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Evaluation of haemato-biochemical parameters using *Cissus quadrangularis* on long bone fracture healing in Wistar rats

SK Yadav, Raju Sharda, Shailendra Kumar Tiwari, Rukmani Dewangan, MO Kalim, MV Kamble and Anupam Soni

Abstract
The study was carried out in twenty-four Wistar rats to evaluate the effect of *Cissus quadrangularis* on long bone fracture healing. The animals were randomly divided in to four equals groups that are Group I, II, III and IV which consisted of six animals in each group. In Group I-the rats were kept as healthy control, Group II the fractured rats were kept as operative control, Group III the fractured rats were subjected to treatment by using *Cissus quadrangularis* (CQ) P.O 400mg/kg body weight daily for 30 days and in Group IV-the fractured rats were subjected to treatment by using tropical application of *Cissus quadrangularis* (CQ) on site daily for 30 days. The, haemato-biochemical parameters were assessed. There were transient changes in hematological parameters which remained within normal physiological limits whereas, significant (p<0.05) changes observed in biochemical parameters. The animals which were given oral administration of *Cissus quadrangularis* in (Group III) resulted in early healing of fractured bone as compared to other groups. Topical application of *Cissus quadrangularis* (group IV) was done as it took slightly longer time. However, both group III and IV were better than the untreated group II (operative control) where no treatment was given. Therefore, it could be concluded that *Cissus quadrangularis* induces early long bone fracture healing.

Keywords: rat, fractured, haematological and biochemical

Introduction
The laboratory rat (*Rattus norvegicus domestica*) is propagated and mainly kept for scientific research purpose (Vandenbergh, 2000) [1]. Rats are more commonly used for research investigation than mice, and they have served as an important animal model for research in psychology and biomedical science. The Wistar rat is outbred of albino rat. The Wistar rat is currently one of the most common rat used for research propose. Wistar rat is characterized by its wide head, long ears, and a tail length that is always less than its body length. Other species of rats such as Sprague Dawley rat and Long–Evans rat are developed from Wistar rats. Wistar rats are more active than Sprague Dawley rats. Hadjod (*Cissus quadrangularis*) is one of the most common species of plants scattered all over India especially in tropical regions (Guhabakshi *et al.,* 2001) [2]. *C. quadrangularis* belongs to the family Vitaceae, which is a perennial plant commonly known as Veldgrap or Devils backbone (Kumbhojkar *et al.,* 1991) [3]. It is known to be 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) [4]. *Cissus quadrangularis* indicates the presence of carotene, phytosterol, terpenoids, β-sitosterol, δ-amyrin, δ amyrone and calcium as confirmed by phytochemical analysis (Mishra *et al.,* 2011) [5]. The stem of *C. quadrangularis*is also an important medicinal plant in Ayurvedic and used as alternative to anthelmintics, dyspeptic, digestive tonic, analgesic in eye and ear diseases, treatment for irregular menstruation and asthma, in complaints of the back and spine (Sen *et al.,* 1966) [6]. The stem juice of this plant is used to treat scurvy, menstrual disorders, otorrhoea and epistaxis. The plant has been well identified in Ayurveda for the treatment of osteoarthritis, rheumatoid arthritis and osteoporosis (Yoganarisimhan, 2000 [7]; Paulsen *et al.,* 2007) [8]. The paste of stem is given in treatment of asthma, burns and wounds, bites of poisonous insects and for saddle sores of horses and camels. The WHO have also recommended to all the member countries to actively promote native medicines and also to initiate steps to conserve and cultivate medicinal plants.

In present times focus on plant research has increased all over the world and a large body of evidence has been collected to its immense potential medicinal plants use in various traditional systems. Medicinal herbs are on lightened due to their widely use for medicinal application and less adverse effects (Mate *et al.*, 2008) [9].

