various qualitative tests for the identification of various plant constituents present in this species.[11-13]

Identification and determination of iron content in plant extract: Spectrophotometric method was performed with UV- VIS Spectrophotometer. Standard stock solutions of iron Fe ions were prepared by dissolving 10 mg of iron Fe (Sigma-Aldrich Co. LLC) in 100 ml deionized water in a volumetric flask (100 ml). The calibration solutions were prepared by pipetting volumes of 0.05, 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 2.50 ml, respectively of the stock standard solution into volumetric flasks (10 ml). Next, of volumes of 1.00 ml of 6M HCl and 1.4 mL of hydroquinone and 0.93 mL of o-phenanthroline solution were added to each volumetric flasks. The absorbance of each solution (working and analyzed solutions) was measured at absorption maximum of 481.0 nm using 10 mm quartz cuvette.[14-15]

Preparation of plant extractgel: The required quantity of preservative methyl paraben (50 mg), glycerine (5 ml) and polymer polyethylene glycol (1 ml) were dissolved in 25 ml of water in a beaker. All materials were stirred at high speed mechanical stirrer by using mechanical stirrer. The weighed quantity of Carbopol 934 (1 gm) and PVP (25 mg) and gelling agents triethanolamine (1 ml) were added slowly in previous liquid mixture during continuous stirring. The plant extract 5% and 10% methanolic and aqueous extract of Trichosanthes dioica fruit was added slowly into polymer gel base containing mixture during

stirring to attain gel structure. The prepared plat extract gel was finally transferred to aluminium collapsible tubes and labeled accordingly required. The marketed povidone formulation was used as reference drug for wound healing.

Pharmacological screening:

Animals: Wistar albino rats (180-220g) of either sex were used for experimental study.The animals were housed in cages at 25 ± 2ºC, awith 12 h light, and 12 h dark cycle. All the animals were acclimatized to laboratory environment for a week before the experiment. They were provided with free access to food and water ad libitum. The animals were cared and used in accordance with the CPCSEA guidelines and experimental protocols approved by institutional animal ethics committee (1196/PO/Re/S/08/CPCSEA).

Acute toxicity studies as per OECD guideline: Acute toxicity studies of methanol and aqueous extracts Trichosanthes dioica were performed in Albino rat dose levels of 50, 300 and 2000 mg/kg as per OECD guide lines. The treated animals did not exhibited any lethal effects or mortality throughout the test period following single oral administration at all selected dose levels of all extracts.

Inducing of Anaemic condition: The iron deficiency as anaemic condition was produced by inducing haloperidol (0.2 mg/kg body weight) drugthrough intraperitoneally in rats within 4 days. Iron deficiency anaemia was induced by haloperidol (0.2 mg/kg body weight) given intraperitoneally for 4 days, assigned as haloperidol control. On day 4, blood samples were collected from the retro-orbital plexus vein of rat eye in vials containing EDTA as anticoagulant and evaluated for haematological parameters (erythrocyte count, haemoglobin count and serum iron, Studies on haloperidol control control showed significant ($P < 0.001$) decrease in haematological parameters as compared to that of control animals which were administered vehicle only. haloperidol control animal showed lowering of the erythrocyte count, haemoglobin concentration lowering of serum iron and serum protein.[16-17]

Anaemic wound healing activity: The excision, incision and dead space wound models were used to evaluate the wound-healing activity of methanol and water extract of Herbal gel formulation. The wistar rats were divided into various groups, each group containing six animals, for excision and incision wound models. 5% and 10%extract containing formulated gel were applied topically to each animal once a day.

