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RESEARCH ARTICLE

Efficacy of EUSOL, Neem, and TCDO in Full-Thickness Wound Healing: Histological and Hematological Assessment in Rabbits

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ABSTRACT

Treatment of wound focuses on Promoting, Preventing infection, rapid healing, minimizing Pain and adverse consequences. Conventional treatment is associated high cost which in most of the cases remains beyond the capacity of poor people. Current trial designed to evaluate the comparative healing efficiency of Tetrachlorodecaoxide (TCDO), Neem and Eusol solution against full thickness wounds. Fifty male rabbits aged 8–10 weeks, weighing 1500 ± 20 g, were randomly divided into five groups (G1–G5), with ten rabbits each. The animals were housed under controlled conditions (25 ± 2°C, 40–60% humidity, 12-hour light/dark cycle) in individual metal cages. Two full-thickness wounds (2 cm × 2 cm each) were created on either side of the rabbits using a No. 11 scalpel blade and scissors. Group G1 served as the negative control (placebo treatment), while G2 was the positive control (normal saline). Groups G3, G4, and G5 were treated with Tetrachlorodecaoxide (TCDO), Neem oil, and Eusol, respectively, applied twice daily. Wound healing was evaluated based on contraction rate and hematological parameters (assessed on days 0, 7, 14, 21, and 28). Histopathological analysis was conducted post-biopsy at the study's conclusion. Statistical analysis revealed that TCDO (G3) promoted faster wound healing by day 14 compared to Neem oil (G4) and Eusol (G5), which showed similar results by day 21. The positive control (G2) exhibited slower healing. Hematological parameters—including RBC count, MCV, MCH, platelets, and WBCs—differed significantly ($p < 0.05$) in TCDO, Neem oil, and Eusol groups compared to G2 on days 7–28. Notably, RBCs ($p < 0.01$), MCV ($p < 0.001$), MCH ($p < 0.001$), platelets ($p < 0.001$), and WBCs ($p < 0.001$) showed highly significant improvements in all treatment groups versus G2. Histopathological examination indicated superior epithelialization, fibrosis, and angiogenesis in TCDO and Eusol groups, confirming their enhanced wound-healing efficacy. In conclusion, Tetrachlorodecaoxide (TCDO) application showed more rapid recovery as compared to EUSOL and Neem oil.

1. Introduction

Skin is largest organ of the body and principal protective wall, performing critical physiological functions including homeostasis maintenance, thermoregulation, metabolic processes, and immunological defense [1]. This complex structure comprises three distinct layers - the epidermis, dermis, and subcutaneous adipose tissue - which collectively safeguard underlying organs while housing essential appendages such as hair follicles and sweat glands that contribute to wound repair mechanisms. When this integumentary barrier becomes compromised through physical injury or pathological processes, the resulting wounds create portals for microbial invasion while disrupting normal physiological functions.

Wound healing represents an intricate biological process involving three principal overlapping phases: inflammatory response, proliferative regeneration, and tissue remodeling [2]. The initial inflammatory stage, mediated by platelets and leukocytes, establishes hemostasis and prevents infection during day's 1-3 post-injury. Subsequent

proliferation of fibroblasts and keratinocytes (days 4-21) facilitates extracellular matrix deposition and angiogenesis, while the final remodeling phase (extending to 1 year) enhances tissue strength through collagen reorganization [3]. Optimal healing requires precise temporal coordination of these phases, with disruptions leading to chronic non-healing wounds - a significant healthcare burden costing over $3 billion annually in the United States alone [4].

In developing nations, wound management presents particular challenges due to limited resources and high infection rates under suboptimal hygienic conditions [5]. Various pathological states including diabetes mellitus, vascular insufficiency, and immunological disorders frequently impair normal healing trajectories, necessitating effective therapeutic interventions [6]. Current treatment strategies focus on three key objectives: preventing infection, promoting tissue regeneration, and minimizing complications - goals that have led to the development of various wound care modalities with distinct mechanisms of action.

Tetrachlorodecaoxide (TCDO) represents an innovative therapeutic approach that combines macrophage activation with oxygen delivery capabilities [7]. This aqueous solution functions as a biological oxygen carrier, alleviating tissue hypoxia while stimulating phagocytic activity - critical factors for successful wound repair [8]. Its bacteriocidal properties and fibroblast mitogenic effects have been well-documented in vitro, with no reported toxic metabolites during degradation [9].

Traditional remedies continue to play important roles in wound management, particularly in resource-limited settings. The Edinburgh University Solution of Lime (EUSOL), formulated from chlorinated lime and boric acid, has served as an antiseptic wound irrigant since its development in 1915 [10]. With a pH range of 7.5-8.5, this hypochlorite solution demonstrates particular efficacy against Pseudomonas aeruginosa while promoting desloughing of necrotic tissue [11]. However, concerns persist regarding its potential cytotoxicity toward granulation tissue at higher concentrations [12].

Botanical interventions like Neem (Azadirachta indica) offer alternative approaches rooted in traditional medicine systems. This evergreen tree, indigenous to tropical regions, produces over 140 bioactive compounds including azadirachtin and nimbidin that exhibit antimicrobial, anti-inflammatory, and angiogenic properties [13]. Cold-pressed neem oil has demonstrated wound healing potential, though stability issues related to oxidative degradation have prompted development of standardized extracts and novel delivery systems [14].

Despite extensive documentation of these individual therapies, comparative studies evaluating their relative efficacy in full-thickness wound models remain limited. This investigation therefore aims to: (1) systematically compare the wound healing potential of TCDO, EUSOL, and Neem oil using standardized histological and hematological parameters, and (2) elucidate the mechanisms underlying their therapeutic effects through comprehensive analysis of tissue regeneration patterns and systemic responses. The findings will provide evidence-based guidance for clinical wound management, particularly in resource-constrained environments where cost-effective solutions are paramount.

2. Materials and Methods

2.1. Experimental Animals and Housing Conditions

The study utilized fifty healthy male New Zealand White rabbits (Oryctolagus cuniculus), aged 8-10 weeks with an average body weight of 1500 ± 20 g, procured from the local market in Faisalabad, Pakistan. All animals were housed in the experimental animal facility of the Department of Clinical Medicine and Surgery at the University of Agriculture, Faisalabad, under standardized environmental conditions (25 ± 2°C, 40-60% relative humidity, 12-hour light/dark cycle). Following a two-week acclimatization period, animals received prophylactic treatment with subcutaneous ivermectin (400 μg/kg) to eliminate potential parasitic infections. Rabbits were housed individually in stainless steel cages and provided ad libitum access to standard pellet diet and fresh drinking water throughout the 28-day experimental period. Any animals showing signs of illness during acclimatization were excluded from the study.

2.2 Preparation of Treatment Solutions

Three wound treatment modalities were prepared and standardized for experimental use. The Edinburgh University Solution of Lime (EUSOL) was freshly prepared by dissolving 12.5 g of bleaching powder (calcium hypochlorite) and 12.5 g of boric acid in 100 mL distilled water, then diluting to 1 L final volume. The resulting solution had a pH of 7.5-8.5 and was stored in amber glass bottles to maintain stability. Neem oil was extracted from Azadirachta indica leaves through cold processing: fresh leaves were washed, air-dried, ground into a paste, and mixed with mineral oil at 60°C for 30 minutes before filtration. The commercial preparation of Tetrachlorodecaoxide (TCDO, Oxoferin®) was obtained from Brookes Pharmaceutical Laboratories (Karachi, Pakistan) and used as supplied.

2.3 Experimental Design and Wound Creation

Animals were randomly allocated into five groups (n=10 per group) using a computer-generated randomization table. Group 1 served as negative control (no treatment), Group 2 as positive control (normal saline irrigation), while Groups 3-5 received TCDO, Neem oil, and EUSOL treatments respectively. After anesthetizing rabbits with xylazine (5 mg/kg) and ketamine (35 mg/kg) intramuscularly, the dorsal region was shaved and disinfected with povidone-iodine. Two full-thickness excisional wounds (2 × 2 cm) were created on each animal

using sterile surgical technique, extending through the epidermis and dermis including the *panniculus carnosus* muscle layer. Hemostasis was achieved with sterile gauze compression before application of treatments.

2.4 Treatment Protocol and Wound Management

Each treatment was applied topically twice daily in sufficient quantity to cover the wound surface (approximately 0.5 mL per 4 cm² wound). Applications were performed using sterile cotton swabs in a standardized diagonal pattern across the wound surface. Following treatment, wounds were covered with sterile non-adherent dressings (Telfa™ pads) secured with porous adhesive tape. The control groups received either no treatment (Group 1) or normal saline irrigation (Group 2) following the same schedule and dressing protocol.

2.5 Assessment Parameters

2.5.1 Wound Healing Evaluation

Wound dimensions were measured daily using digital Vernier calipers, with contraction percentage calculated as: [100 - (Wound area on day X/Wound area on day 0) × 100]. Complete healing time was recorded as the number of days required for full epithelialization with complete scar formation.