It was also reported in the ancient Ayurvedic time as a general tonic with analgesic and specific bone fracture healing properties. Modern research has shed light on *Cissus* ability to speed bone healing by acting as a glucocorticoid antagonist. Since, anabolic and androgenic compounds are well known to act as antagonist to the glucocorticoid receptor as well as promote bone growth 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. *Cissus quadrangularis* is found to contain vitamins and steroids, which are found to have specific effect on bone fracture healing. (Udupa and Prasad, 1964)[10]. 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) [9].

Materials and Methods

The present study was conducted in the Department of Veterinary Surgery and Radiology, College of Veterinary Science and A.H, Anjora, Durg, (C.G.). The study period was 5 months. The total 24 Wistar rats were selected and divided into 4 treatment groups. The experimental groups were mentioned in table.1.

Table 1: Showing experimental groups 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 400mg/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

Parameters Studied

Haematological profile

One ml blood was collected from retro orbital sinus in sterilized glass vials containing EDTA (Ethylene Diamine Tetra Acetic Acid, 2 gm/ml blood) on 0, 7, 14th and 28th day for haematological examinations. The estimation of haematological parameters was done by automated haematology blood cell counter (B.C- 2800 Vet, Mindray). The parameters which were estimated are mentioned below

a) **Haemoglobin (Hb):** The values were expressed in gm/dl.
b) **Packed Cell Volume (PCV):** The values were expressed in percentage.
c) **Total Erythrocyte Count (TEC):** The results were expressed in millions /cu.mm of blood.
d) **Total Leukocyte Count (TLC):** The result were expressed in thousand/cu.mm of blood.
e) **Differential Leukocyte Count (DLC):** The blood smears were prepared immediately after the blood collection. The smear was fixed in methanol for 1minute and was stained with Geimsa stain for 30 minutes. The counts were expressed in percentage.

Biochemical Parameters: The blood samples (1ml) from the rat were collected and serum was separated at 0, 7th, 14th and 28th days for estimation of biochemical parameters. These parameters were estimated by standard methods using Semi-Automated Biochemistry Analyzer (Diasil-100 Systronics make).The parameters which were estimated are as follows:

a. Alkaline phosphatase (U/L).
b. Serum calcium (mg/dl)
c. Serum phosphorus (mg/dl)

Statistical analysis: The mean and standard error of recorded values were calculated, Data was analysed by using analysis of variance (ANOVA) for knowing any difference existing among the groups using standard procedures as out lined by Snedecor and Cochran (1994).

Results and Discussion

Haematological parameters

Haemoglobin

The values (Mean ± SE) of haemoglobin (gm/dl) at various time intervals in different groups are shown in Table.1 and represented in Fig.1. Non-significant differences were recorded in haemoglobin values among the groups and within the groups at different time interval. In present study, haemoglobin showed non-significant decrease on 7th post-operative day in group II, III, and IV, followed by fluctuation of the haemoglobin values within normal physiological limits up to the end of study. The non-significant decrease might be attributed to presence of more inflammation till 7th day in group II, III and IV resulting, due to the physical stress at the time of creating fracture. Tembhurne *et al.* (2010) [11] also mentioned marginal decrease of haemoglobin during femoral fracture healing in canines. Similar observations have also reported by Aithal *et al.* (1998) [12] and Rajhans (2013) [13] in dogs and Gupta (2015) [14] in goats.

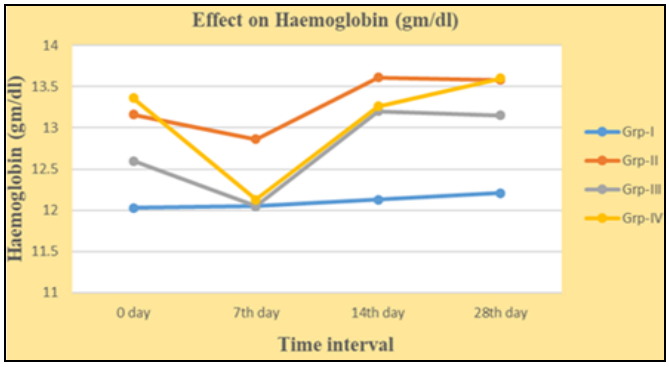

Fig 1: Mean value of Haemoglobin (gm/dl) at various time interval in different groups.