Group I: The animals of received gel base (control)

Group II: Treated group with a marketed povidone formulation

Group III: Methanolic extract of Trichosanthes dioica fruit (TDME5 %) gel

Group IV: Methanolic extract of Trichosanthes dioica fruit (TDME10 %) gel

Group V: Aqueous extract of Trichosanthes dioica fruit (TDWE5 %) gel

Group VI: Aqueous extract of Trichosanthes dioica fruit (TDWE10 %) gel

Excision wound model: The animals were divided into three groups with six in each were anaesthetized by open mask method with anesthetic ether before wound creation. The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 300 mm2 full thickness of skin from a predetermined shaved area. The wounds were left undressed to the open environment. The ointment base, standard drug ointment (povidone) and methanolic extract of TDR ointment (5%, w/w) was applied topically to the control group, standard group and treated group respectively, till the wound was completely healed. In this model, wound contraction and epithelialization period was monitored. Wound contraction was measured as percent contraction in each 2 days after wound formation. From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination. [(18)]

Incision wound model: In incision wound model**,** all the animals of each group were anaesthetized under light ether anesthesia. Two full thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from midline on each side of the depilated back of rat. After the incision was made the both edges of skin kept together and stitched with black silk surgical thread (no. 000) and a curved needle (≠11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then ointment base, standard ointment and extracts ointment were applied daily up to 10 days; when wounds were cured thoroughly the sutures were removed on the day 10 and tensile strength of cured wound skin was measured using tensiometer. [(19)]

Wound healing evaluation parameters:

Measurement of wound contraction: An excision wound margin was traced by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wounds was traced on a transparent paper in every 2 days, throughout the monitoring period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated. [(20)] The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 300 mm2, as 100%, by using the following formula as % wound contraction = initial wound size − specific day wound size initial wound size × 100

Epithelialization period: It was evaluated by noting the number of days required for the Escher to fall off from the wound surface exclusive of leaving a raw wound behind.

Measurement of tensile strength: The force required to open the healing action is known as tensile strength. It is used to measure the completeness of healing. It also indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. The sutures were removed on the 9th day after wounding and the tensile strength was measured on 10th day. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer, which is based on method of **Kuwano et al. (1994**) [(21)]In this method, wound-breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen.

Hydroxyproline estimation: Hydroxyproline is an uncommon amino acid present in the collagen fibres of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60–70 ◦C to constant weight and were hydrolysed in 6N HCl at 130 ◦C for 4 h in sealed glass tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 Mperchloric acid and color was developed with the help of Ehrlich reagent at 60 ◦C. The absorbance was measured at 557 nm using a uv spectrophotometer (Shimadzu, Japan). The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure l-hydroxyproline.

Results and Discussion:

Preliminary phytoprofiles screening of fruits of Trichosanthes Dioica: Extraction process of plant metabolites in the crude drugs. Extractive values are useful for the evaluation of nature of the active phytoconstituents present in the drug especially when the constituents of a drug cannot be readily estimated by any other means Petroleum ether (4.23 %), Methanol Extract (9.02 %) and Water Extract (10.18 %)**.** Methanol and aqueous Extracts of Trichosanthes dioica were obtained by continuous soxhlet were subjected to qualitative phytochemical tests to identify the presence of secondary metabolite (viz., alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. Aqueous extract obtained by maceration method are also subjected to qualitative phytochemical tests. Phytochemical screening of Trichosanthes dioica showed that Methanol extract showed the presence of carbohydrates, glycosides, alkaloids, tannins, flavanoids, protein and amino acids and saponins whereas Water extract showed the presence of carbohydrates, tannins, flavanoids, protein and amino acids and saponins.

Preliminary phytoprofiles screening of fruits of Trichosanthes Dioica: Extraction process of plant metabolites in the crude drugs. Extractive values are useful for the evaluation of nature of the active phytoconstituents present in the drug especially when the constituents of a drug cannot be readily estimated by any other means

Identification and determination of iron content in plant extract: Spectrophotometric method was performed with a UV- Visible Spectrometer (Shimadzu) for determination of iron content in extracts of Trichosanthes dioica**.** Before spectrophotometric analysis, intensity of color was increased by addition of potassium hydroquinone and o-phenanthroline solution. calibration curve of iron prepared in concentration range of 0.5 µg/ml to 2.5 µg/ml were found to be linear. The iron content in the plant extract was determined by UV visible spectrophotometer. The iron content in methanolic extract of Trichosanthes dioica was found 210.73±0.02mg/100g, whereas aqueous extract contain 175.73±0.14 mg/100g.