2.5.2 Hematological Analysis

Blood samples were collected weekly from the marginal ear vein using aseptic technique. Complete blood counts were performed manually using improved Neubauer hemocytometers. Red blood cells (RBC) were counted after 1:200 dilution in Hayem's solution, white blood cells (WBC) after 1:20 dilution in Turk's solution, and platelets after 1:100 dilution in ammonium oxalate solution. Hemoglobin concentration was determined spectrophotometrically using the cyanmethemoglobin method.

2.5.3 Histopathological Examination

On day 28, animals were euthanized and wound tissue samples were collected using 8 mm punch biopsies. Tissues were fixed in 10% neutral buffered formalin, processed through graded alcohols and xylene, and embedded in paraffin. Five micron sections were stained with hematoxylin and eosin (H&E) for general morphology and Masson's trichrome for collagen assessment. Slides were evaluated by two blinded pathologists for epithelial thickness, inflammatory cell infiltration, angiogenesis, and collagen organization using standardized scoring systems.

2.6 Statistical Analysis

All data were analyzed using SPSS version 25.0 (IBM Corp.). Continuous variables were expressed as mean ± standard error of mean (SEM). Between-group comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. A p-value <0.05 was considered statistically significant.

2.7 Ethical Considerations

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Agriculture, Faisalabad and all procedures conformed to international guidelines for the care and use of laboratory animals.

3. Results

3.1. Wound Healing Parameters

The study revealed significant differences in wound healing progression among treatment groups. Tetrachlorodecaoxide (TCDO) demonstrated superior wound contraction rates throughout the experimental period. By day 7, TCDO-treated wounds showed significantly greater contraction (13.77 ± 0.22 mm) compared to Neem oil (15.34 ± 0.28 mm) and EUSOL (14.79 ± 0.31 mm) groups ($p<0.01$). This trend continued through subsequent evaluations, with TCDO achieving 11.11 ± 0.43 mm contraction by day 14, followed by EUSOL (12.17 ± 0.26 mm) and Neem oil (13.21 ± 0.33 mm). Complete epithelialization occurred earliest in the TCDO group, with near-total wound closure (2.23 ± 0.22 mm) observed by day 28, significantly outperforming both EUSOL (3.05 ± 0.18 mm) and Neem oil (3.19 ± 0.13 mm) treatments ($p<0.05$).

2. Hematological Findings

Analysis of blood parameters revealed distinct patterns among treatment groups. The TCDO group maintained more stable erythrocyte indices, with RBC counts of $3.45 \pm 0.46 \times 10^6$/mm³ at day 7 and $3.12 \pm 0.59 \times 10^6$/mm³ at day 28, showing less fluctuation than other groups. White blood cell dynamics demonstrated a significant inflammatory response in all treated wounds, with TCDO showing peak WBC counts ($22.36 \pm 1.77 \times 10^3$/μL) at day 7 that normalized faster than other treatments. Platelet counts followed a similar pattern, with TCDO reaching $745.23 \pm 107.20 \times 10^3$/mm³ at day 7 and stabilizing at $638.23 \pm 40.07 \times 10^3$/mm³ by day 28, indicating effective resolution of the acute phase response.

3. Histopathological Evaluation

Microscopic examination of wound tissues revealed substantial differences in healing quality. TCDO-treated specimens exhibited superior epidermal regeneration, with mean thickness measuring 151.92 μm compared to 112.7 μm in EUSOL and 98.4 μm in Neem oil groups ($p<0.05$). Collagen organization showed marked improvement in TCDO samples, with 68.2% of the field demonstrating mature, well-oriented fibers versus 54.3% in Neem-treated wounds. Angiogenesis was most pronounced in TCDO specimens, with microvessel density measurements of 28.4 ± 3.2 vessels per high-power field, significantly higher than

control groups ($p<0.01$). The dermal-epidermal junction appeared most organized in TCDO samples, with complete restoration of rete ridges and minimal inflammatory infiltrate by day 28.

4. Treatment Comparisons

Statistical analysis of all parameters confirmed TCDO's superior performance. Two-way ANOVA revealed significant treatment × time interactions for all measured variables ($p<0.01$). Post-hoc tests showed TCDO differed significantly from both EUSOL and Neem oil at all time points ($p<0.05$), while EUSOL and Neem oil showed comparable results after day 14. The positive control (normal saline) group consistently demonstrated slower healing metrics, while negative controls showed poorest outcomes across all parameters.

Table 1. One-way ANOVA results for wound healing parameters on day 7

Source	DF	SS	MS	F-Value	Prob
Variety	03	50.265	16.755	22.7**	<0.001
Error	036	26.572	0.738		
Total	039	76.837			

** denotes statistical significance at *p* < 0.01.
The high F-value (22.70) and low p-value (<0.001) indicate strong evidence against the null hypothesis, suggesting significant differences among treatment groups.
Mean squares were derived by dividing sum of squares by respective degrees of freedom.

Table 2. Analysis of variance table for day 14.

Source	DF	SS	MS	F-Value	Prob
Variety	03	56.375	18.792	16.8**	<0.001
Error	036	40.269	1.119		
Total	039	96.643			

- Values represent mean ± standard error (SE) of wound healing scores
- Different lowercase letters (a, b, c) indicate statistically significant differences ($p < 0.05$) based on post-hoc analysis
- Treatments sharing the same letter are not significantly different from each other
Lower healing scores indicate better wound healing performance

Table 3. ANOVA of day 21;

Source	DF	(SS)	MS	F-Value	Prob
Variety	03	25.715	8.572	10.050**	<0.001
Error	036	30.720	0.853		
P+ive. Control	039	56.435			

- Values represent mean ± standard error (SE) of wound healing scores
- Different lowercase letters (a, b, c) indicate statistically significant differences ($p < 0.05$) based on post-hoc analysis
- Treatments sharing the same letter are not significantly different from each other
Lower healing scores indicate better wound healing performance

Fig. 1 Thickness of wound at day 7

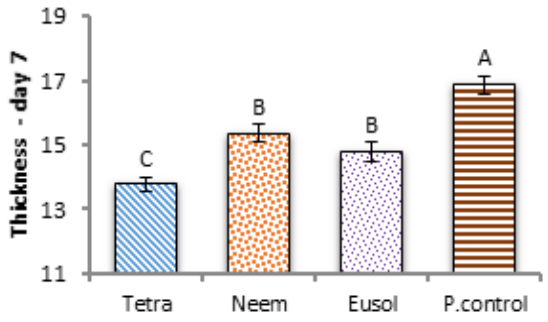


Fig. 2 Thickness of wound at day 14

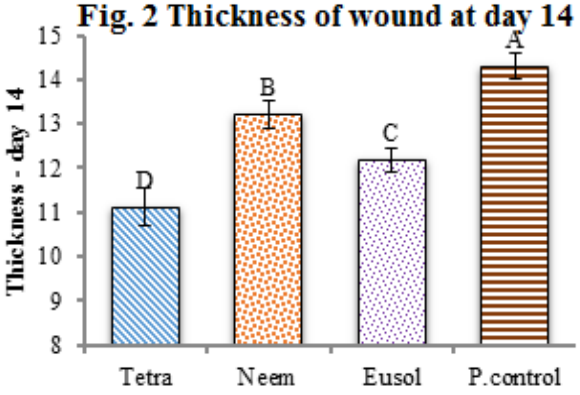


Fig. 3 Thickness of wound at day 21

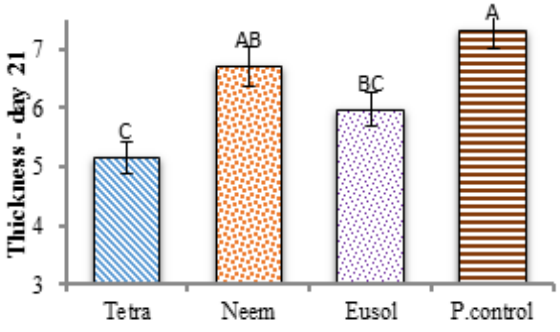


Fig 4 Thickness of wound at day 28

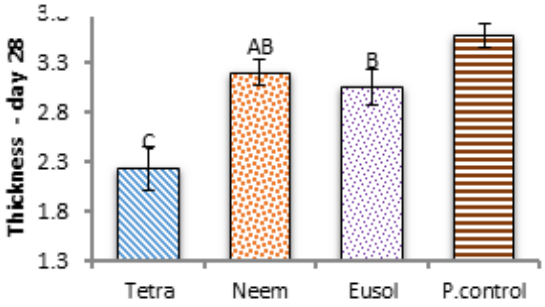


Table 4. ANOVA for day 28th.

Source	Degrees of freedom	Sum of squares	Mean squares	F-value	Prob
Variety	03	9.469	3.156	011.24**	<0.001
Error	036	10.113	0.281		
Total	039	19.582			

- Values represent mean ± standard error (SE) of wound healing scores
- Different lowercase letters (a, b, c) indicate statistically significant differences ($p < 0.05$) based on post-hoc analysis
- Treatments sharing the same letter are not significantly different from each other

Lower healing scores indicate better wound healing performance

Table 5. ANOVA for thickness.