Packed cell volume (%)

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

represented in Fig.2.Non significant differences were recorded in packed cell volume observations among the groups and within the groups at different time interval. In present study, packed cell volume showed non-significant decrease on 7th post-operative day followed by fluctuation of the values within normal physiological limits up to 28 days post treatment in group II, III and IV. This non-significant decrease in packed cell volume might have resulted due to physical stress, haemodilution and anaesthesia at the time of fracture creation. The above findings are in accordance with the findings of Aithal *et al*. (1998) [12], and Rajhans (2013) [13] in dogs. Tembhurne *et al.* (2010) [11] also mentioned marginal decrease of packed cell volume during femoral fracture healing in canines.

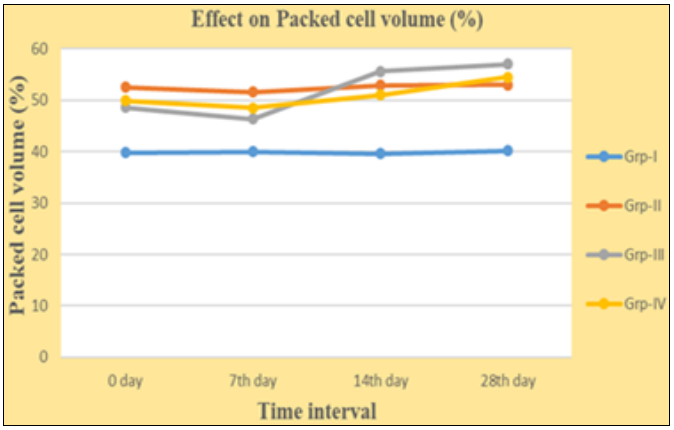

Fig 2: Mean value of packed cell volume (%) at various time interval in different groups.

Total Erythrocyte count (10^6 /mm^3)

The values (Mean ± SE) of total erythrocyte count (10^6 /mm^3) at various time intervals in different groups are shown in Table.1 and represented in Fig.3. Non-significant differences were recorded in total erythrocyte count values among the groups and within the groups at different time interval. In present study, total erythrocyte count showed non-significant decrease on 7th post-operative day, followed by non-significant increase within normal physiological limits by 14thand 28th post-operative day in rats of group II, III and IV. The transient fall in total erythrocyte count may be attributed to mild haemorrhage and trauma during fracture creation as also reported by Lobo *et al.* (2013) [15]. The present observation also correlates with the findings of Aithal *et al.* (1998) [12] during supracondylar fracture healing in dogs.

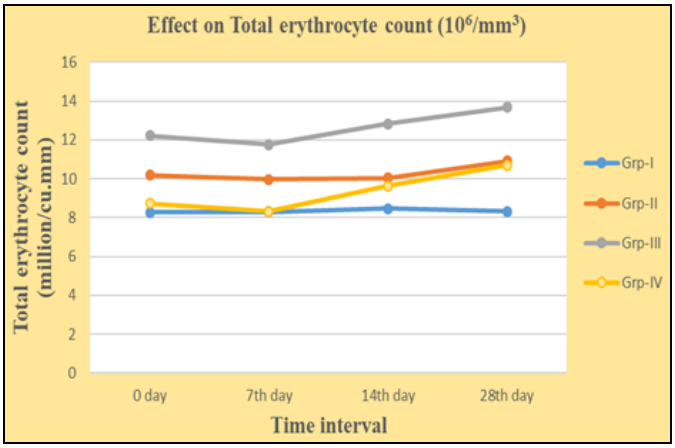

Fig 3: Mean value of Total erythrocyte count (106/mm3) at various time interval in different groups.