Anaemic wound healing activity:

Wound contraction: Group treated with methanolic extract of T. Diocia (10%) showed complete wound closer in 15 days, while it was about 24 days in control rats. The result concluded that methanolic extract

of of T. Diocia (10%) showed best effect than aqueous extract and significant with treated group, **(Table 1 and Figure 1-2)**

Epithelialization period: The epithelialization time was measured from the first day. The epithelialization time was found to be significantly ($P < 0.01$) reduced in Group treated with methanolic extract of T. Diocia (10%). The treatments with methanolic extract of T. Diocia (10%) received gel base (10%) showed complete epithilaztion process withinn 13 days while while it was about 22 days in control rats. **(Table 2 and Figure 3)**

Hydroxyproline content: Group treated with methanolic extract of T. Diocia (10%) showed significant increase in hydroxyproline content when compared to control group ($P < 0.01$). (Table 3 and Figure 4)

Tensile strength: Tensile strength for Group treated with methanolic extract of T. Diocia (10%) on 10th day was found to be significant ($P < 0.01$) than control group. **(Table 3 and Figure 5)**

Conclusion: Wound healing is the process of repair that follows injury to the skin and other soft tissues. Wounds exhibit impaired healing, have failed to progress through the normal stages of healing. Such wounds results pathologic inflammation due to delayed, incomplete, and healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or anaemic condition. Tricosanthese Doica is commonly found plant in India and used to treat various diseases. In present work Tricosanthese Doica fruit were selected for wound healing activity in anaemic animal using excision and incision model. Phytochemical screening of Trichosanthes dioica showed that Methanol extract showed the presence of carbohydrates, glycosides, alkaloids, tannins, flavanoids, protein and amino acids and saponins whereas Water extract showed the presence of carbohydrates, tannins, flavanoids, protein and amino acids and saponins. The iron content in methanolic extract of Trichosanthes dioica was found 210.73±0.02mg/100g, whereas aqueous extract contain 175.73±0.14 mg/100g.Methanolic extract of Tricosanthese Doica fruit showed significant wound healing property in both model. It exhibit be fast epithelization, wound contraction and better tensile strength.

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Table 1: Effect of plant extract gel formulation on % of wound contraction of excision anaemic wound models in rats

Group	0 Days	3 Days	6 Days	9 Days	12 Days	15 Days	18 Days	21 Days	24 Days
Group I	0	27.34	34.28	47.64	51.4	76.15	85.43	93.76	100
Group II	0	52.24	74.32	99.15	100				
Group III	0	19.12	39.14	58.21	65.11	71.02	91.02	100	
Group IV	0	34.21	51.24	72.61	83.45	100			
Group V	0	17.43	37.56	51.22	61.21	71.24	87.32	96.11	100
Group VI	0	27.12	47.24	68.21	78.21	95.14	100		

