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Assessment of haematological parameters on long bone fracture healing in wistar rats using *Cissus quadrangularis*

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Abstract
Twenty-four Wistar rats were used in the study to assess *Cissus quadrangularis* impact on the healing of long bone fractures. The animals were split into four equal groups, Groups I, II, III, and IV, with six animals in each group, at random. Rats in Group I were maintained as a healthy control, rats in Group II were maintained as an operative control, rats in Group III were treated with *Cissus quadrangularis* (CQ) P.O. 400 mg/kg body weight daily for 30 days, and rats in Group IV were treated with tropical application of *Cissus quadrangularis* (CQ) on site daily for 30 days. Assessments were made of the haematological parameters. Haematological parameters experienced brief variations that stayed within typical physiological ranges. Compared to other groups, the animals in Group III that received oral administration of *Cissus quadrangularis* saw early recovery of their damaged bones. Since it took a little longer, topical treatment of *Cissus quadrangularis* (group IV) was carried out. Nevertheless, compared to group II (surgical control), which received no treatment, groups III and IV both performed better. It follows that *Cissus quadrangularis* promotes early, prolonged bone fracture repair.

Keywords: Haematological parameters, long bone fracture healing, wistar rats, *Cissus quadrangularis*

Introduction
Cissus quadrangularis (Linn) is sometimes referred to as *Vitis* quadrangularis or "Hadjod" Commonly used by humans in India for the treatment of fractures. These plants are widely referred to as Vedhari in Gujrati, Perandi in Tamil, Nalleru in Telugu and Veldgrap, Edible Stemmed Vine in English, Hadjod in Hindi, Kandvel in Marathi, Haddjor in Punjabi, Hadbhanga in Oria, and Vedhari in Sanskrit (Ghouse, 2015) [5]. It has also been observed that the roots of Parijat (*Nyctanthes arbor tristis*) and the leaves of *Coriandrum sativum* (Dhaniya) exhibit osteo-inductive qualities in the healing of bone fractures (Singh *et al*., 1998 and Kumar *et al*., 2002) [21, 10].

The laboratory rat (*Rattus norvegicus domestica*) is propagated and primarily kept for research purposes (Vandenbergh, 2000) [24]. Rats are more commonly used for research than mice, and they've been a crucial animal model for studies in psychology and health care. The Wistar rat descends from the albino rat. The first rat developed to serve as a model organism at a time when laboratories primarily used the common house mouse was developed at the Wistar Institute in 1906. All laboratory rat strains descend from the original colony established by physiologist Henry Donaldson, scientific administrator Milton J. Greenman, and genetic researcher/embryologist Helen Dean King.

One of the most common rats used for research proposals is the Wistar rat. The wistar rat has a wide head, long ears, and a tail that always exceeds its total length. The Wistar rats are the source of other rats, such as the Sprague Dawley and Long–Evans rats. The Wistar rats are more energetic than the Sprague Dawley rats. Domestic rats are calmer and less likely to bite, they can tolerate more crowding, breed earlier and produce more offspring, and their brain, liver, kidneys and heart are comparatively smaller than wild rats. Hadjod (*Cissus quadrangularis*), one of the most common species of plants scattered all over India especially in tropical regions, is one of the most common species of plants scattered all over India especially in tropical regions. *C. quadrangularis* belongs to the family Vitaceae, which is a perennial plant commonly known as Veldgrap or Devils backbone.

It is known as an ancient medicinal plant with optimum healing properties in white tissue area of the body such as tendon and ligament etc. (Justin and Joseph, 2011) [8].

The presence of carotene, phytosterol, terpenoids, -sitosterol, -amyrin, -amyrone and calcium is confirmed by phytochemical analysis. In Ayurvedic medicine, the stem of *C. quadrangularis* is used as an alternative to anthelmintics, dyspeptic, digestive tonic, analgesic in eye and ear diseases, treatment for irregular menstruation and asthma, and complaints of the back and spine. *Cissus quadrangularis* is an herb, reaching a height of approximately 1.5 m and having quadrangular-sectioned branches with internodes 8 to 10 cm long and 1.2 to 1.5 cm wide. It has quadrangular-sectioned branches with internodes 8 to 10 cm. There are hot, dry regions of India where *Cissus quadrangularis* thrives. It is also found on the lower slopes of the Western Ghats, and is widely distributed across drier areas of Arabia and Africa (Justin and Joseph, 2011) [8].