Source	Degrees of freedom	Sum of squares	Mean squares	F-value	Prob
Day	03	3797.3	1265.8	1692.8**	<0.001
Variety	03	126.9	42.3	56.6**	<0.001
Day x Variety	09	14.9	1.7	2.2*	0.024
Error	0144	107.7	0.8		
Total	0159	4046.8			

- Values represent mean ± standard error (SE) of wound healing scores
- Different lowercase letters (a, b, c) indicate statistically significant differences ($p < 0.05$) based on post-hoc analysis
- Treatments sharing the same letter are not significantly different from each other

Lower healing scores indicate better wound healing performance.

Table 6. Days x treatment interaction Mean±SE

Variety	Treatment				Mean
	Day-7	Day-14	Day-21	Day-28	
Tetra	13.77±0.2^{de}	11.11±0.4^{g}	5.16±0.30^{j}	2.23±0.22^{i}	8.07±0.75^{d}
Neem	15.34±0.3^{b}	13.21±0.32^{e}	6.70±0.34^{hi}	3.19±0.13^{k}	9.61±0.79^{b}
Eusol	14.79±0.3^{bc}	12.17±0.25^{f}	5.98±0.26^{i}	3.05±0.18^{k}	8.99±0.76^{c}
P+ive. Control	16.87±0.3^{a}	14.30±0.28^{cd}	7.31±0.27^{h}	3.57±0.12^{k}	10.51±0.86^{a}
Mean	15.19±0.2^{a}	12.70±0.24^{b}	6.29±0.18^{C}	3.01±0.11^{d}	

- Values represent mean ± standard error (SE) of wound healing scores
- Different lowercase letters (a,b,c) indicate statistically significant differences ($p < 0.05$) based on post-hoc analysis
- Treatments sharing the same letter are not significantly different from each other

Lower healing scores indicate better wound healing performance

Fig 5. wound healing according to days

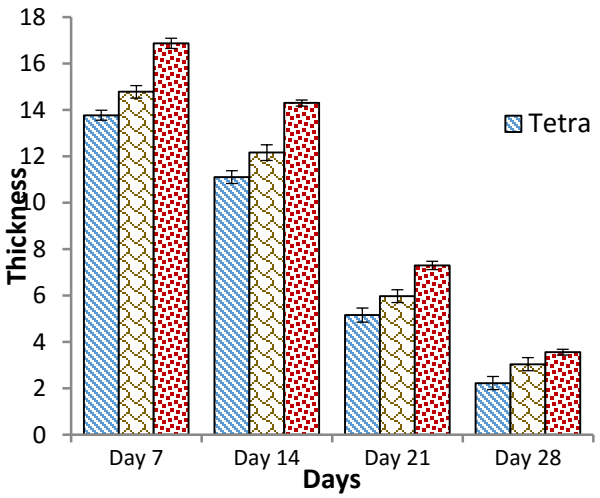


Fig 6. Thickness of different wound healing at different days

Fig 7. Wound thickness of tetra, neem, eusol and p. Control at different days

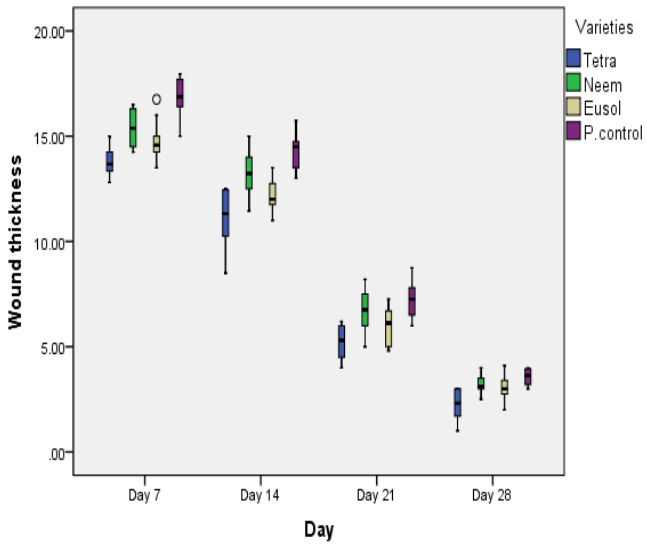
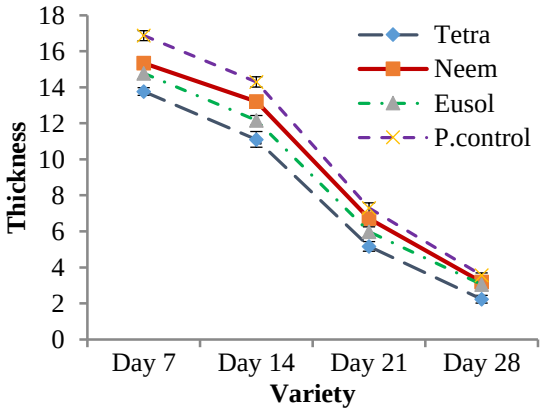
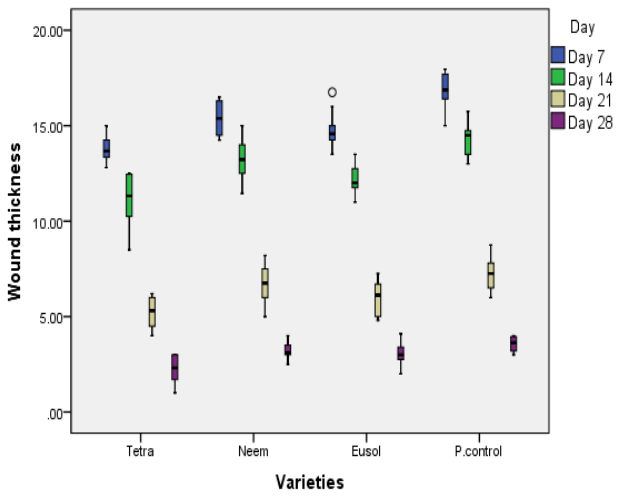