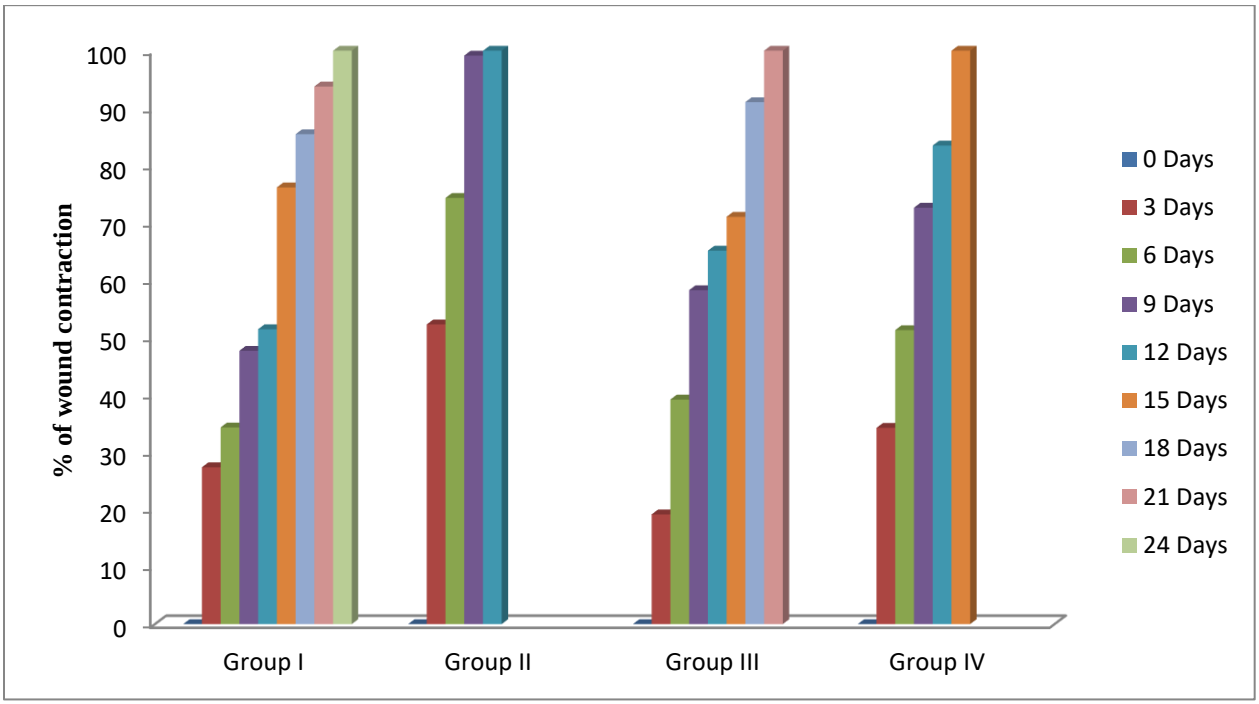

Fig. 1: Effect of methanolic extract of T. Diocia gel formulation on % of wound contraction of excision anaemic wound models in rats