Asthisamhari, also known as *Cissus quadrangularis*, is a succulent plant found in tropical and subtropical regions. It's found in the hottest parts of India, as well as in neighboring countries like Pakistan, Bangladesh, Srilanka, and Malaysia. Plain coastal areas can be cultivated with it. Cuttings of the plant are used to reproduce the plant. Scurvy, menstrual issues, otorrhoea, and epistaxis can be treated with the stem juice of this plant. Osteoarthritis, rheumatoid arthritis and osteoporosis are treated with the plant in Ayurveda. The paste of the stem is given in treatment of asthma, burns and wounds, bites of poisonous insects and saddle sores of horses and camels.

In India, *Cissus quadrangularis* (Linn) is commonly used for fracture healing, and is also known as Hadjod and Vitis quadrangularis. These plants are also known as Vajravalli in Sanskrit, Hadjod in Hindi, Kandvel in Marathi, Haddjor in Punjabi, Hadbhanga in Oria, Vedhari in Gujrati, Perandi in Tamil, Nalleru in Telugu, and Veldgrap, the edible stamen in English (Ghouse, 2015) [5]. The leaves of *Coriandrum sativum* (Dhaniya) and the roots of Parijat (*Nyctanthes arbour tristis*) have been reported to possess osteo-inductive properties in bone fracture healing.

The WHO has also recommended to all member countries to actively promote native medicines and also to initiate steps to conserve and cultivate medicinal plants. There is a large body of evidence that has been collected to its immense potential medicinal plants use in various traditional systems. Medicinal herbs are on lightened due to their widely used for medicinal application and less adverse effects.

In ancient Ayurvedic times, it was reported to have analgesic and specific bone fracture healing properties. Modern studies have revealed that *Cissus* is capable of accelerating bone regeneration by acting as a glucocorticoid antagonist. It's well-known that anabolic and androgenic substances act as antagonists to the glucocorticoid receptor and boost bone development and fracture healing. In addition to speed up the remodelling process of bone healing, *Cissus* also leads to much faster increase in bone tensile strength. The *Cissus quadrangularis* plant is rich in vitamin C and beta-carotene. As analyzed, *Cissus quadrangularis* contains ascorbic acid 479 mg and carotene 267 units per 100 gm of freshly prepared paste in addition to calcium oxalate. *Cissus quadrangularis* is found to contain vitamins and steroids, which are found to have specific effect on bone fracture healing. (Udupa and Prasad, 1964) [23]. The intramuscular administration of alcoholic extract of this plant promotes healing of fractured bones. The methanolic extract of *Cissus quadrangularis* promotes healing process of fractured bone and increases calcium level for callus formation (Mate *et al*., 2008) [14].

Methods and Materials

The present investigation was carried out within the Department of Veterinary Surgery and Radiology, College of Veterinary Science and A.C.G. The duration of the study was five months.

Design experimentation

The study was conducted on 24 Wistar rats, which were divided into four equal groups and subjected to the following treatment:

Table 1: Showing experimental design of study

S. No	Group	No. of animals	Treatment
1.	I	6	Healthy control
2.	II	6	Immobilization of fractured bone using splint and antiseptic dressing of wound daily for 30 days
3.	III	6	Immobilization of fractured bone using splint and antiseptic dressing of wound. Application of *Cissus quadrangularis* P O 400 mg/kg b.wt daily for 30 days
4.	IV	6	Immobilization of fractured bone using splint and antiseptic dressing of wound. Tropical application of *Cissus quadrangularis* on site daily for 30 days

Preparation of the extract of *Cissus quadrangularis*

The fresh stems of the *Cissus quadrangularis* plant were procured from the nearby market, and any extraneous materials were cleared, then chopped into small pieces, and finally dried under the sun. A motorized mixer was used to grind the dried plant material into a fine powder. Each set of Soxhlet apparatus was filled with 100 grams of finely powdered material taken in a thimble. A solution of methanol and water in ratio 7:3 was put into the soxhlet in the required amount. The heater plate was connected to the electricity supply and watched for the extraction process while it was connected to the electricity supply. The final filter was poured into a tray for methanol evaporation after 24-48 hours. Methanol was evaporated by placing the tray on a hot water bath at 400. The semi-solid extract was weighed to obtain the exact weight of the extract.