Total Leukocyte Count (10^3/mm^3)

The values (Mean ± SE) of total leukocyte count (10^3/mm^3) at various time intervals in different groups are shown in Table.1 and represented in Fig.4. Non-significant differences were recorded in total leukocyte count among the groups and within the groups at different time interval.

In present study, total leukocyte count showed non-significant increase on 7th post-operative day and gradual decrease at 14th and 28th days which was indicative of returns to normal condition after fracture. However, non-significant higher values in group II, III and IV at 7th day might be attributed to the systemic inflammatory changes after fracture as supported by the findings of Maiti *et al.* (1999) [16] during repair of experimental fractures under influence of anabolic steroid in dogs.

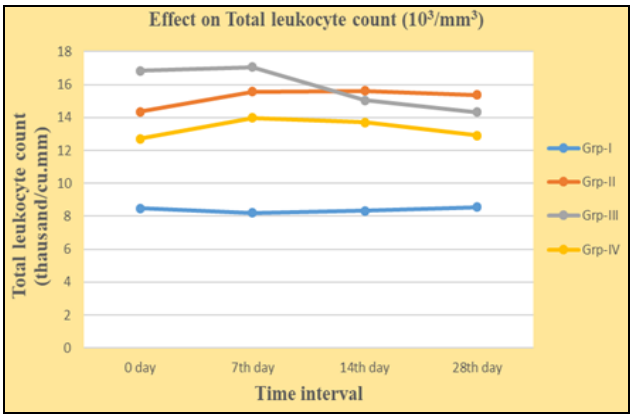

Fig 4: Mean value of Total leukocyte count (103/mm3) at various time interval in different groups.

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. Non-significant differences were recorded in lymphocyte among the groups and within the groups at different time interval.

In present study, lymphocyte count showed non-significant decrease on 7th post treatment day followed by non-significant increase within normal physiological limits on 14th and 28th post-operative days in group II, III and IV. The decrease in lymphocyte count on 7th day can be attributed to inflammatory reaction caused as a result of surgical intervention. Similar findings were also observed by Kaneko (1997) [17] in domestic animals after surgical intervention.

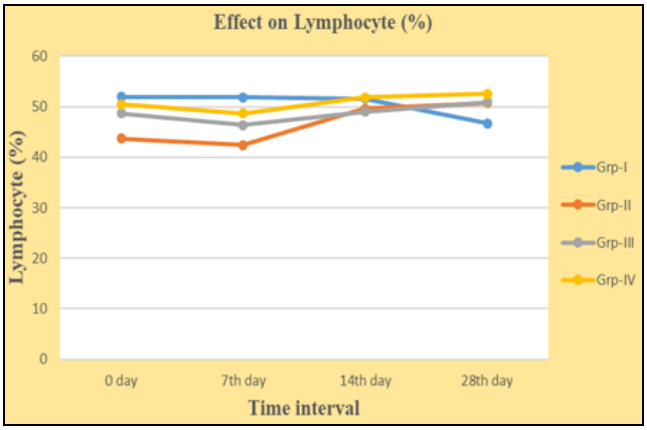

Fig 5: Mean value of Lymphocyte (%) at various time interval in different groups.

Monocyte (%)

The values (Mean ± SE) of monocyte (%) at various time intervals in different groups are shown in Table.1 and represented in Fig.6. Non-significant differences were recorded in monocyte among the groups and within the group at different time interval. In present study, mean monocyte count showed non-significant variation at 7, 14 and 28 days respectively in group II, III and IV. However, the marginal changes remained within normal physiological limit. Similar findings were also reported by Chaurasia *et al.* (2019) [18] during diaphyseal fracture of long bone repaired by using different biomaterials in dog.