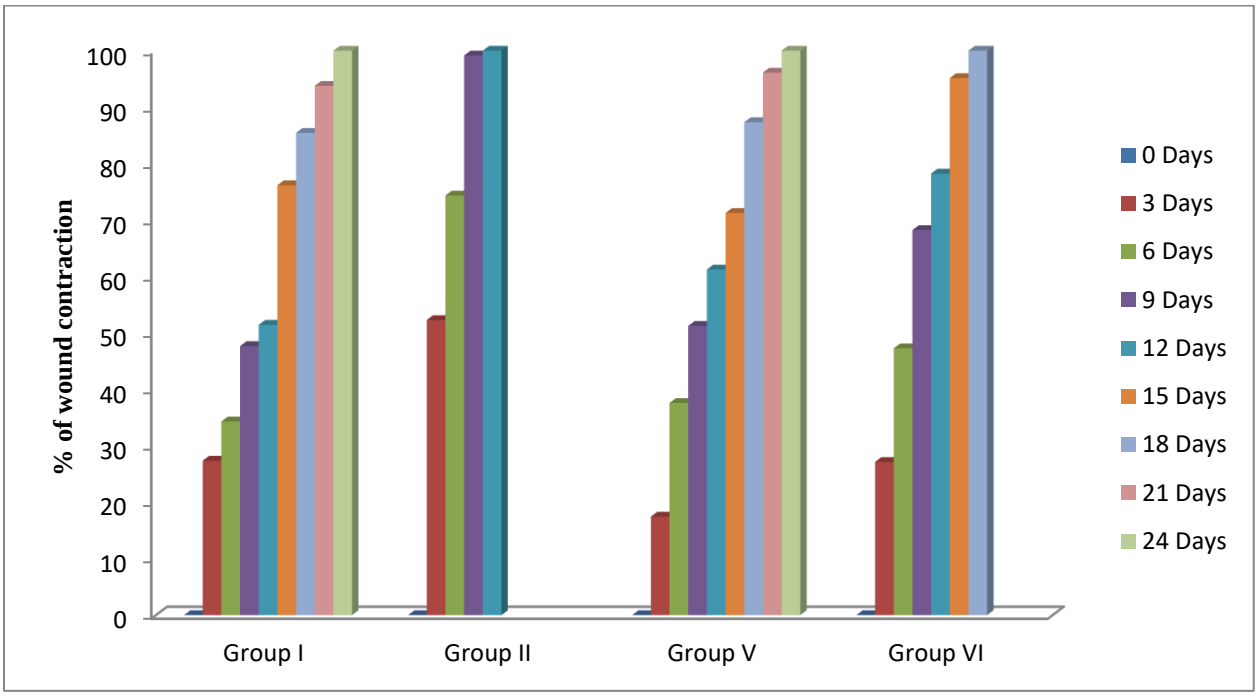

Fig. 2: Effect of aqueous extract of T. Diocia gel formulation on % of wound contraction of excision anaemic wound models in rats

Table 2: Effect of plant extract ointment on Epithelialization time of excision anaemic wound models in rats

Group	Epithelialization time (Days)
Group I	22
Group II	10
Group III	20
Group IV	13
Group V	21
Group VI	16