Preparation of working solution and ointment

A daily fresh solution was prepared using distilled water to measure the required amount of extract of *Cissus quadrangularis* in rats. For preparation of *Cissus quadrangularis* ointment. Various ingredients such as wool fat, hard paraffin, white/yellow soft paraffin and extract of *Cissus quadrangularis* were used.

Determination of LD_{50} toxicity

Acute toxicity of the plant *Cissus quadrangularis* was determined by the calculation of LD_{50} which represents the dose that can be fatal to 50 percent of any rat group. Determination of LD_{50} in rats was done to determine the proper treatment dose that should be used in present experiment. For this procedure, five Wistar rats were used. 2000 mg/kg b.wt methanolic extract of *Cissus quadrangularis* was administered orally to each rats. Monitoring was done up to five days. If 50% rats did not die, then 1/5th of limit dose would be used as therapeutic dose. In present study, no rats were affected as per the trial dose and its 1/5th limit dose was used for treatment purpose.

Treatment
Twenty-four Wistar rats, weighing between 150 and 200 grams, of either sex, were used in the investigation. They were acquired from the College of Veterinary Science and Animal Husbandry's Small Animal House in Anjora, Durg. In individual cages, the animals were acclimated to conventional environmental and nutritional conditions. They were fed standard laboratory food and given unlimited access to water. Prior to surgery, medio-lateral radiographic images of the tibia were taken and each rat had a clinical orthopaedic evaluation. Animals in all three groups were given general anesthesia and given a mixture of Xylazine[1] (20 mg/kg b.wt) and Ketamine[2] (50 mg/kg b.wt) intramuscularly. A drill machine with a proper size bit was used to induce a fracture in the tibia bone.

Animal preparation: Following general anesthesia, the surgical region was made clean by using a razor to remove hair and applying an antibacterial lotion (Betadine). After that, a drill machine and the appropriate drill bit were used to generate the fracture in the tibia bone. Using an x-ray, the fracture of the tibial bone was confirmed.

Parameters Studied
Haematological profile
On days 0,7,14, and 28 for haematological tests, one milliliter of blood was drawn from the retroorbital sinus and placed in sterile glass vials containing EDTA (Ethylene Diamine Tetra Acetic Acid, 2 gm/ml blood). The automated haematological blood cell counter (B.C-2800 Vet, Mindray) was used to estimate the haematological parameters. The calculated parameters are listed as follows:

a) **The readings for hemoglobin (Hb):** Were given in grammes per deciliter.
b) **Packed Cell Volume (PCV):** A percentage was used to express the values.
c) **Total Erythrocyte Count (TEC):** Millions/cu.mm of blood was used to express the results.
d) **Total Leukocyte Count (TLC):** The results were given in milliliters per milliliter of blood.
e) **Differential Leukocyte Count (DLC):** Right away following blood collection, blood smears were made. The smear was fixed in methanol for 1minute and was stained with Geimsa stain for 30 minutes. The counts were expressed in percentage.

Analytical statistics
The data was analyzed using analysis of variance (ANOVA) to determine whether there were any differences between the groups utilizing conventional procedures as outlined by Snedecor and Cochran (1994) [26]. The mean and standard error of the recorded values were determined.

