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Research Article

FRACTURE HEALING ACTIVITY OF ETHANOLIC EXTRACT OF *LEPIDIUM SATIVUM L.* SEEDS IN INTERNALLY FIXED RATS' FEMORAL OSTEOTOMY MODEL

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ABSTRACT

The present study was designed to investigate of possible potential fracture healing activity of 400 mg/kg ethanolic extract of *Lepidium sativum* L. seeds in internally fixed rats' femoral osteotomy model. The experimental protocol designed as the animals were divided into two groups (n=6) like control and test groups and was administrated vehicle and extract, respectively. X–Ray method was use for of assessment of bone healing activity. It was taken for each animal of control and test group at 2nd, 4th and 8th weeks. After fourth week, X-ray photographs shows that test group had significantly larger callus formation and more disposing of osseous material as compare to control group. After eight weeks, X-Ray photographs indicated that the fractures bone of test group animals had seen as complete joined but not in control group. The present study data revealed that ethanolic extract of *Lepidium sativum* seeds may be significant fracture healing activity.

Keywords: *Lepidium sativum*; osteotomy, K- wire, Calcification; x-Rays

INTRODUCTION

The *Lepidium sativum* (L) (family- Brassicaceae) is a native shrub. It seeds contain volatile essential aromatic oils, active principle and fatty oils and carbohydrate, protein, fatty acid, Vitamin: β-carotene, riboflavin, and niacin, and ascorbic acid, Flavonoids, Isothiocynates glycoside [1]. A literature survey reveals that it has been reported that bronchodilator effect of *Lepidium sativum* against allergen induced bronchospasm in guinea pigs. [2], hypoglycemic activity of *Lepidium sativum* L. aqueous extract in normal and diabetic rats [3], and Antihypertensive effect of *Lepidium sativum* L. in spontaneously hypertensive rats [4]. Some diagnostic agent, drugs and enzyme that effected or having fracture healing activity like diagnostic ultrasound treatment increases the bone fracture-healing rate in an internally fixed rat femoral osteotomy model [5]. Cyclo-oxygenaes-2 function is essential for bone fracture healing [(6)], treatment of zinc increase in bone protein components and enhancement fractures healing in rat [(7)] and inhibition of fracture healing by indomethacin in rats [(8)].

Lepidium sativum seeds were publicly used in Saudi Arabia as a traditional medicine, mostly for the treatment of recent traumatic fracture [(9, 10)]. Good results of healing of fractures were observed over decades in the hands of traditional folk medicine practitioners.

The aim of the present study is to investigate the effect of *Lepidium sativum* seeds on fracture healing activity in albino rats.

MATERIALS AND METHODS

Collection and authentication of plant materials

The mature *Lepidium sativum* seeds were collected from Mandsaur district, M.P. The plant was identified by Dr. H.S. Chattree (Ex professor of botany), P. G. College of Mandsaur, and M.P. And voucher specimen (BRNCP /L/02/2006) was submitted in department of pharmacognosy; BRNCP, Mandsaur, M.P.

Sample preparation and extraction

The trampled *Lepidium sativum* L. seeds were extracted by soxhlet apparatus using ethyl alcohol as a solvent. The extract was dried in rotator evaporator under reduced pressure.

Phytochemical screening

Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides, amino acid & protein and flavonoids [(11, 12, 13, 14, 15, and 16)] (Shown table no. 1).

a

b

c

a = I.P. injection of ketamine HCl for anaesthesia; b = Right femoral Osteotomy rat by threaded blade; c= Fixation of fractured bone by kirschner (k) wire

Animals

Adult male wistar rats having weight around 150-200 g were maintained at 25 ± 2°C, kept in well ventilated animal house under photoperiodic condition in large polypropylene cages, standard food and water *ad libitum*. The experiment was carried out in accordance to the guidelines mentioned in the CPCSEA, and Institutional Animal Ethical Committee approved the experiment protocols (Reg.No.-918/ac/05/CPCSEA).