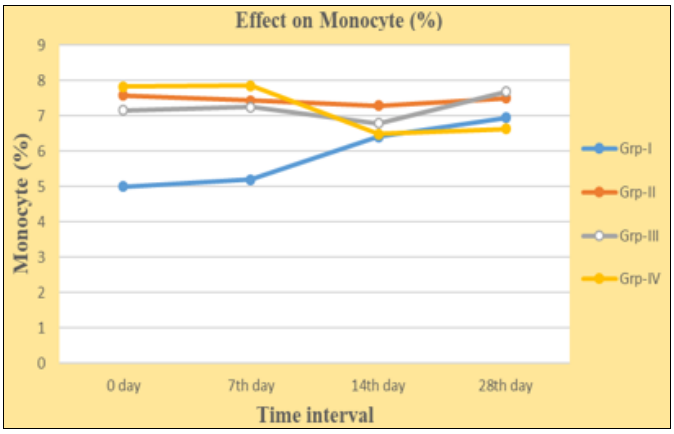

Fig 6: Mean value of Monocyte (%) at various time interval in different groups.

Granulocyte (%)

The values (Mean ± SE) of granulocyte (%) at various time intervals in different groups are shown in Table.1 and represented in Fig.7.Non-significant differences was recorded in granulocyte among the groups and within the groups at different time interval. In present study, granulocyte count showed non-significant increase on 7th post treatment day followed by returns within normal physiological limits on 14th and 28th post-operative days in group II, III and IV respectively. Increase in the value of granulocytes on 7th post-operative day might be due to fact that neutrophils are considered as the first line of defense of the body in response to any surgical intervention. Similar findings were also observed by Schalm *et al.* (1975) [19] and Sastry (1989) [20] in dogs.

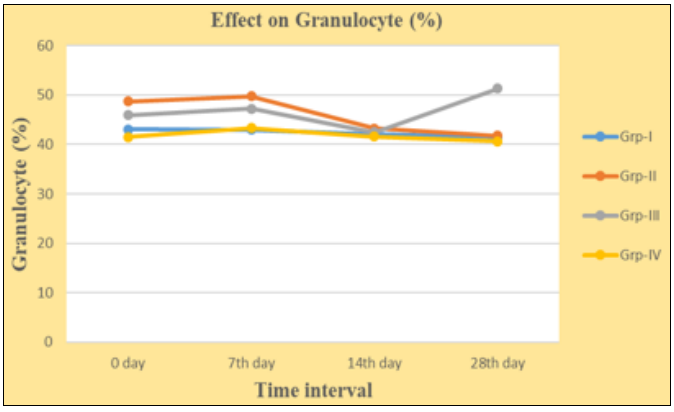

Fig 7: Mean value of Granulocyte (%) at various time interval in different groups.

Biochemical parameters

Alkaline phosphatase (ALP) (U/L)

The values (Mean ± SE) of alkaline phosphatase (ALP) (U/L) at various time intervals in different groups are shown in Table.2 and represented in Fig.8. The Alkaline phosphatase level showed non-significant difference in group I, however, in group II, III and IV showed significant increase on 14th and 28th day. The values ranged from (178.81±7.11 to 295.4±3.69) in all the four groups at various time intervals. Alkaline phosphatase is involved in bone formation and healing of fractures. The enzyme, secreted by the osteoblast cells accelerates the process of mineralization either by increasing the local concentration of inorganic phosphate or by activating the collagen fibers to induce deposition of calcium salts at fracture site. *Cissus quadrangularis* leads to increased alkaline phosphatase levels during fracture healing in dogs (Mishra *et al.*, 2010) [21].

In the present study, the marked elevation of alkaline phosphatase in early postoperative period could be attributed to adrenal hyper function due to stress, skin and muscle trauma and increased osteogenic activity and deposition of calcium salts at the site of fracture as opined by Wilkinson (1980) [22] and Pardeshi and Ranganath (2009) [23]. While, Mahendra *et al.* (2007) [24] reported significant increase in serum alkaline phosphatase activity throughout the observation period in dogs during femoral fracture repaired by using of polymetha acrylate.