Results and Discussion
Haematological parameters
Haemoglobin: The values (Mean±SE) of hemoglobin (gm/dl) at different time intervals in different groups indicated in Table 1 and Fig. 1. At various time intervals, there were non-significant variations in the hemoglobin levels between the groups and within the groups. In the current investigation, group II, III, and IV's hemoglobin levels decreased non-significantly on the seventh post-operative day. Up to the study's conclusion, hemoglobin levels fluctuated within normal physiological bounds. The non-significant decrease may be explained by the fact that group II, III, and IV had more inflammation up to day seven as a result of the physical stress that caused the fracture. Tembhurne *et al.* (2010) [22] also noted a little drop in hemoglobin during the healing of canine femur fractures.

Packed cell volume (%)
The values (Mean±SE) of packed cell volume (%) at various time interval in different groups are shown in Table.1 and represented in Fig.2.

When comparing the packed cell volume measurements between the groups and within the groups at various time intervals, no significant changes were found. In the current investigation, group II, III, and IV saw fluctuations in packed cell volume within normal physiological bounds for up to 28 days following treatment, with a non-significant decrease observed on the seventh post-operative day. The non-significant reduction in packed cell capacity could perhaps be attributed to physical strain, haemodilution, and anesthesia during the fracture formation process. The aforementioned results are consistent with those of Rajhans (2013) [17] and Aitha *et al*. (1998) [1] in dogs. The minimal decrease in packed cell volume during the healing of canine femur fractures was previously mentioned by Tembhurne*et al*. (2010) [22].

Total Erythrocyte count (10^6 /mm^3)
The values (Mean±SE) of total erythrocyte count (10^6 /mm^3) at various time interval in different groups are shown in Table.1 and represented in Fig.3.

At various time intervals, there were non-significant variations in the total erythrocyte count values between the groups and within the groups. In the current investigation, rats in groups II, III, and IV had non-significant decreases in total erythrocyte count on the seventh post-operative day, and non-significant increases within normal physiological limits by the fourteenth and twenty-eighth post-operative days. According to Lobo *et al*. (2013) [12], there may have been little bleeding and trauma during the formation of the fracture, which could have caused the brief decrease in the total erythrocyte count. The current discovery aligns with the results of Aithal *et al*. (1998) [1] regarding the healing process of supracondylar fractures in dogs.

Total Leukocyte Count (10^3/mm^3)
The values (Mean±SE) of total leukocyte count (10^3/mm^3) at various time interval in different groups are shown in Table.1 and represented in Fig.4.

At various time intervals, there were no discernible variations in the total leukocyte count between the groups or within the groups. The total leukocyte count in the current study increased non-significantly on the seventh post-operative day and gradually decreased on the fourteenth and twenty-eighth days, indicating a return to normal following a fracture. The results of *Maiti et al*. (1999) [13] during the repair of experimental fractures in dogs under the influence of anabolic steroids, however, support the idea that the non-significantly higher values in groups II, III, and IV at the seventh day could be ascribed to the systemic inflammatory alterations following fracture.

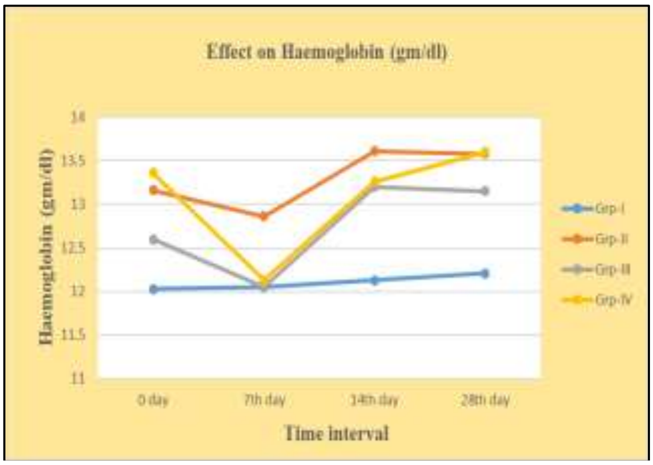

Fig 1: Mean value of Haemoglobin (gm/dl) at various time interval in different groups.