Experimental design

The acute toxicity study of ethanolic extract of *Lepidium sativum* seeds L. was not occurred at 2000mg/kg (as per the OECD - 420) on male Wistar rats. One fifth dose of 2000mg/kg was selected regarding toxicity study. The experimental animals were divided into two groups each of six animals. Group-I served as control and received normal saline throughout the experiment. Group-II (test) received (400mg/kg p.o.) ethanolic extract for 8 weeks. They were undertaken overnight fasting and anaesthetized by 40 mg/kg per i.p. ketamine hydrochloride, under sterile operating condition. Surgically, 1 to 1.5 cm. incision was made over the lateral thigh of right leg. The femur was exposed after blunt dissection and an osteotomy was performed with threaded blade. The fractured right femur bone was fixed with intramedullary kirschner (k) wire [(5)].

Method of assessment

X-Ray photographs

X–Ray method was use for of assessment of bone healing. The X-rays photographs were taken by Mediatronics 100 mA, machine. X-ray photographs were taken for each animal of control and test group at 2nd, 4th and 8th weeks. The fracture healing activity is assessed on basis of calcification.

Statistical analysis

The study was ended and the animals were sacrificed followed by collection of data, tabulation, and statistical analysis with the SPSS package.

RESULTS

Phytochemical screening of ethanolic extracts of *Lepidium sativum* L. Seeds

The powder of extract was used to perform various phytochemical tests. So various phytochemical was present in ethanolic extract of *Lepidium sativum* seeds (shown table no.2).

X-Ray photographs of right femur bone of rats after 8th weeks of osteotomy:

The results of X-ray photographs taken at 2nd, 4th and 8th week demonstrated a continuous significant increase in callus formation in test group when compared to control group. (Shown fig. no. 1, 2, 3,).

Statistical analysis

Its data shown that the P values of the control and test groups at 4 weeks were P = NS, P = NS, and P < .001 in the longitudinal lateral (LL), longitudinal medial (LM), and circumferential (CM) callus measurements, respectively.

The P values at 8 weeks had more significant areas of P < .045 and P < .005 in the LM and CM callus, respectively. The LL callus remained no significant (P = NS).

DISCUSSION

The phytochemical study of the ethanolic ELS seeds has revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, and amino acids like glutamine, cysteine, and glycine. Hence fracture healing activity of *Lepidium sativum* seeds were possible by one or more of these constituents, such as fatty acids, [(17)] protein [(18)], or through their activities [(19)] as well as possible incorporation in the biological activities similar to the stable isotope tracers. [(20)].

The X-ray photographs taken at 2nd, 4th and 8th week demonstrated a continuous significant increase in callus formation in test group when compared to control group. Finally, the full recovery in test group was obtained in the 8th week.

This result of calcification may be due to ethanolic extract of *Lepidium sativum* (ELS) acting as antinflammatory agent and may produce collagen. This observation was supported by the presence of glycine residues at every third position of triple helix (Gly- X- Y) n portion of alpha chain and form fibril. The fibril is constituent of collagen I and collagen II. The Collagen I is approximately 90 % of total bone protein [(21)].

Kolodziejski et al., (1969) said their activity due to fatty acid, the present study data cannot support it because of ethanolic extract of *Lepidium sativum* was defatted.

Burghardt et al. (1994), & Koropp et al. (1994) said that their activity due to protein, the present study data also support it because of cysteine, glycine, and glutamate were observed in ethanolic extract *Lepidium sativum* seeds. Collagen synthesis was occurrence of glycine residues at every third position of triple helix (Gly- X- Y) n. Osteoblasts are synthesized by collagens which form a matrix that traps cells. Osteoblasts were gradually differentiated to become osteocytes this may be possible mechanism of fracture healing of *Lepidium sativum* seeds. The present study data revealed that ethanolic extract of *Lepidium sativum* L. seeds may contribute to significant fracture healing activity.

Table 1: Phytochemical tests of ethanolic extract *Lepidium sativum* seeds

S.No.	Name of components	Name of chemical tests	Observation
1.	**Test of glycosides**		
1.1	Cardiac glycosides	Legal test	Pink colour
		Keller - Killiani test	Reddish-Brow Colour appears at junction of two layers
1.2	Test of anthroquinone glycoside	Brontrager test	Ammonia layer turn pink to red Colour
1.3	Test of cynogegenetic.	Sodium picrate test	Filter paper turns Brink-red.
1.4	Test of Flavonoids	Shinoga test	Pink Colour
1.5	Test of Saponin	Foam and heamolytic test	Persistent foam & heamolytic zone appears
2.	**Test of alkaloids**		
2.1		Dragendraff's test	Orange-brown Ppt
2.2		Wagner test	Reddish-brown Ppt
2.3		Hager test	Yellow Ppt.
2.4		Mayer	Ppt
3.	**Test of Tannins and Phenolic Compounds**		
3.1		5% FeCl3 Solution	Deep blue-black ppt.
3.2		Dil. Iodine Solution	Transient red colour
3.3		Dil. HNO3 Test	Reddish to Yellow colour (+ve)
4.	**Test of protein and amino acid Compounds**		
4.1		Biuret test	Violet colour(+ve)
4.2		Millions test	Red colour(+ve)
4.3	Test of sulpher contain aminoacid.	PbS Ppt	Brownish blue colour(+ve)
4.4	Test of Tyrosine	3 drops millon's reagent	Dark red colour