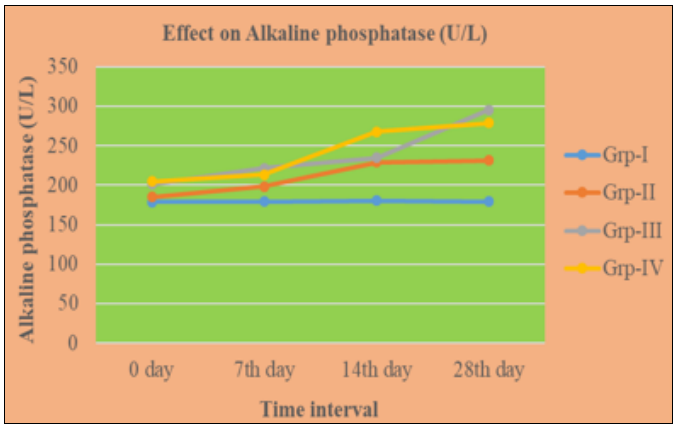

Fig 8: Mean value of alkaline phosphatase (U/L) at various time interval in different groups.

Serum Calcium (mg/dl)

The values (Mean ± SE) of serum calcium (mg/dl) at various time interval in different groups are shown in Table.2 and represented in Fig.9. The serum calcium level showed non-significant difference between group I and II, but significant difference between group III and IV at various time intervals. The values ranged from (8.66±0.19 to12.09±0.78) in all the four groups at various time intervals.

In present study, non-significant fluctuations of mean serum calcium values were observed on 7, 14 and 28-day interval in group II. Significant increase of serum calcium on 7th post-operative day and non-significant decrease of serum calcium on 14 and 28 post treatment days was noticed in group III and IV. The initial increase followed by decrease in the level of serum calcium to a greater extent in the *Cissus quadrangularis* treated group III and IV rats could be due to faster healing process with more mobilization of calcium in the formation of bridging callus. Similar findings were observed by Maiti *et al.* (1999) [16] study with use of *Cissus Quadrangularis* in accelerating healing process of experimentally fractured radius-ulna in dogs.

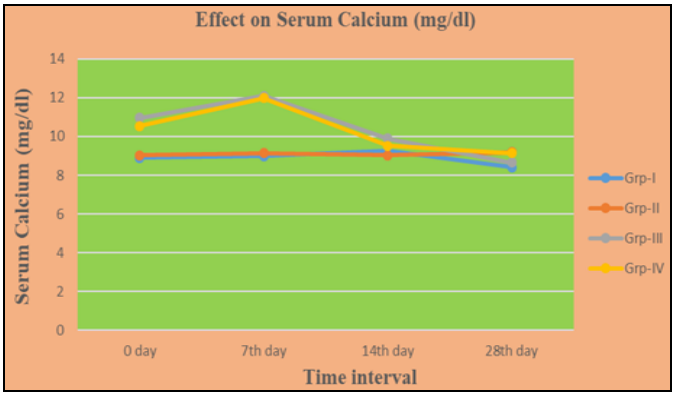

Fig 9: Mean value of Serum Calcium (mg/dl) at various time interval in different groups.

Serum phosphorus (mg/dl)

The values (Mean ± SE) of serum phosphorus (mg/dl) at various time interval in different groups are shown in Table.2 and represented in Fig.10.The serum phosphorus level showed non-significant difference between group I and II, and showed significant difference between group III and IV, at various time intervals. The values ranged from (12.72±0.29 to15.31±0.58) in all the four groups at various time intervals. In present study, non-significant increase of serum phosphorus was observed on 7 and 14 followed by decline on 28 post treatment days in group II. To its contrary significant increase of serum phosphorus on 7, 14 and 28 post treatment days was noticed in group III and IV. The increase of serum phosphorus might be attributed to osteoclastic activity leading to reabsorption of dead bone, there by resulting in increased level of serum phosphorus. Above findings are in accordance with the finding of Pandey and Udapa (1981) [25] in dogs, Rani and Ganesh (2003) [26] in goats and Jaware (2012) [27] in bovine who also reported higher value of serum phosphorus in the initial stage of fracture healing.