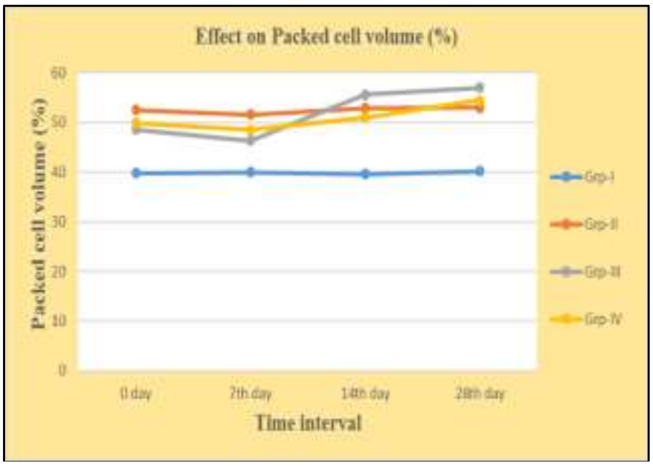

Fig 2: Mean value of Packed cell volume (%) at various time interval in different groups.

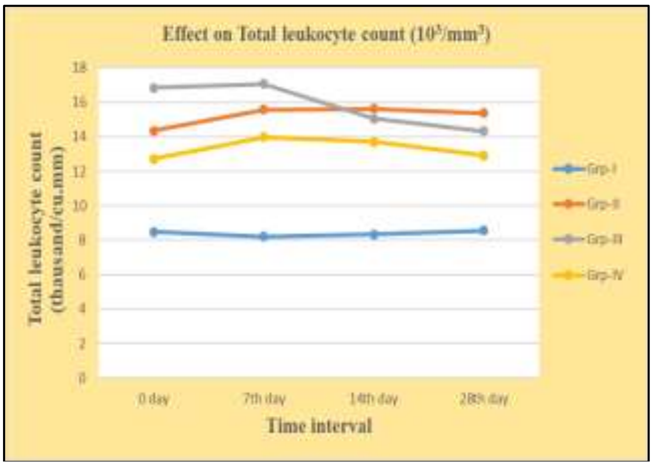

Fig 4: Mean value of Total leukocyte count (103/mm3) at various time interval in different groups.

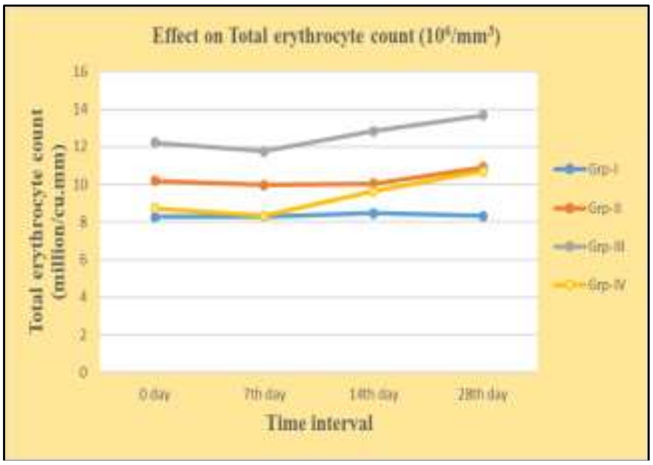

Fig 3: Mean value of Total erythrocyte count (106/mm3) at various time interval in different groups.