4.5	Test of Tryptophan	Few drops Glyoxalic acid + conc. H2SO4	reddish ring appeared at junction of two layer
4.6	Test of Glycine	Aq CuSO4 + Fecl3	Red colour
4.7	Test of Cysteine		Black Colour
4.8	Test of Glutamine	CuSO4	Deep blue colour
5.	**Test of Steroid**	Salkowshi reaction	Chloroform layer appear, red and blue
6.	**Test of reducing glycoside**	Benedict's test	Green and red Colour
7.	**Test of non reducing Starch**	Iodine test	Blue colour

Table 2: Phytochemical screening of ethanolic extract of *Lepidium sativum* seeds

S.No.	Name of Tests	Results
1.	**Glycosides**	+ve
	Cardiac glycosides	+ve
	Anthroquinone glycoside	+ve
	Cynogegenetic	+ve
	Flavonoids	+ve
	Coumarin glycoside	-ve
	Saponin glycoside	-ve
2.	***Alkaloids***	+ve
	Dragendraff's test	+ve
	Wagner test	+ve
	Hager test	+ve
	Mayer test	+ve
3.	**Tannins and Phenolic Compound**	+ve
4.	**Proteins**	+ve
	Sulpher contain aminoacid	+ve
	Biuret test	+ve
	Millions test	+ve
	Tyrosine	-ve
	Tryptophan	-ve
	Glycine	+ve
	Cysteine	+ve
	Glutamine	+ve
5.	Steroid	+ve
6.	Reducing glycoside	+ve
7.	Non reducing starch	-ve