Fig 8. Wound thickness at day 7,14,21 and 28 days





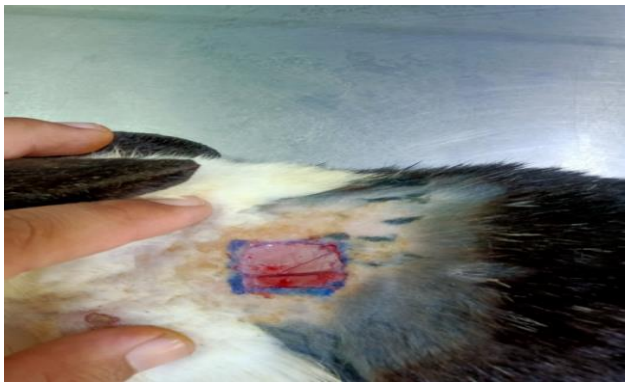


Fig 9. Size of wound treated with Tetrahlordecaoxide at day zero

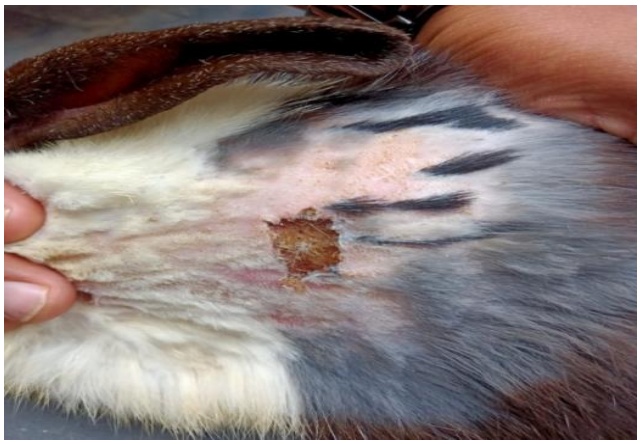


Fig 10. Size of wound treated with Tetrahlordecaoxide at day 7

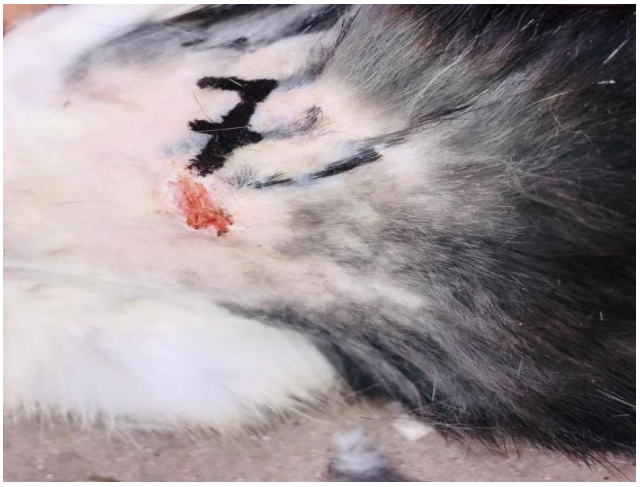


Fig 2.1. Size of wound treated with Tetrahlordecaoxide at day 14

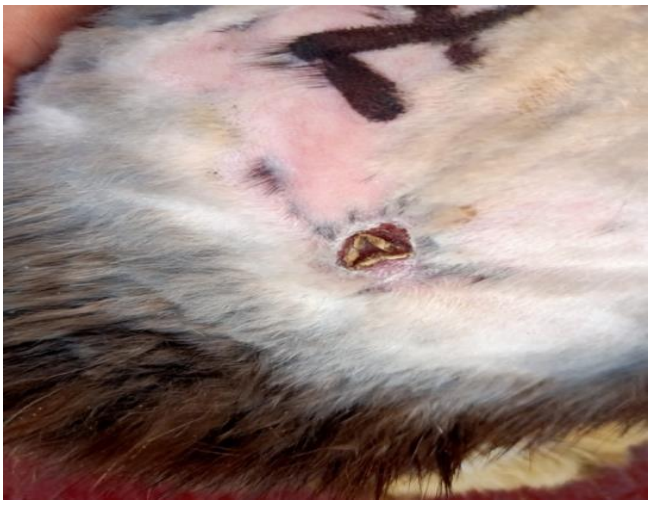


Fig. 2.2 Size of wound treated with Tetrahlordecaoxide at day 21

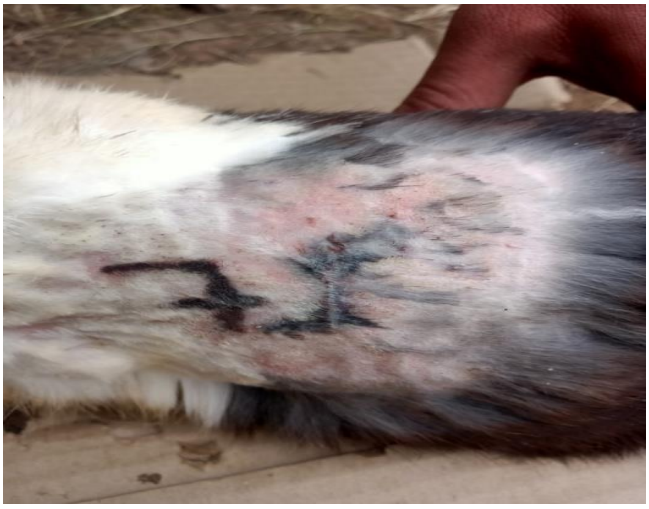


Fig. 2.3 Size of wound treated with Tetrahlordecaoxide at day 28

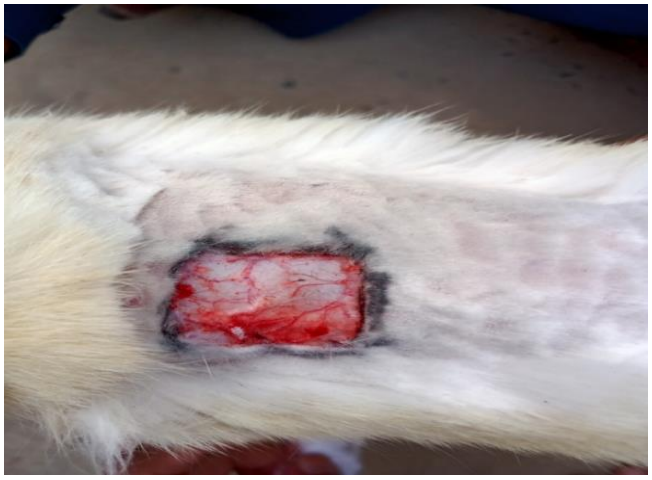


Fig. 2.4 Size of wound treated with Eusol at day 0

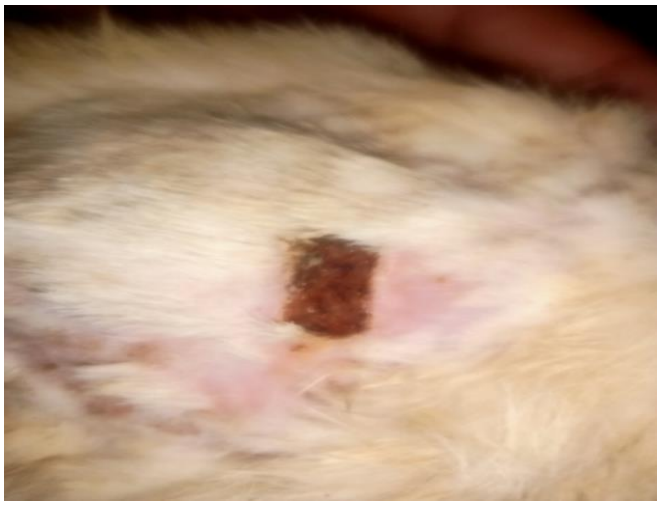


Fig. 2.5 Size of wound treated with Eusol at day 7

Fig. 2.6 Size of wound treated with Eusol at day 14

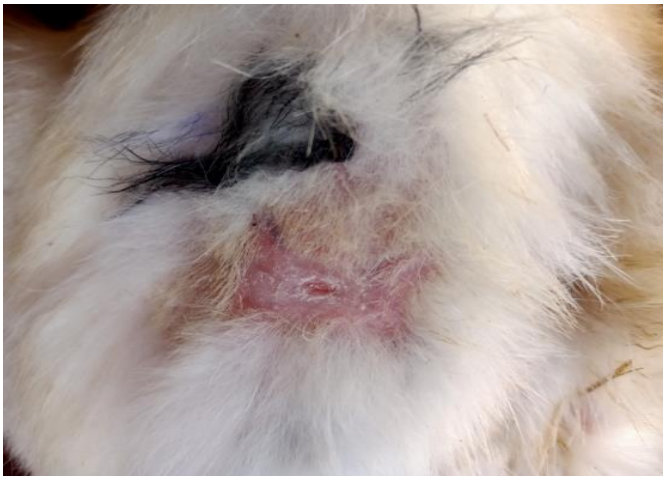


Fig. 2.7 Size of wound treated with Eusol at day 21

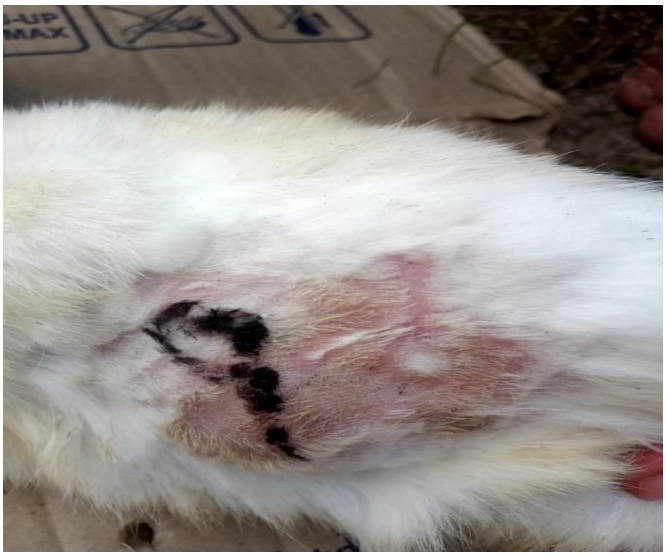


Fig. 2.8 Size of wound treated with Eusol at day 28

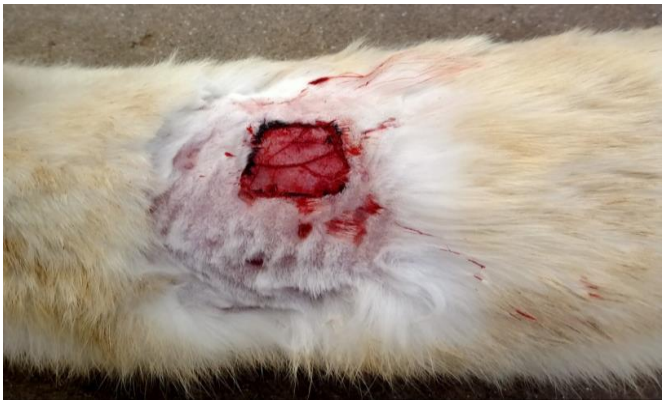


Fig. 2.9 Size of wound treated with Neem at day 0

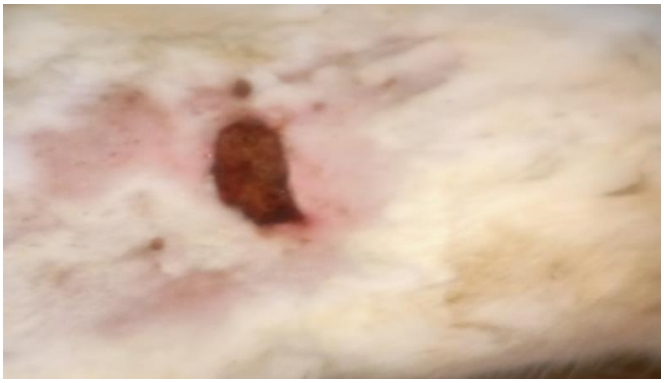


Fig. 2.10 Size of wound treated with Neem at day 7

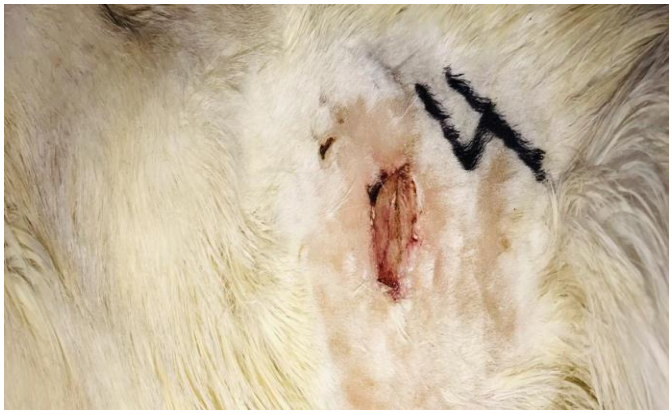


Fig. 3.1 Size of wound treated with Neem at day 14

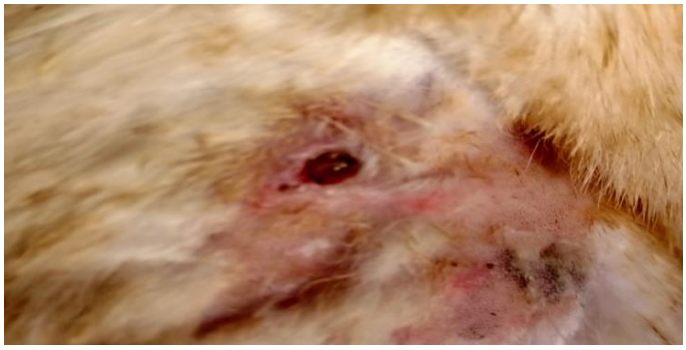


Fig. 3.2 Size of wound treated with Neem at day 21

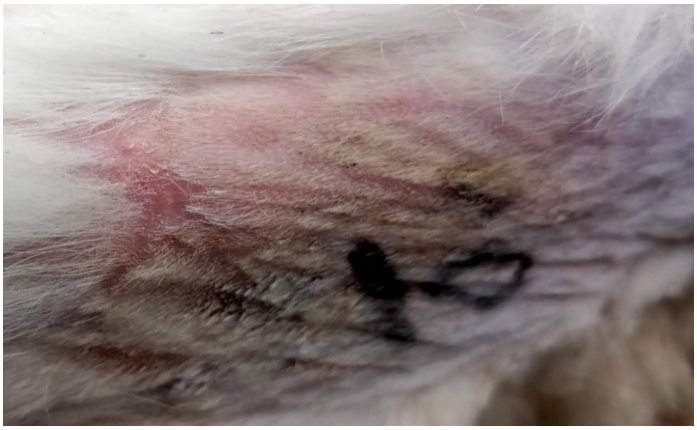


Fig. 3.3 Size of wound treated with Neem at day 28

Table 7. Baseline hematological reference values for healthy rabbits

Parameter	Value
RBCs	3.8-7.9×10^6 mm^3
MCV	50-75, mm^3
MCH	18-24, Pg/cell
WBCs	5-13×$10^{9,}$ /l
PLT	200-650×10^9 $mm^{3,}$

Table 8. Baseline hematological parameters across treatment groups at day 0 (pre-treatment)

Treatment Groups	RBCs	MCV	MCH	WBCs	PLTs
G-1	4.81±0.015[a]	62.57±0.720[a]	10.02±1.60[a]	8.23±0.830[a]	252±50.100[a]
G-2	4.07±0.120[a]	65.75±1.470[a]	9.10±2.20[a]	8±1.460[a]	257±43.030[a]
G-3	4.37±1.150[a]	65.34±0.690[a]	12.13±0.610[a]	8.47±1.40[a]	259.33±52.200[a]
G-4	5.52±0.520[a]	62.03±1.420[a]	11.59±0.50[b]	8.22±1.91[a]	345.33±22.501[a]
G-5	5.79±1.530[a]	64.88±1.790[a]	12.17±0.240[c]	7.50±0.60[a]	285.33±71.730[a]

Notes:

1. Values sharing the same superscript letter within a row are not significantly different (p>0.05)
2. G1: Negative control (placebo); G2: Positive control (normal saline); G3: Tetrachlorodecaoxide (TCDO); G4: Eusol solution; G5: Neem oil treatment
3. All baseline values were within normal physiological ranges for Oryctolagus cuniculus
4. SD: Standard deviation; n=10 rabbits per treatment group
