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Effect of *Cissus quandrangularis* on physiological parameters of long bone (Tibia) fracture healing in Wistar rats

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Abstract
The present study was conducted in the Department of Veterinary Surgery and Radiology, College of Veterinary Science and A.H, Anjora, Durg, (C.G.). A total of 24 Wistar rats were categorized into four equal groups. In present study, physiological parameters such as rectal temperature (°F) showed non-significant increase in group II, III and IV up to 2nd day post treatment which subsequently reduced to normal value by 3rd day post treatment and remained near base value up to 28 days of observation. While, the heart rate and respiration rate differed non significantly among the groups and within the groups at different time intervals when compared to base value.

Keywords: Radiological, tropical, fracture bone, rat, minutes, orally

Introduction
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. This breed was developed at the Wistar Institute in 1906 for use in biological and medical research, and is the first rat developed to serve as a model organism at a time when laboratories primarily used the common house mouse (*Mus musculus*). 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. Wistarrats are more active than Sprague Dawley rats.
Hadjod(*Cissusquadrangularis*) is one of the most common species of plants scattered all over India especially in tropical regions (Guhabakshi*et al.*, 2001)[1]. *C.quadrangularis* belongs to the family Vitaceae, which is a perennial plant commonly known as Veldgrap or Devils backbone. 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. *Cissusquadrangularis* indicates the presence of carotene, phytosterol, terpenoids, β-sitosterol, δ-amyrin, δ amyrone and calcium as confirmed by phytochemical analysis. 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. The stem juice of this plant is used to treat scurvy, menstrual disorders, otorrhoea and epistaxis.

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.). A total of 24 Wistar rats were selected and divided into four equal groups. Each group having 6 rats and experimental design as seen in table.1.

Parameters Studied
Physiological Parameters**:** These parameters were recorded before creating defect in tibia of rats and on 0, 1st, 2nd, 3rd, 7th, 14th, and 28th day post operatively.

- **Rectal temperature (°F):** The rectal temperature was recorded with clinical thermometer and it was expressed in °F.
- **Heart Rate (beats per minute):** The heart rate was recorded by auscultation with help of stethoscope.

- **Respiration rate (breaths per minute):** It was recorded by counting the thoracic respiratory movements per minute.

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) [2].

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 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

Result and discussion

Physiological parameters

Rectal temperature (°F): The values (Mean ± SE) of rectal temperature (°F) at various time intervals in different groups are shown in Table.2 and Fig.1. The mean rectal temperature (°F) in group I at 0,1,2,3,7,14 and 28 days was 98.38 ± 0.14, 98.33 ± 0.10, 98.5 ± 0.16, 98.51 ± 0.10, 98.35 ± 0.10, 98.33 ± 0.14 and 98.38 ± 0.10 respectively. In Group II the mean rectal temperature (°F) at 0,1,2,3,7,14 and 28 days was 98.56 ± 0.10, 99.28 ± 0.12, 99.35 ± 0.15, 99.06 ± 0.04, 98.85 ± 0.05, 98.38 ± 0.04 and 98.28 ± 0.07 respectively. Whereas rats of in Group III the mean rectal temperature (°F) at 0,1,2, 3,7,14 and 28 days was 98.21 ± 0.14, 98.99 ± 0.11, 98.67 ± 0.10, 98.50 ± 0.13, 98.41 ± 0.14, 98.38 ± 0.15 and 98.28 ± 0.10 respectively. In Group IV the mean rectal temperature (°F) at 0,1,2,3,7,14 and 28 days was 98.13 ± 0.15, 98.93 ± 0.13, 98.73 ± 0.12, 98.08 ± 0.13, 98.05 ± 0.14, 98.25 ± 0.15 and 98.61 ± 0.12 respectively.

Table 2: Effect on rectal temperature (^{0}F) in all the four groups at various time interval (Mean ± S.E.)