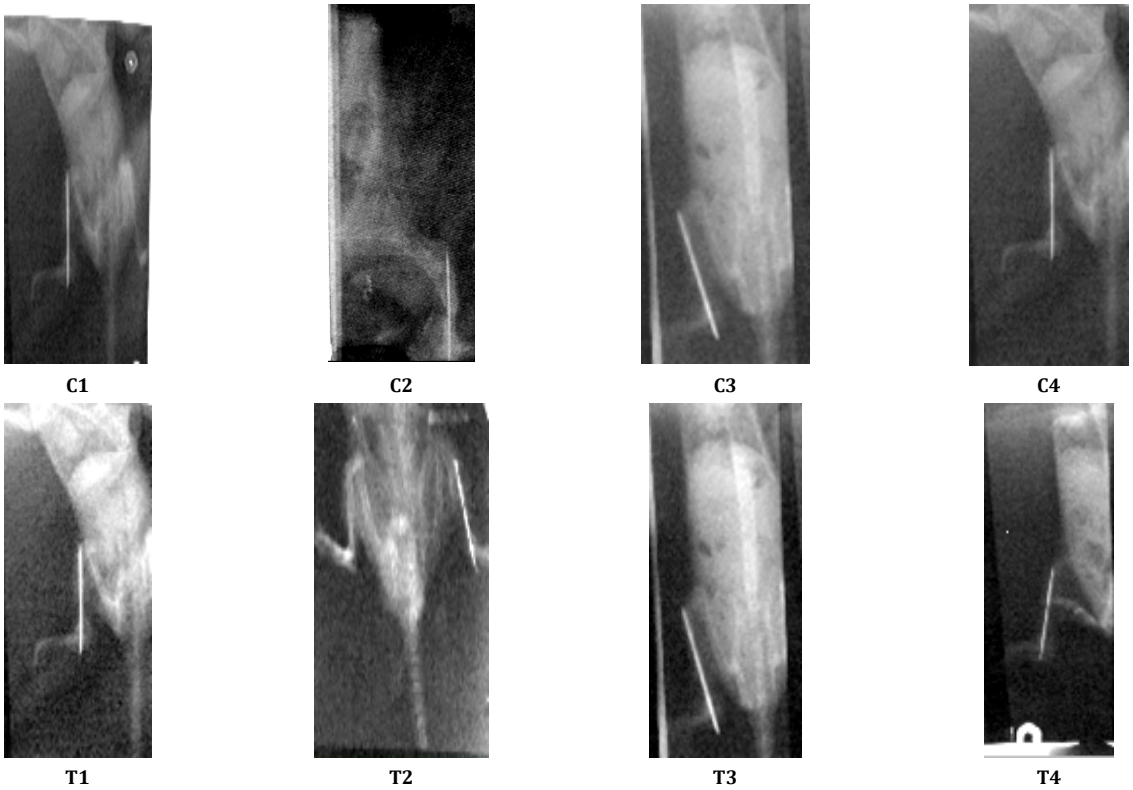


Fig 1: X-Ray photographs of right femur bone rats after 4th weeks of osteotomy

(Control groups= C1, C2, C3, C4 and test group= T1, T2, T3, T4)