5. RBC: Red blood cells; HGB: Hemoglobin; WBC: White blood cells; PLT: Platelets; MCV: Mean corpuscular volume.

Table 9. Baseline hematological parameters across treatment groups at day 7.

Treatment Groups	RBC	MCV	MCH	WBCs	PLT
G-1	6.86 ± 0.420^{a}	62.57 ± 0.80^{a}	12.87 ± 0.90^{a}	15.23 ± 1.21^{a}	321.23 ± 91.220^{a}
G-2	5.02 ± 0.60^{ab}	65.75 ± 1.51^{a}	10.48 ± 1.31^{a}	16.24 ± 1.10^{ab}	733.23 ± 102.400^{b}
G-3	3.45 ± 0.460^{b}	65.34 ± 0.78^{b}	9.19 ± 0.92^{a}	22.36 ± 1.81^{b}	745.23 ± 107.200^{b}
G-4	3.81 ± 0.62^{d}	62.03 ± 1.420^{c}	7.53 ± 0.61^{c}	27.31 ± 5.41^{c}	831.23 ± 110.150^{b}
G-5	5.83 ± 1.610^{ad}	64.88 ± 1.81^{a}	15.87 ± 1.51^{d}	9.71 ± 0.520^{bd}	310 ± 76.010^{a}

Statistical legend:

- Values with different superscript letters within a parameter row indicate significant differences ($p \leq 0.05$, one-way ANOVA with Tukey's post-hoc test)
- Identical superscripts denote non-significant differences between groups

Table 10. Baseline hematological parameters across treatment groups at day 14.

Treatment Groups	RBC	MCV	MCH	WBCs	PLT
G-1	7.68 ± 0.510^{a}	65.21 ± 0.840^{a}	15.43 ± 2.12^{a}	13.33 ± 0.60^{a}	327.33 ± 13.12^{a}
G-2	4.86 ± 0.640^{b}	63.10 ± 1.82^{a}	14.58 ± 1.28^{a}	16.10 ± 1.350^{b}	632.65 ± 810^{b}
G-3	3.73 ± 0.020^{bc}	58.50 ± 1.40^{b}	8.12 ± 0.47^{b}	20.42 ± 2.220^{c}	824.33 ± 93.2^{bc}
G-4	2.45 ± 0.30^{c}	52.61 ± 1.34^{c}	6.80 ± 0.570^{c}	29.33 ± 0.450^{d}	984 ± 60.60^{c}
G-5	5.82 ± 0.92^{b}	65.39 ± 0.71^{a}	16.82 ± 1.210^{a}	7.85 ± 0.50^{e}	302.33 ± 82.61^{a}

Statistical legend:

- Values with different superscript letters within a parameter row indicate significant differences ($p \leq 0.05$, one-way ANOVA with Tukey's post-hoc test)
- Identical superscripts denote non-significant differences between groups

Table 2.1. Baseline hematological parameters across treatment groups at day 21.

Treatment Groups	**RBC**	**MCV**	**MCH**	**WBCs**	**PLT**
G-1	6.78 ± 0.230^{a}	66.21 ± 1.560^{a}	13.51 ± 0.860^{a}	12.33 ± 10^{a}	309 ± 39.480^{a}
G-2	5.34 ± 1.020^{abc}	65.22 ± 1.640^{a}	12.4 ± 1.020^{a}	17.17 ± 1.43^{ab}	353.23 ± 52.290^{a}
G-3	3.39 ± 0.420^{b}	60.74 ± 1.540^{b}	9.34 ± 0.840^{c}	29.49 ± 0.860^{c}	777.57 ± 42.060^{b}
G-4	2.82 ± 0.040^{c}	44.09 ± 2.260^{c}	5.69 ± 0.270^{d}	29.14 ± 3.160^{d}	910.55 ± 58.160^{bc}
G-5	5.88 ± 1.230^{a}	66.11 ± 0.580^{a}	15.61 ± 0.750^{a}	8.15 ± 0.730^{e}	305.56 ± 83.080^{a}

Statistical legend:

- Values with different superscript letters within a parameter row indicate significant differences ($p \leq 0.05$, one-way ANOVA with Tukey's post-hoc test)
- Identical superscripts denote non-significant differences between groups

Table 2.2. Baseline hematological parameters across treatment groups at day 28.