Parameter	Groups	Times intervals (days)						
		0	1	2	3	7	14	28
Rectal temperature (^{0}F)	I	$98.38^{aAB} \pm 0.14$	$98.33^{aA} \pm 0.10$	$98.5^{aA} \pm 0.16$	$98.51^{aB} \pm 0.10$	$98.35^{aAB} \pm 0.10$	$98.33^{aA} \pm 0.14$	$98.38^{aA} \pm 0.10$
	II	$98.56^{aB} \pm 0.10$	$99.28^{bB} \pm 0.12$	$99.35^{aA} \pm 0.15$	$99.06^{aB} \pm 0.04$	$98.85^{aB} \pm 0.05$	$98.38^{aA} \pm 0.04$	$98.28^{aA} \pm 0.07$
	III	$98.21^{aAB} \pm 0.14$	$98.99^{bAB} \pm 0.11$	$98.67^{abA} \pm 0.10$	$98.50^{bB} \pm 0.13$	$98.41^{bB} \pm 0.14$	$98.38^{abA} \pm 0.15$	$98.28^{abA} \pm 0.10$
	IV	$98.13^{abA} \pm 0.15$	$98.93^{cAB} \pm 0.13$	$98.73^{bcA} \pm 0.12$	$98.08^{aA} \pm 0.13$	$98.05^{aA} \pm 0.14$	$98.25^{bcA} \pm 0.15$	$98.61^{cA} \pm 0.12$

*Superscript abc indicates significant (p<0.05) value within groups at different time interval from base value.
*Superscript ABC indicates significant (p<0.05) value within groups at different time interval from base value.

Non significant difference was recorded in mean rectal temperature (°F) among the groups and within the group at different time intervals. In present study, rectal temperature (°F) showed non-significant increase in group II, III and IV up to 2nd day post treatment which subsequently reduced to normal value by 3rd day post treatment and remained near base value up to 28 days of observation. This increase might have been due to physical stress and anaesthesia induced at the time of creating fracture in tibial bone. Above findings are in accordance with the Kelly (1974)[3] and Maiti *et al.* (2011) [4] who also reported increased rectal temperature during their study on evaluation of herb, *Cissus quadrangularis* in accelerating healing process of femur osteotomesis in dogs.

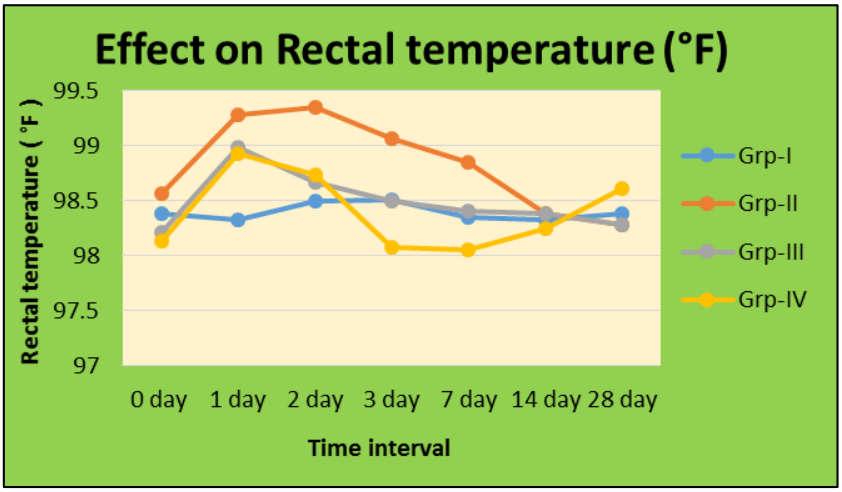

Fig 1: Mean value of Rectal temperature (°F) at various time interval in different groups

Heart rate (beats/min): The values (Mean ± SE) of heart rate (beats/min) at various time intervals in different groups are shown in Table.3 and Fig.2. The mean heart rate (beats/min) in group I at 0,1,2,3,7,14 and 28 days was 283.67 ± 4.1, 279.17 ± 3.6, 278.16 ± 5.2, 282.17 ± 3.5, 276.67 ± 3.87, 277.50 ± 3.28 and 278.17 ± 2.0 respectively. In Group II the

mean heart rate (beats/min)at 0,1,2,3,7,14 and 28 days was 273.66 ± 2.82, 276.5 ± 2.17, 274.16 ± 2.31, 280.33 ± 1.28, 274.83 ± 1.92, 280 ± 2.46 and 283.66 ± 1.25 respectively. In Group III the mean heart rate (beats/min) at 0,1,2,3,7,14 and 28 day was 274.83 ± 2.85, 280.33 ± 1.51, 281.50 ± 0.56, 274.33 ± 2.07, 278.0 ± 1.15, 281 ± 0.77 and 280.16 ± 1.09 respectively and in Group IV the mean heart rate (beats/min) at 0,1,2,3,7,14 and 28 days was 281.16 ± 5.0, 292 ± 2.90, 288 ± 5.05, 295.16 ± 2.63, 295.17 ± 2.19, 295.83 ± 2.21 and 296.67 ± 2.24 respectively.