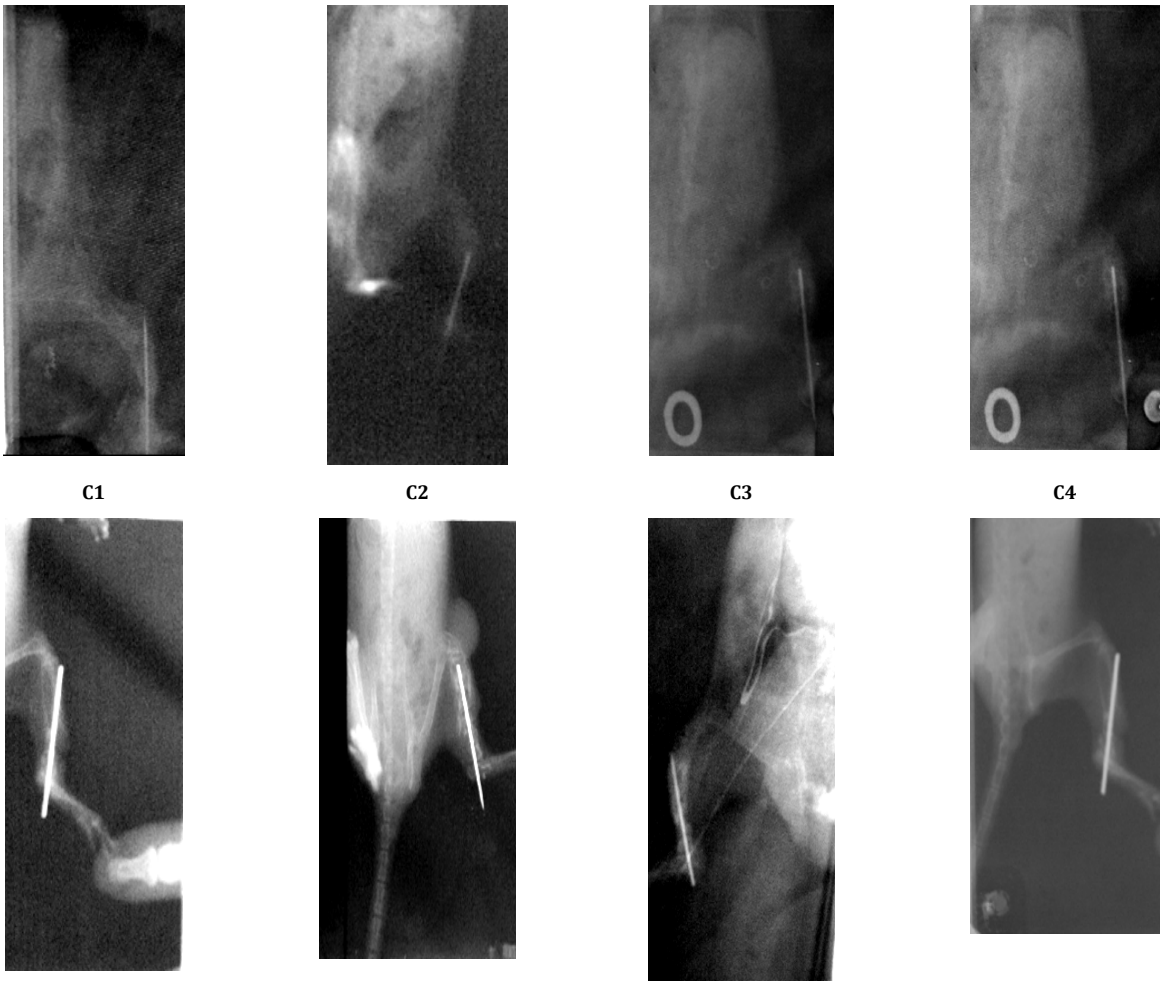
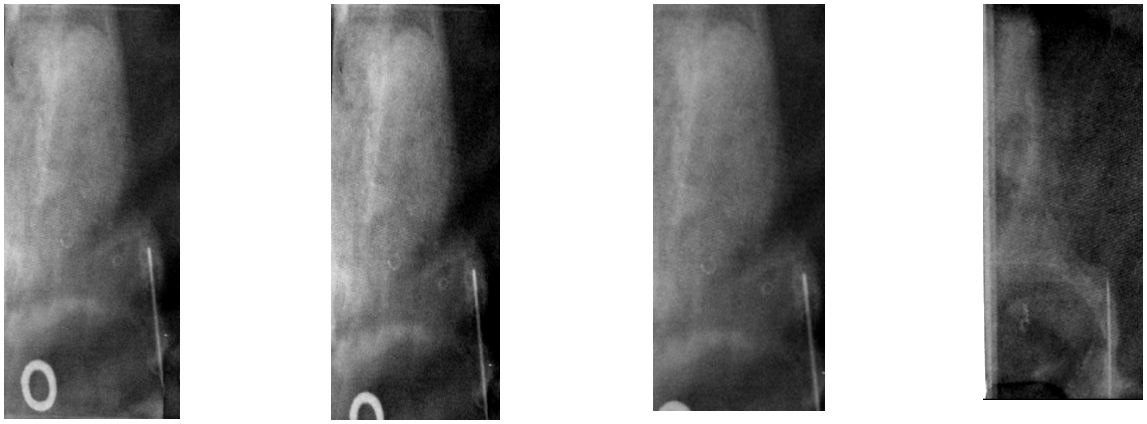
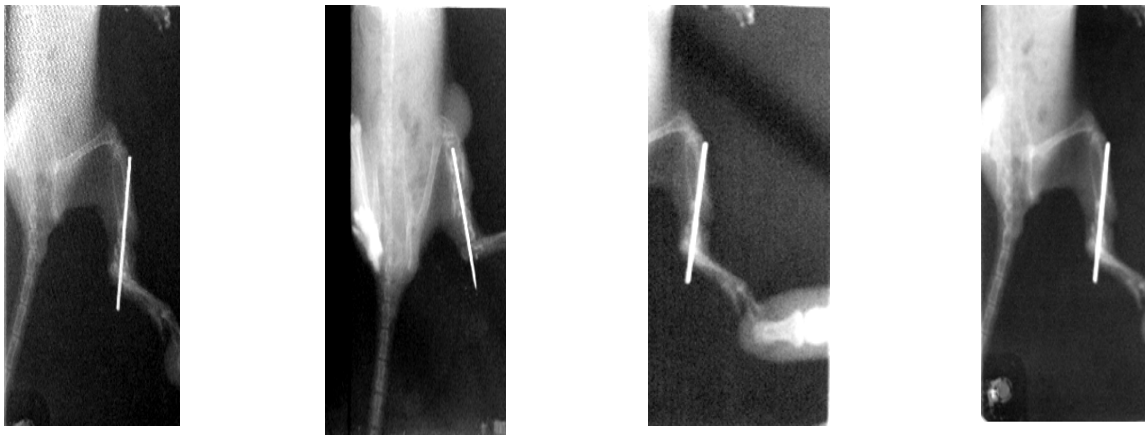


Fig. 2: X-Ray photographs of right femur bone rats after 4th weeks of osteotomy:

(Control groups= C1, C2, C3, C4 and test group= T1, T2, T3, T4)





T1 **T2** **T3** **T4**

Fig. 3: *X -Ray photographs of right femur bone of rats* after 8th weeks of osteotomy

(Control groups= C1, C2, C4, C4 and test group= T1, T2, T3, T4)

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