Treatment Groups	RBC	MCV	MCH	WBCs	PLT
G-1	6.89±0.210^{a}	67.19±0.580^{a}	10.98±0.480^{a}	10.33±1.120^{a}	301±62.32 ^{a}
G-2	5.4±1.060^{ab}	66.55±0.860^{a}	9.29±1.10^{ab}	13.67±3.470^{a}	278.33±51.50^{a}
G-3	3.12±0.590^{b}	61.47±1.160^{b}	7.79±1.100^{b}	19.54±1.880^{bc}	638.23±40.07^{b}
G-4	3.59±0.460^{b}	51.26±1.590^{c}	5.3±0.780^{c}	24.72±1.110^{c}	819.23±63.57^{c}
G-5	5.56±1.130^{ab}	66.11±0.210^{a}	16.3±0.460^{d}	8.30±0.70^{a}	304.32±85.12^{a}

Statistical legend:

- Values with different superscript letters within a parameter row indicate significant differences ($p \leq 0.05$, one-way ANOVA with Tukey's post-hoc test)
- Identical superscripts denote non-significant differences between groups

Table 2.3. Baseline hematological parameters across treatment groups at day 0, 7, 14, 21 & 28.

Groups	**RBCs –Day-0**	**RBCs-Day-7**	**RBCs-Day-14**	**RBCs-Day-21**	**RBCS-Day-28**
G-1	4.81±0.005^{a}	6.86±0.420^{a}	7.68±0.470^{a}	6.78±0.230^{a}	6.89±0.210^{a}
G-2	4.07±0.120^{a}	5.02±0.590^{ab}	4.86±0.640^{b}	5.34±1.020^{abc}	5.4±1.060^{ab}
G-3	4.37±1.150^{a}	3.45±0.460^{b}	3.73±0.020^{bc}	3.39±0.420^{b}	3.12±0.590^{b}
G-4	5.52±0.520^{a}	3.81±0.560^{d}	2.45±0.290^{c}	2.82±0.040^{c}	3.59±0.460^{b}
G-5	5.79±1.530^{a}	5.83±1.610^{ad}	5.82±0.860^{b}	5.88±1.230^{a}	5.56±1.130^{ab}

- Values with different superscript letters within a parameter row indicate significant differences ($p \leq 0.05$, one-way ANOVA with Tukey's post-hoc test)
- Identical superscripts denote non-significant differences between groups.

Fig. 4.3.4 level of RBC at different days (Haematological analysis of RBCs at day 0, 7, 14, 21 & 28).

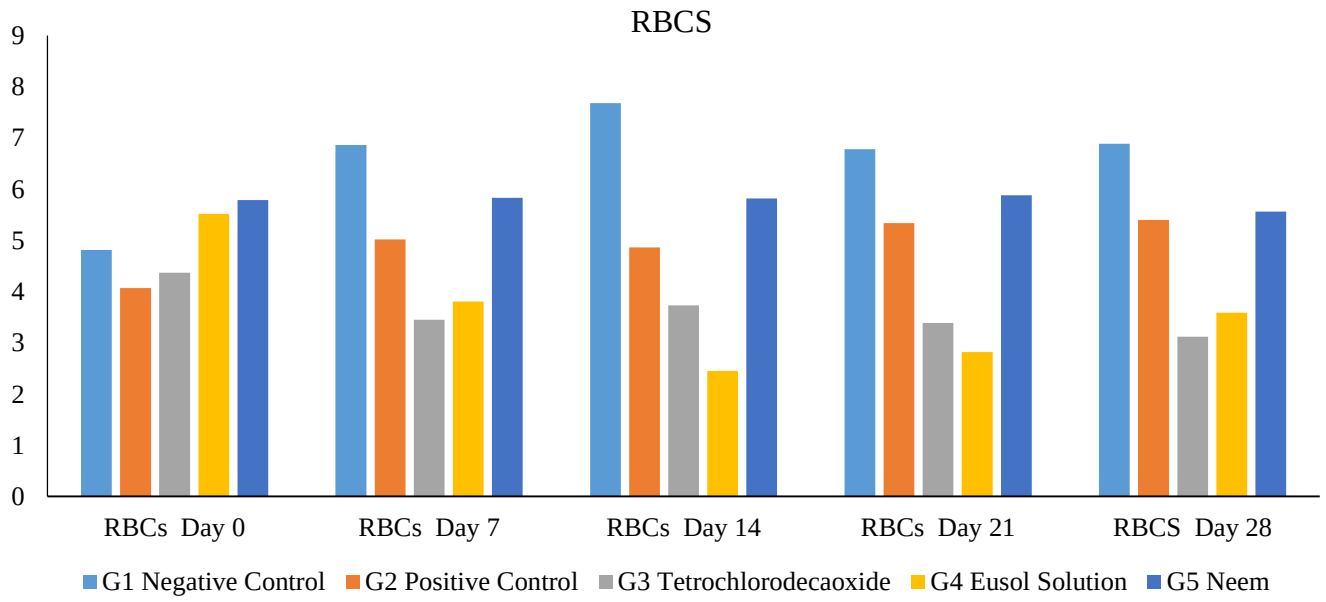


Fig. 3.5 level of MCV at different days

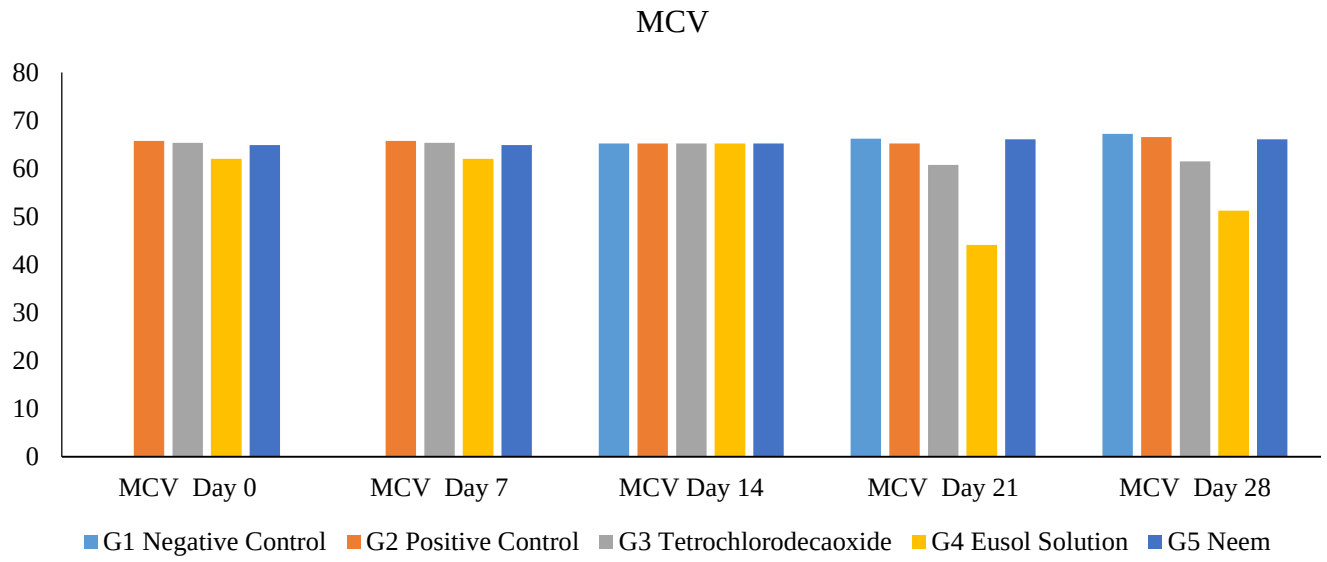


Fig. 3.6 level of MCH at different days

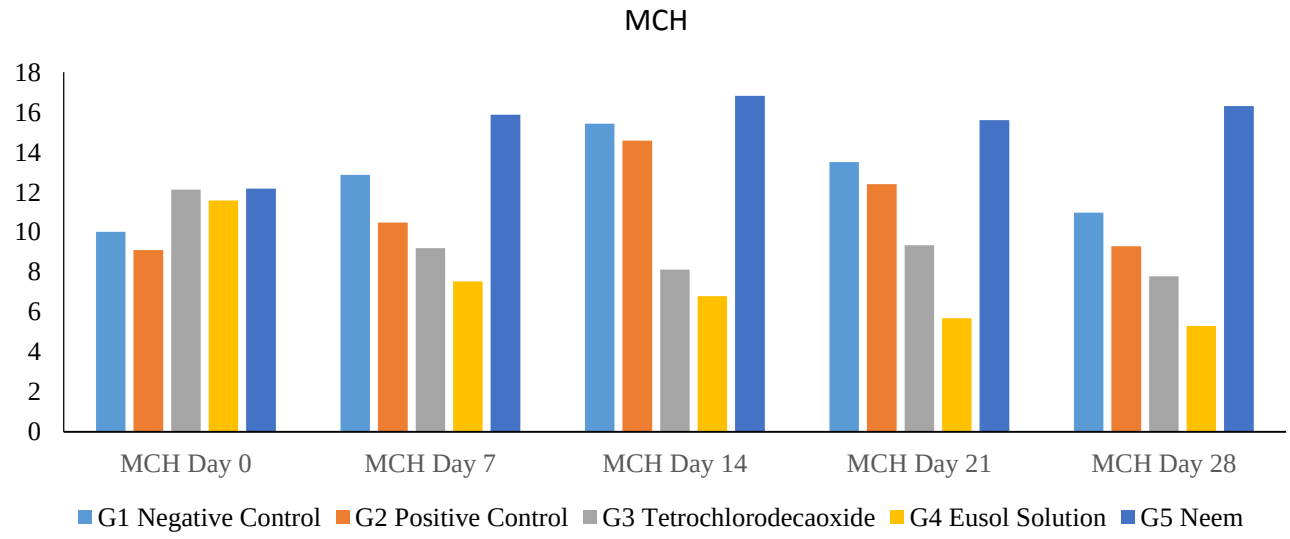


Table 2.4. Temporal changes in mean corpuscular volume (MCH) across treatment groups