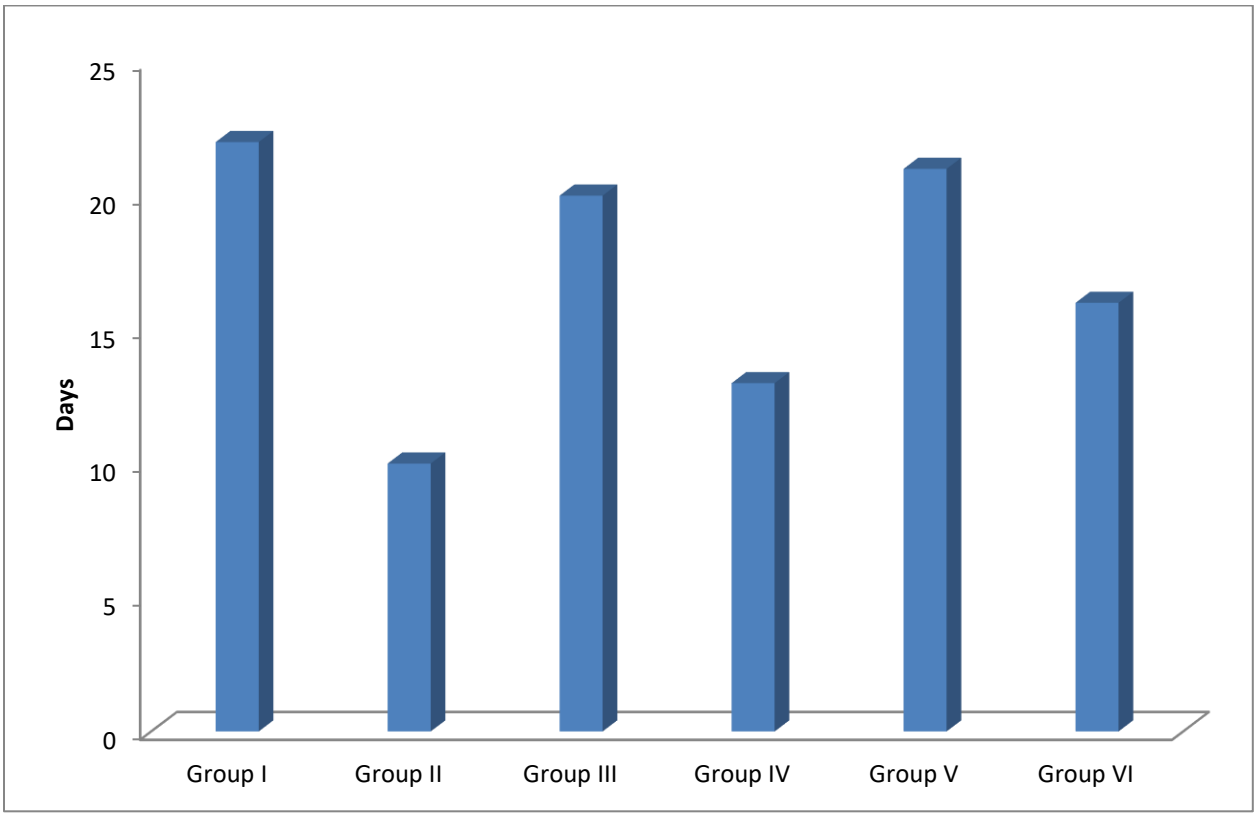

Fig. 3: Effect of methanolic extract and aqueous extract of T. Diocia gel formulation on Epithelialization time of excision anaemic wound models in rats

Table 3: Effect of plant extract gel fromulation on Hydroxyproline and Tensile strength of incision anaemic wound models in rats

Group	Hydroxyproline (mg/g tissue)	Tensile strength (g/mm2)
Group I	23.11 ± 0.001	391.21 ± 4.21

Group II	70.22 ± 0.002	595.04 ± 3.01
Group III	34.27 ± 0.001	449.11 ± 2.14
Group IV	58.22 ± 0.002	501.01 ± 3.51
Group V	24.73 ± 0.001	431.26 ± 2.02
Group VI	47.97 ± 0.003	469.27 ± 3.14