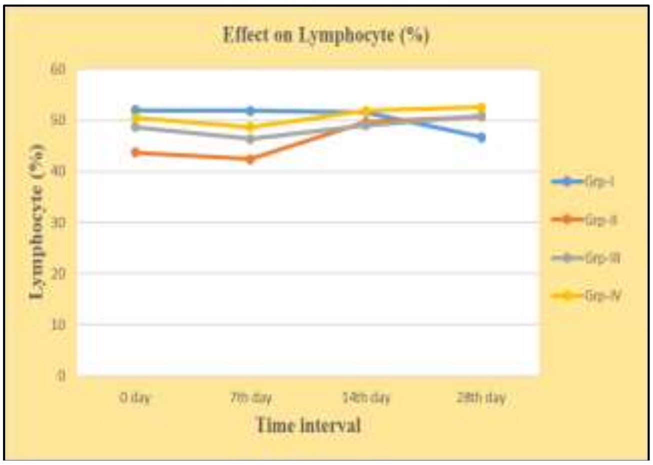

Fig 5: Mean value of Lymphocyte (%) at various time interval in different groups

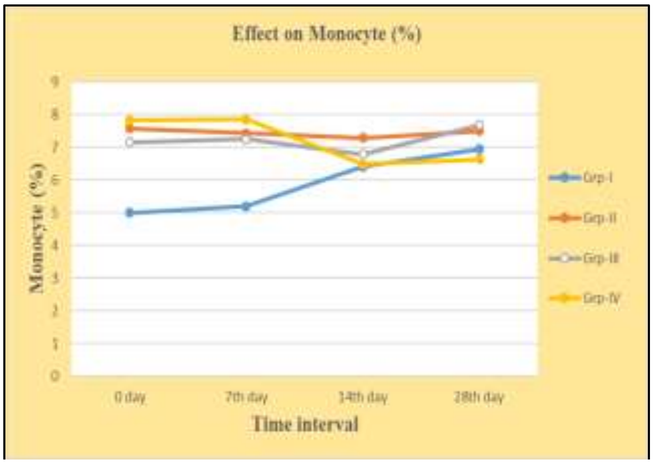

Fig 6: Mean value of Monocyte (%) at various time interval in different groups

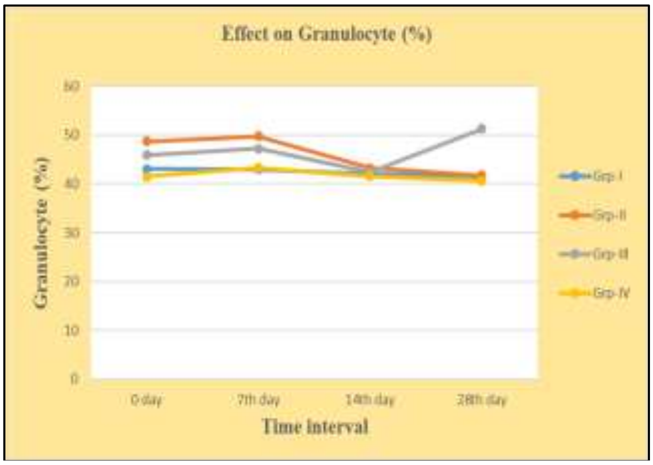

Fig 7: Mean value of Granulocyte (%) at various time interval in different groups.

Differential Leukocyte Count (%)

Lymphocyte (%)

The values (Mean±SE) of lymphocyte (%) at various time intervals in different groups are shown in Table.1 and represented in Fig.5.

Between groups and within groups, non-significant variations in lymphocyte counts were observed at various time intervals. In the current investigation, group II, III, and IV lymphocyte counts demonstrated a non-significant drop on the seventh post-treatment day, followed by a non-significant increase within normal physiological limits on the fourteenth and twenty-eighth post-operative days. The inflammatory response brought on by the surgical procedure can be blamed for the reduction in lymphocyte count on the seventh day. Kaneko (1997) [9] also noticed comparable results in house animals following surgery.

Monocyte (%)

The values (Mean±SE) of monocyte (%) at various time interval in different groups are shown in Table.1 and represented in Fig.6.

Monocyte levels within and between groups at various time intervals showed no discernible variations.

In the current investigation, group II, III, and IV's mean monocyte counts varied non-significantly at 7, 14, and 28 days, respectively. The slight alterations, meanwhile, stayed within the typical physiological range. Similar results after diaphyseal fracture of long bone healed by utilizing various biomaterials in dogs were also reported by Chaurasia *et al*. (2019) [2].

Granulocyte (%)

The values (Mean±SE) of granulocyte (%) at various time interval in different groups are shown in Table.1 and represented in Fig.7.

The present study found that there were non-significant differences in granulocyte counts between the groups and within the groups at different time intervals. The granulocyte count increased non-significantly on the seventh post-treatment day, and then returned to normal physiological limits on the fourteenth and twenty-eighth post-operative days in groups II, III, and IV, respectively. The reason for the increase in granulocyte counts on the seventh post-operative day could be that neutrophils are thought to be the body's first line of defense in response to any surgical intervention. Similar findings were also noted in dogs by Schalm *et al*. (1975) [19] and Sastry (1989) [18].