Groups	MCH-Day-0	MCH-Day-7	MCH-Day-14	MCH-Day-21	MCH-Day-28
G-1	10.02±1.560[a]	12.87±0.860[a]	15.43±2.120[a]	13.51±0.860[a]	10.98±0.480[a]
G-2	9.10±2.150[a]	10.48±1.250[a]	14.58±1.280[a]	12.4±1.020[a]	9.29±1.10[ab]
G-3	12.13±0.610[a]	9.19±0.860[a]	8.12±0.470[b]	9.34±0.840[c]	7.79±1.100[b]
G-4	11.59±0.460[b]	7.53±0.570[c]	6.80±0.570[c]	5.69±0.270[d]	5.3±0.780[c]

G-5	12.17±0.240^{c}	15.87±1.460^{d}	16.82±1.210^{a}	15.61±0.750^{a}	16.3±0.460^{d}

- Values with different superscript letters within a parameter row indicate significant differences ($p \leq 0.05$, one-way ANOVA with Tukey's post-hoc test)
- Identical superscripts denote non-significant differences between groups

Table 2.5. Temporal changes in WBCs across treatment groups.

Groups	WBCs Day 0	WBCs Day 7	WBCs Day 14	WBCs Day 21	WBCs Day 28
G-1	8.23±0.830^{a}	15.23±1.150^{a}	13.33±0.60^{a}	12.33±10^{a}	10.33±1.120^{a}
G-2	8±1.460^{a}	16.24±1.060^{ab}	16.10±1.350^{b}	17.17±1.43^{ab}	13.67±3.470^{a}
G-3	8.47±1.350^{a}	22.36±1.770^{b}	20.42±2.220^{c}	29.49±0.860^{c}	19.54±1.880^{bc}
G-4	8.22±1.860^{a}	27.31±5.390^{c}	29.33±0.450^{d}	29.14±3.160^{d}	24.72±1.110^{c}
G-5	7.50±0.560^{a}	9.71±0.520^{bd}	7.85±0.50^{e}	8.15±0.730^{e}	8.30±0.70^{a}

- Values with different superscript letters within a parameter row indicate significant differences ($p \leq 0.05$, one-way ANOVA with Tukey's post-hoc test)
- Identical superscripts denote non-significant differences between groups.

Fig. 3.7 level of WBCS at different days

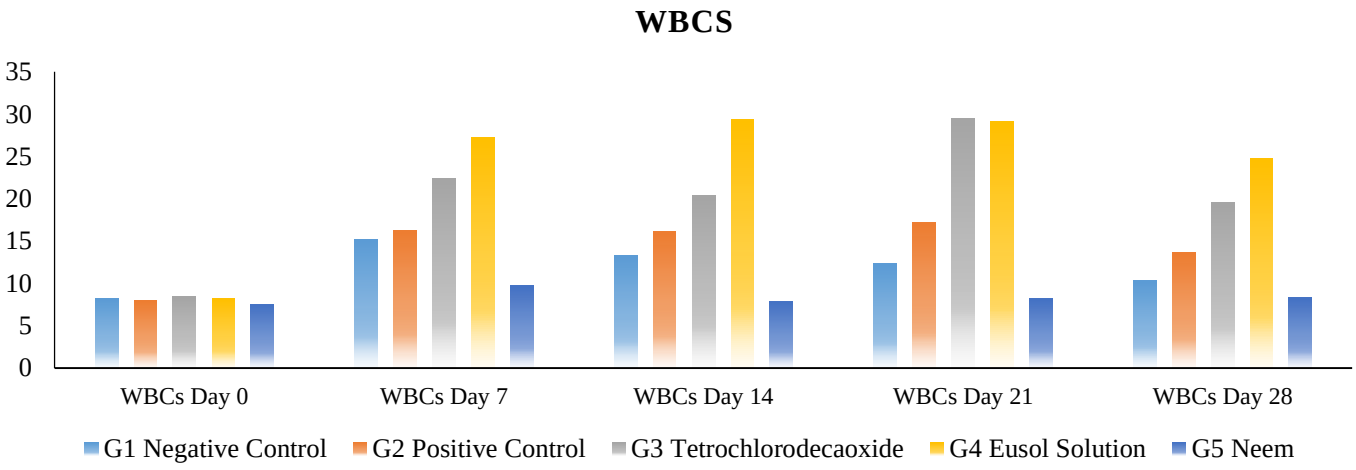


Table:2.6 Temporal changes in PLTs across treatment groups.

Groups	PLTs-Day-0	PLTs-Day-7	PLTs-Day-14	PLTs-Day-21	PLTs-Day-28
G-1	252±50.100^{a}	321.23±91.220^{a}	327.33±13.050^{a}	309±39.480^{a}	301±62.320^{a}
G-2	257±43.030^{a}	733.23±102.400^{b}	632.65±810^{b}	353.23±52.290^{a}	278.33±51.500^{a}
G-3	259.33±52.170^{a}	745.23±107.200^{b}	824.33±93.200^{bc}	777.57±42.060^{b}	638.23±40.070^{b}
G-4	345.33±22.500^{a}	831.23±110.150^{b}	984±60.590^{c}	910.55±58.160^{bc}	819.23±63.570^{c}
G-5	285.33±71.730^{a}	310±76.010^{a}	302.33±82.560^{a}	305.56±83.080^{a}	304.32±85.120^{a}

Fig. 3.8 level of PLT at different days

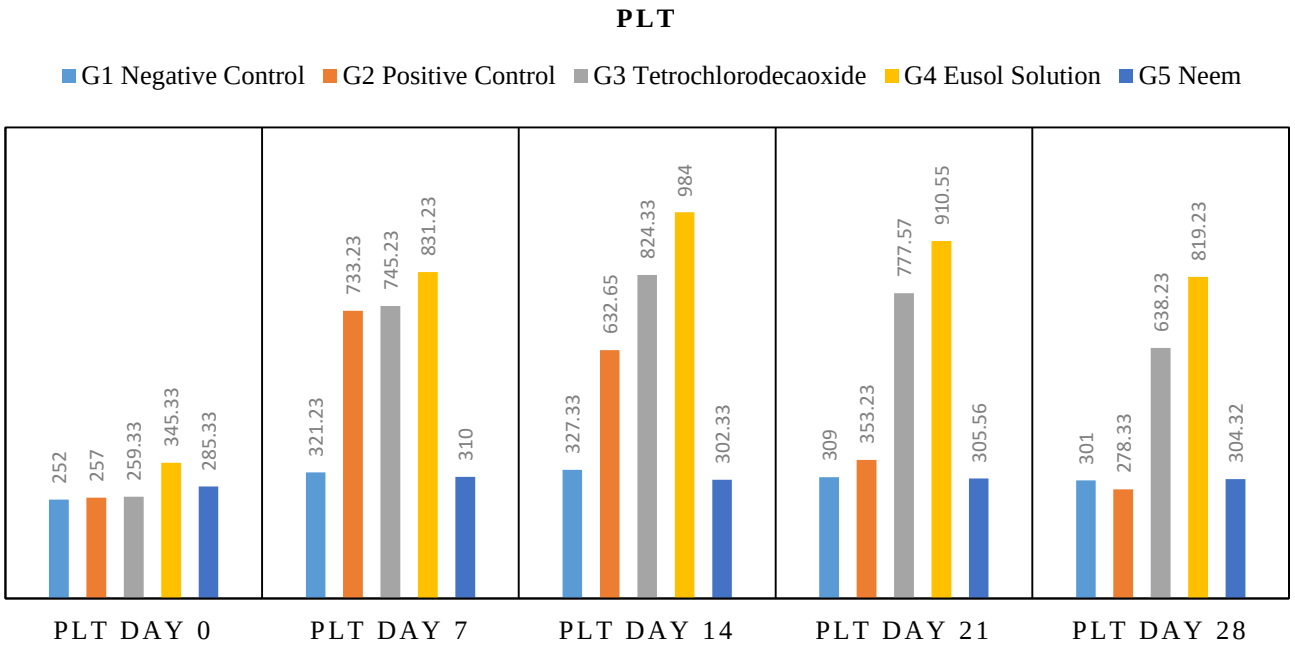


Fig 3.9Photomicrograph of slide. Healed wound tissue treated withTetrachlorodecaoxide (TCDO)