Table 3: Effect on heart rate (beats/ mins) in all the four groups at various time interval (Mean ± S.E.)

Parameter	Groups	Times intervals (days)						
		0	1	2	3	7	14	28
Heart rate (Beats/ min)	I	$283.67^{aA} \pm 4.01$	$279.17^{aA} \pm 3.6$	$278.16^{aAB} \pm 5.2$	$282.17^{aB} \pm 3.5$	$276.67^{aA} \pm 3.87$	$277.50^{aA} \pm 3.28$	$278.17^{aA} \pm 2.21$
	II	$273.66^{aA} \pm 2.82$	$276.5^{aA} \pm 2.17$	$274.16^{aA} \pm 2.31$	$280.33^{abAB} \pm 1.28$	$274.83^{aA} \pm 1.92$	$280^{abA} \pm 2.46$	$283.66^{bB} \pm 1.25$
	III	$274.83^{aA} \pm 2.85$	$280.33^{bA} \pm 1.51$	$281.50^{bAB} \pm 0.56$	$274.33^{aA} \pm 2.07$	$278.0^{abA} \pm 1.15$	$281^{bA} \pm 0.77$	$280.16^{bAB} \pm 1.09$
	IV	$281.16^{aA} \pm 5.0$	$292^{bB} \pm 2.90$	$288^{abB} \pm 5.05$	$295.16^{bC} \pm 2.63$	$295.17^{bB} \pm 2.19$	$295.83^{bB} \pm 2.21$	$296.67^{bC} \pm 2.24$

*Superscript abc indicates significant (p<0.05) value within groups at different time interval from base value.
*Superscript ABC indicates significant (p<0.05) value within groups at different time interval from base value.

Non significant differences were recorded in heart rate among the groups and within the groups at different time intervals when compared to base value. In present study, heart rate (beats/min) fluctuated within normal physiological limits post treatment in group II, III and IV. The present findings simulate with the observations recorded by Varshney *et al.* (2004)[5] and Maiti *et al.* (2011)[4] after evaluating *Cissus quandgularis* accelerating healing effect on femur osteotomies in dogs.

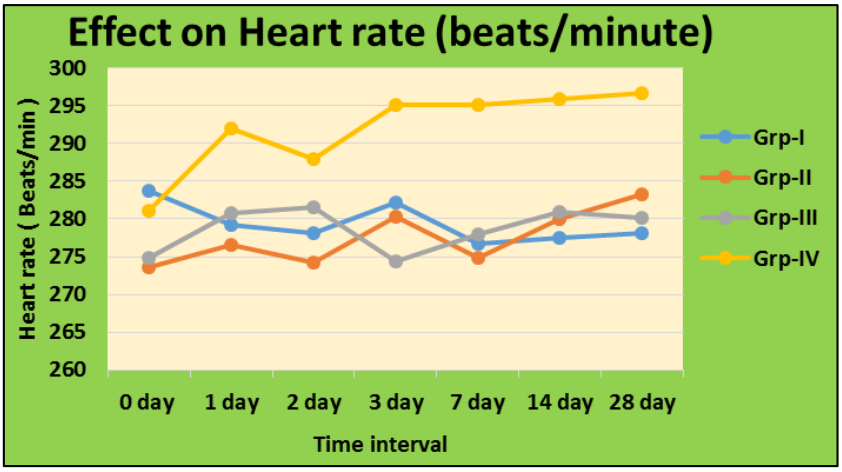

Fig 2: Mean value of Heart rate (beats/minute) at various time interval in different groups

Respiration rate (breaths/min): The values (Mean ± SE) of respiration rate (breaths/min) at various time intervals in different groups are shown in Table. 4 and Fig. 3.
The mean respiration rate (breaths/min) in group I at 0,1,2,3,7,14 and 28 days was 81.33 ± 4.09, 83.16 ± 3.59, 80.33 ± 5.19, 82.0 ± 3.49, 82.16 ± 3.87, 79.83 ± 3.28 and 82.16 ± 2.21 respectively. In Group II the mean respiration rate (breaths/min) at 0,1,2,3,7,14 and 28 days was 80.5 ± 2.82, 82.0 ± 2.17, 79.83 ± 2.31, 75.66 ± 1.28, 80.50 ± 1.92, 80.56 ± 2.46 and 80.43± 1.25 respectively. In Group III the mean respiration rate (breaths/min) at 0,1,2,3,7,14 and 28 days was 76.83 ± 2.85, 80.0 ± 1.51, 81.5 ± 0.56, 80.0 ± 2.07, 79.86 ± 1.15, 79.0 ± 0.77 and 78.55 ± 1.01 respectively and in Group IV the mean respiration rate (breaths/min) at 0,1,2,3,7,14 and 28 days was 82.16 ± 5.0, 86.50 ± 2.88, 85.70 ± 5.05, 85.33 ± 2.63, 85.0 ± 2.19, 83.83 ± 2.21 and 84.16 ± 2.24 respectively.