Table 2: Haematological parameters

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

Table 3: Biochemical parameters

Parameters	Groups	Time interval (days)			
		0	7	14	28
ALP (U/L)	I	178.81 ± 7.11^{aA}	179.13 ± 9.33^{aA}	180.21 ± 12.20^{aA}	179.36 ± 11.76^{aA}
	II	185.13 ± 17.42^{aA}	198.56 ± 8.17^{aA}	229.1 ± 11.11^{bB}	231.46 ± 9.61^{bB}
	III	201.35 ± 12.59^{aA}	221.46 ± 9.32^{abA}	234.98 ± 8.75^{bB}	295.4 ± 3.69^{cC}
	IV	204.85 ± 13.25^{aA}	213.48 ± 7.42^{aA}	267.65 ± 5.95^{bC}	278.58 ± 6.63^{bC}
Ca (mg/dl)	I	8.90 ± 0.14^{aAB}	8.98 ± 0.11^{aA}	9.01 ± 0.19^{aA}	8.92 ± 0.54^{aA}
	II	9.04 ± 0.19^{aAB}	9.13 ± 0.37^{aA}	9.03 ± 0.10^{aA}	9.18 ± 0.54^{aA}
	III	10.95 ± 0.39^{bB}	12.09 ± 0.78 ^{dB}	9.89 ± 0.13^{cB}	8.66 ± 0.19^{aA}
	IV	10.53 ± 0.25^{cA}	11.98 ± 0.43^{aB}	9.52 ± 0.13^{bB}	9.13 ± 0.86^{aA}
P (mg/dl)	I	13.29 ± 0.44^{aA}	13.18 ± 0.37^{aA}	13.67 ± 0.55^{bA}	13.52 ± 0.40^{bAB}
	II	13.09 ± 0.29^{aA}	13.33 ± 0.29^{aA}	13.49 ± 0.38^{aA}	12.72 ± 0.29^{aA}
	III	12.84 ± 0.33^{aA}	14.17 ± 0.42^{bB}	14.88 ± 0.23^{bB}	15.31 ± 0.58^{bC}
	IV	12.83 ± 0.29^{aA}	14.00 ± 0.58^{bB}	14.12 ± 0.27^{bAB}	14.46 ± 0.38^{bBC}

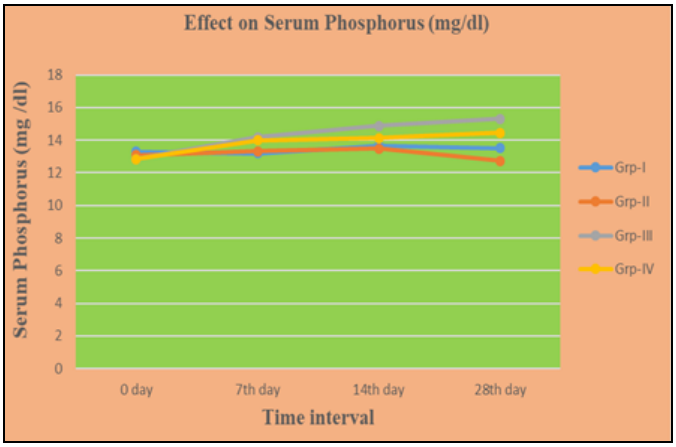

Fig 10: Mean value of Serum Phosphorus (mg/dl) at various time interval in different groups.

Conclusion

On the basis of above findings it can be concluded that, the haemato-biochemical parameters showed non-significant alterations during the process of bone healing in *Cissus quadrangularis* treated animals.

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