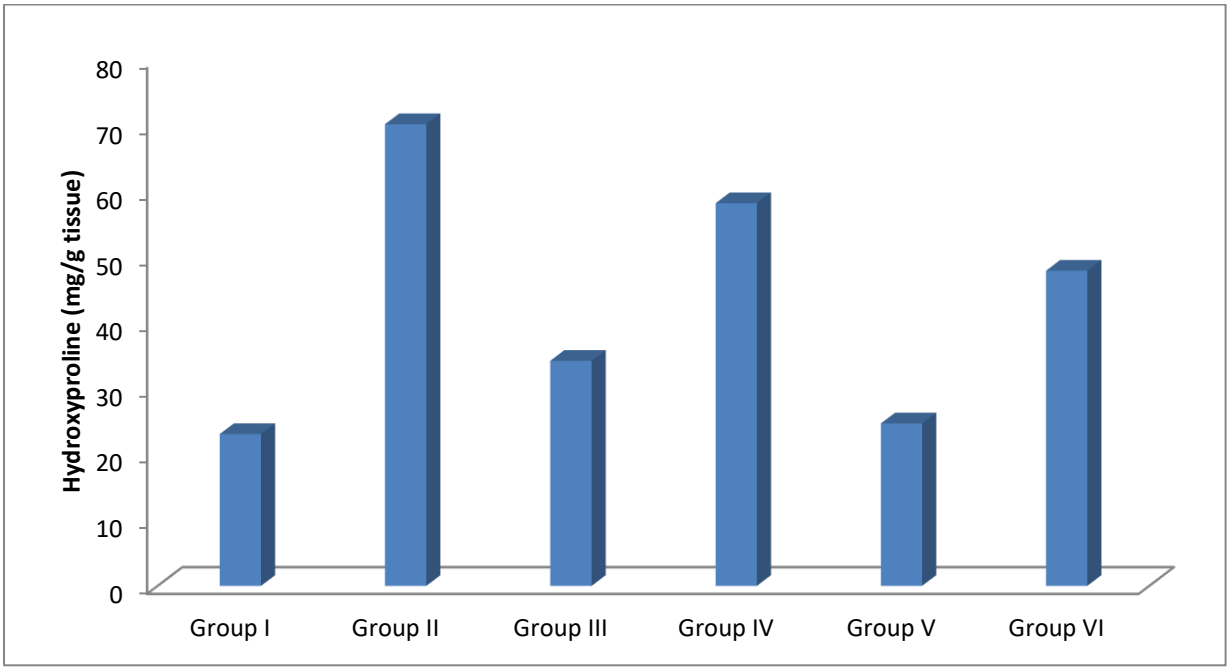

Fig. 4: Effect of methanolic extract and aqueous extract of T. Diocia gel formulation on Hydroxyproline on incision anaemic wound models in rats

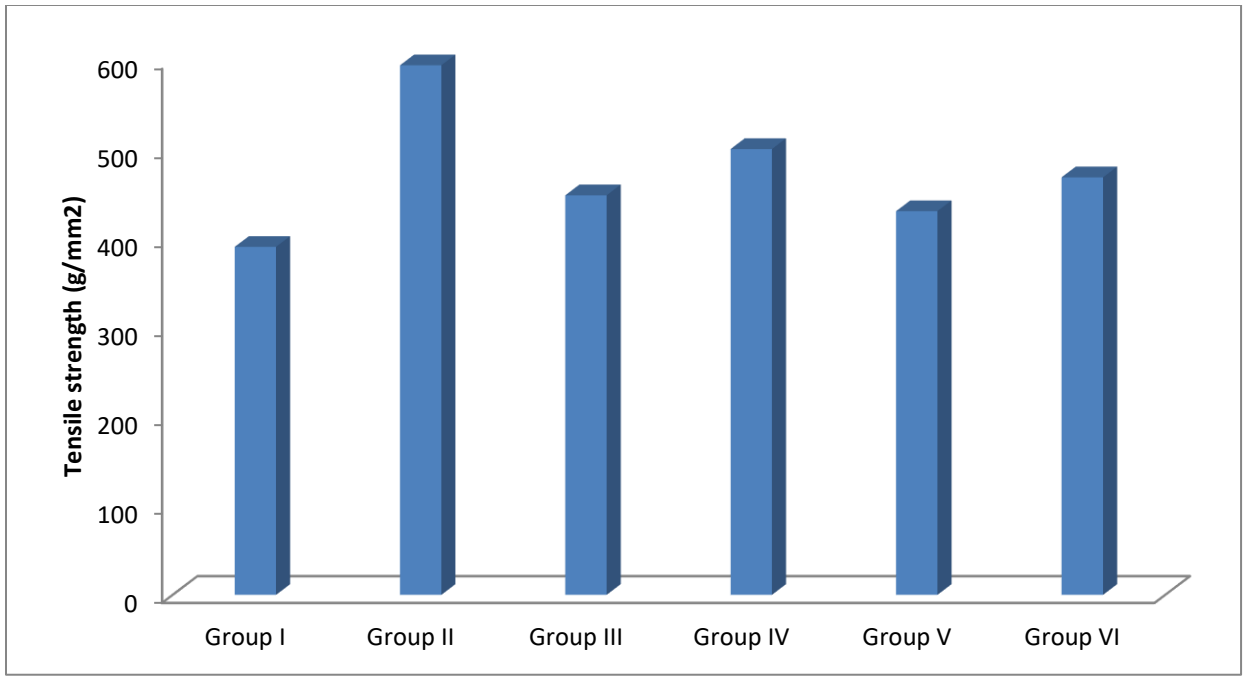

Fig. 5: Effect of methanolic extract and aqueous extract of T. Diocia gel formulation on Tensile strength on incision anaemic wound models in rats