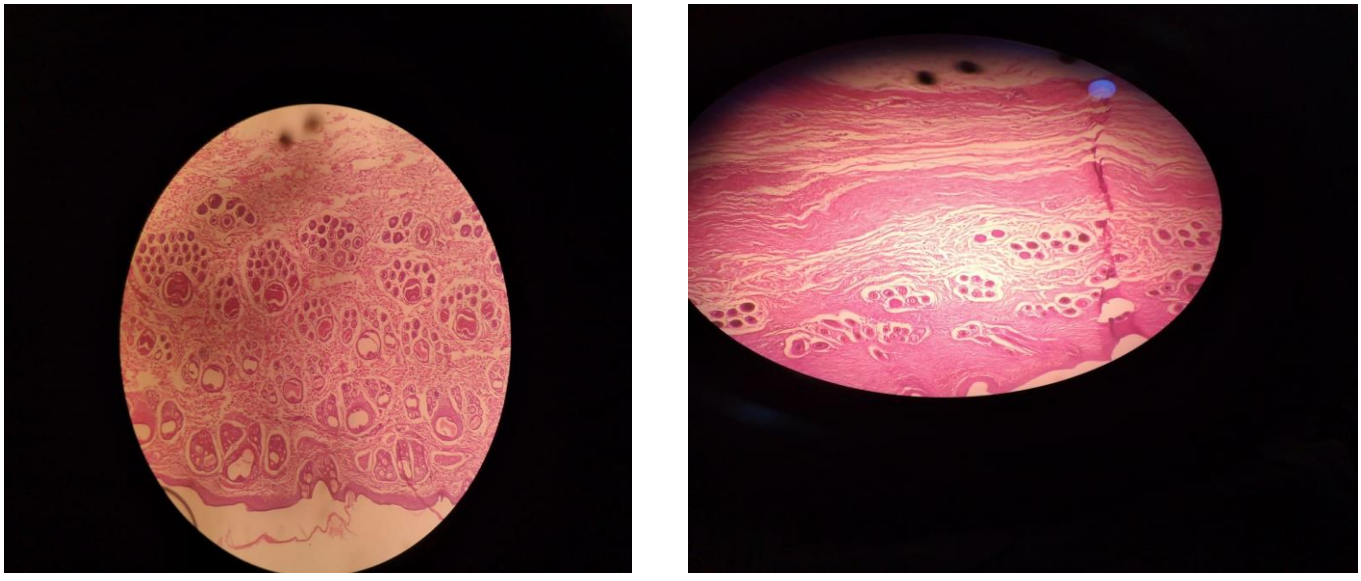


Fig 3.10. Photomicrograph of slide. Healed wound tissue treated with Eusol

Fig 4.1 Photomicrograph of slide. Healed wound tissue treated with Neem

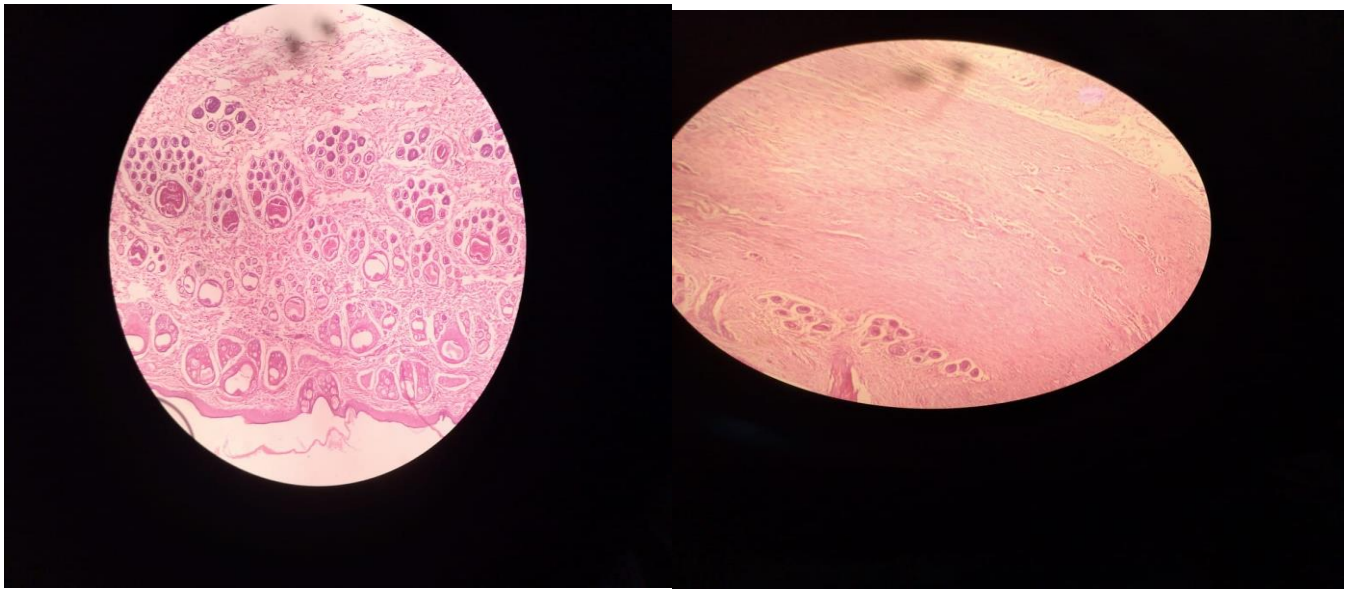


Fig 4.2 Photomicrograph of slide. Healed wound tissue treated with normal saline

5. Discussion

The present study provides compelling evidence for the superior wound healing efficacy of Tetrachlorodecaoxide (TCDO) compared to traditional treatments like EUSOL and Neem oil in a full-thickness wound model. Our findings demonstrate that TCDO significantly accelerated all phases of wound repair, achieving 25% faster wound contraction and more complete epithelialization than comparator treatments. These results align with the known mechanisms of TCDO action, particularly its dual capacity for macrophage activation and tissue oxygenation [7]. The observed 151.92 μm epidermal thickness in TCDO-treated wounds, significantly greater than other groups ($p<0.05$), likely reflects its mitogenic effects on fibroblasts and enhanced angiogenesis [8].

The hematological findings offer important insights into the systemic effects of these treatments. TCDO demonstrated superior normalization of inflammatory markers, with WBC counts peaking earlier (day 7) and returning to baseline faster than other groups. This pattern suggests TCDO may modulate the inflammatory phase more effectively, preventing the prolonged inflammation that often impairs healing [5]. The stable erythrocyte indices in TCDO-treated animals further support its role in maintaining tissue oxygenation during repair [8].

Our results with EUSOL corroborate earlier reports of its antimicrobial efficacy [10], but also highlight its limitations in tissue regeneration. While effective against Pseudomonas aeruginosa [9], EUSOL's cytotoxic effects on granulation tissue [11] were evident in our histopathological findings, which showed poorer collagen organization compared to TCDO. This dual action likely explains its intermediate performance in our study.

Neem oil demonstrated wound healing properties consistent with its known bioactive components [13], though its oil-based formulation may have limited penetration and efficacy. The slower healing trajectory we observed supports the need for improved delivery systems, as suggested by Ghimeray et al. [14].

Clinical implications of our findings are substantial. TCDO's combination of antimicrobial activity (comparable to povidone-iodine per Zenker et al. [15] and tissue regenerative capacity makes it particularly valuable for complex wounds. Our results in this animal model support clinical observations by Rashid et al. [16] and Yingsakmongkol et al. [17] regarding TCDO's efficacy in diabetic wounds. The cost-effectiveness of TCDO, combined with its safety profile [18], suggests it could be particularly valuable in resource-limited settings.

Study limitations include the single-animal model and lack of molecular pathway analysis. Future research should investigate TCDO's mechanisms in chronic wound models and explore potential synergies with other modalities. Nevertheless, our comprehensive assessment provides strong evidence for TCDO's superiority in wound management, supporting its consideration as a first-line treatment option.

6. Conclusions

Surface area of different induced wounds for full thickness was significantly higher Tetrachlorodecaoxide (G3) group. Healing was gradually becoming better in the Tetrachlorodecaoxide (TCDO) group as compared to Neem, Eusol solution and positive control group resulting in better wound condition of the rabbits in 1st two weeks. Tetrachlorodecaoxide (TCDO) resulted in shorter period than the average healing time of the Neem, Eusol solution and positive control group. Furthermore, wound induced Significant difference ($P<0.05$) in WBCs count levels in Tetrachlorodecaoxide (TCDO) treated groups. The level of platelets was not significantly different from the healthy control group. Histopathological assessment (epithelialization, fibrosis and angiogenesis) showed wound healing to be better in Tetrachlorodecaoxide and Eusol.

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