Table 2: Haematological parameters

Parameters	Groups	Time intervals in Days			
		0	7	14	28
Hb (gm/dl)	I	12.03±0.85aA	12.05±0.47aA	12.13±0.56aA	12.21±0.41aA
	II	13.16±0.50aA	12.86±0.85aA	13.61±0.36aA	13.58±0.36aA
	III	12.6±0.46aA	12.05±0.37aA	13.2±0.45abA	13.15±0.73bA
	IV	13.36±0.49aA	12.13±0.67aA	13.26±0.33abAB	13.6±0.21bA
PCV (%)	I	39.8±1.50aA	39.96±1.25aA	39.65±1.50aA	40.2±1.59aA
	II	52.53±1.65aB	51.63±1.35aB	52.93±1.0aB	53.03±0.76aB
	III	48.6±1.25aB	46.33±1.63aB	55.63±1.52bB	57.01±1.36bBC
	IV	49.91±2.11aB	48.56±2.33aB	51.01±2.01aB	54.55±2.55aC
TEC (millions/cu.mm)	I	8.27±0.65aA	8.31±0.43aA	8.48±0.51aA	8.33±0.40aA
	II	10.18±0.26bB	9.96±0.78aB	10.05±0.32abA	10.91±0.31aA
	III	12.23±0.77aB	11.76±1.27aB	12.84±1.09aB	13.68±1.19aB
	IV	8.74±0.51aAB	8.32±0.42aA	9.63±0.67aA	10.70±0.60bB
TLC (thousand/cu.mm)	I	8.48±0.56aA	8.21±0.27aA	8.33±0.47aA	8.56±0.31aA
	II	14.35±0.78aB	15.58±0.83aB	15.63±0.68aB	15.36±0.68aC
	III	16.85±1.0bB	17.07±0.78aB	15.06±0.48aB	14.33±0.42aBC
	IV	12.7±1.0aB	13.98±1.07aB	13.7±1.0aB	12.91±0.83aB
DLC					
Lymphocytes (%)	I	51.93±1.42aA	51.85±1.03aA	51.58±1.07aA	46.68±4.68aA
	II	43.73±3.27aA	42.42±1.02aA	49.61±0.99abA	50.71±0.99bA
	III	48.7±1.30aA	46.36±1.13aA	49.06±2.09aA	50.96±0.75aA
	IV	50.51±1.17aA	48.65±1.09aA	51.9±1.13aA	52.56±1.02aA
Monocyte (%)	I	5.0±0.55aA	5.2±0.65aA	6.41±0.46bA	6.95±0.34bA
	II	7.58±0.46aB	7.43±0.37aB	7.28±0.27aA	7.5±0.56aA
	III	7.16±0.47aB	7.25±0.67aB	6.78±0.49aA	7.68±0.40aA
	IV	7.83±0.42aB	7.85±0.52aA	6.48±0.48aA	6.63±0.35abA
Granulocyte (%)	I	43.06±1.48aA	43.00±1.17aA	42.0±0.86aA	41.53±1.13aA
	II	48.68±3.48bA	49.78±2.17aA	43.23±0.87abA	41.78±0.70aA
	III	45.96±2.73aA	47.28±3.85aA	42.48±0.81aA	41.35±9.38aA
	IV	41.48±1.27aA	43.32±2.13aA	41.61±0.98aA	40.63±1.06aA

Conclusions

Based on the observations and analysis conducted during this study, it is possible to draw the conclusion that the groups treated with *Cissus quadrangularis* experienced accelerated fracture healing, suggesting that the plant's anti-inflammatory, analgesic, and osteoinducing qualities contributed to early bone regeneration. In terms of hematological parameters, there were non-significant variations between the groups and within the group at various time intervals in mean hemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, lymphocyte, monocyte, and granulocyte. Nonetheless, the slight variation noted during the early research phase persisted within typical physiological bounds.

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