Table 4: Effect on respiration rate (breaths/ mins) in all the four groups at various time interval (Mean ± S.E.)

Parameter	Groups	Times intervals (days)						
		0	1	2	3	7	14	28
Respiration rate (Breaths/ min)	I	$81.33^{aA} \pm 4.09$	$83.16^{aA} \pm 3.59$	$80.33^{aAB} \pm 5.19$	$82.0^{aB} \pm 3.49$	$82.16^{aA} \pm 3.87$	$79.83^{aA} \pm 3.28$	$82.16^{aA} \pm 2.21$
	II	$80.5^{aA} \pm 2.82$	$82.0^{aA} \pm 2.17$	$79.83^{aA} \pm 2.31$	$75.66^{aAB} \pm 1.28$	$80.50^{aA} \pm 1.92$	$80.56^{aA} \pm 2.46$	$80.43^{aB} \pm 1.25$
	III	$76.83^{aA} \pm 2.85$	$80.0^{abA} \pm 1.51$	$81.5^{bAB} \pm 0.56$	$80.0^{abA} \pm 2.07$	$79.86^{bA} \pm 1.15$	$79.0^{abA} \pm 0.77$	$78.55^{bAB} \pm 1.01$
	IV	$82.16^{aA} \pm 5.0$	$86.50^{aB} \pm 2.88$	$85.70^{aB} \pm 5.05$	$85.33^{aC} \pm 2.63$	$85.0^{aB} \pm 2.19$	$83.83^{aB} \pm 2.21$	$84.16^{aC} \pm 2.24$

*Superscript abc indicates significant (p<0.05) value within groups at different time interval from base value.
*Superscript ABC indicates significant (p<0.05) value within groups at different time interval from base value.

Non significant differences were recorded in respiration rate among the groups and within the group at different time intervals. In present study, respiration rate (breaths/min) fluctuated within normal physiological limits post treatment in group II, III and IV which corroborates with the findings recorded by Varshney *et al.* (2004) [5] using *Cissus Quandargularis* following fracture healing in dogs.

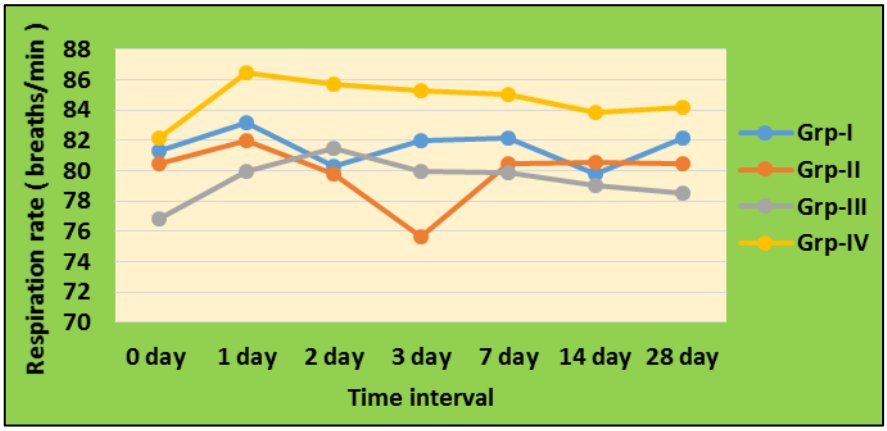

Fig 3: Mean value of Respiration rate (breaths/minute) at various time interval in different groups

Conclusion

It is concluded that the oral administration of *Cissus quandrangularis* showed accelerated fracture healing with complete bridging of fracture gap as compared to its topical application as evident by radiographs. The physiological parameters showed non-significant alterations during the process of bone healing in *Cissus quandrangularis* treated animals.

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Conflict of interest: The authors declare that they have no conflict of interest.

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