

## Wound healing activity of human placental extracts in rats

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**KEY WORDS** placental extracts; wound healing; animal disease models; lethal dose 50

### ABSTRACT

**AIM:** To study wound healing activity of the human placental extract (HPE) in rats. **METHODS:** Full thickness wounds were inflicted on depilated dorsum of Charles foster rats with 8 mm Acu-punch biopsy. The HPE was applied both at topical and im routes (2.5 mL/kg). Effects were compared on the basis of physical criteria, biochemical criteria, and histopathological study with respect to untreated control, vehicle control (1.5 % benzyl alcohol), and framycetin topical treated groups. **RESULTS:** Significant lowering of wound size ( $P < 0.05$ ), wound index ( $P < 0.05$ ), and number of days required for complete healing ( $P < 0.01$ ); significant gain in tensile strength ( $P < 0.01$ ); appreciable increase of tissue DNA, total protein, and collagenesis were observed in HPE treated group. **CONCLUSION:** Human placental extract systematically helps collagenesis leading to potent healing of wounds.

### INTRODUCTION

It is reported that one of the major components of human placental extract (HPE) is polydeoxyribonucleotide (PDRN)<sup>[1]</sup>. It is enriched in enzymes like alkaline and acid phosphatase, glutamic oxaloacetic acid transaminase (GOT); RNA, DNA, and ATP; vitamins like B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, pantothenic acid, biotin, PABA, folic acid, B<sub>12</sub>, choline, and inositol; amino acids like alanine, aspartic acids, cystein, histidine, leucine, lysin, phenyl alanine, proline, serine, threonine, tryptophan, valine, and tyrosine; steroids like 17-

ketosterol, cholestrin, and cholesterol; fatty acids and elements like Na, K, Ca, Mg, Cu, Fe, P, and Si<sup>[2]</sup>. All these components may exert multiple biological activities. The crude extract of human placenta shows corticotropin releasing factor (CRF)-like activity<sup>[3]</sup>. It has been proved to be promoter of human epidermal keratinocyte proliferation<sup>[4]</sup>. HPE is being widely marketed in India for wound healing, immunotropic and anti-inflammatory activities. The present programme was undertaken to evaluate pharmacologically the wound healing activity of HPE in animal model.

### MATERIALS AND METHODS

**Test drug** Human placentas weighing between 400–600 g collected at the time of full term spontaneous delivery were immediately placed under ice, then the amniotic membrane and umbilical cord were removed, minced into small pieces and washed with cold normal saline. Aqueous extract with these pieces of placenta was prepared, sterilized and sealed in ampules (2 mL) under inert condition. Each mL of the extract in the ampule was derived from 0.1 g of placenta. This extract contains protein (0.95 g/L), DNA (2.8 mg/L), RNA (1.6 mg/L), Na<sup>+</sup> ion (27.9 mmol/L), K<sup>+</sup> ion (3.07 mmol/L), and Cl<sup>-</sup> ion (15.1 mmol/L).

**Acute toxicity (LD<sub>50</sub>)** HPE (Placentrex<sup>®</sup>, M/S Albert David Ltd, Calcutta, India), the aqueous extract of human placenta, was administered at 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mL per 20 g of mice in intra peritoneal route. The studies were carried out continuously for 72 h.

**Animal** Charles foster male albino rats weighing 180–200 g were taken. Animals were housed in aseptic polypropylene cages in normal room temperature and atmosphere. They were supplied pellet diet and water *ad libitum*.

**Preparation of animal** For this purpose animals were anaesthetized with injection thiopentone sodium at a

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Received 2001-02-05

Accepted 2001-06-14

dose of 35 mg/kg in ip route. The wide area of the dorsum of each rat was then depilated with a pair of sterilized scissors and scalpel blade. Thereafter, the depilated dorsum of each rat was cleanly dressed with 70 % ethyl alcohol.

**Infliction of wound** Two full thickness punch wounds of 8 mm diameter were inflicted on either side of the midline of depilated dorsum to each anaesthetised animal with acu-punch biopsy instrument (Acuderm Inc, Ft Lauderdale, FL 33309, USA).

**Treatment** Animals were divided into four groups each comprising of ten animals. Each group received different treatments like HPE topical, HPE in im route at a dose of 2.5 mL/kg, framycetin antibiotic cream (Soframycin, Roussel India, Thane 400613, India) in topical route, and one group left untreated for control. Four animals from each group were sacrificed on d 5 for different biochemical and histological estimation. The rest six animals of individual group were continued treatment. The dose of HPE in im route was determined as about 1/10th of the safe dose used for LD<sub>50</sub> study in mice using the calculation according to Frederich *et al*<sup>(5)</sup>.

**Wound contraction** The wound contraction size was measured with mm scale daily.

**Wound index** Wound index was measured daily with an arbitrary scoring system<sup>(6)</sup>(Tab 1).

Tab 1. Arbitrary scoring system for the measurement of wound index.

Gross changes	Wound index
Complete healing of wounds	0
Incomplete but healthy healing	1
Delayed but healthy healing	2
Healing has not yet been started but the environment is healthy	3
Formation of pus-evidence of necrosis	4
Total	10

**Tensile strength** Tensile strength of healed wound tissue of different treatment groups were measured after complete healing of each wound with tensiometer (M/S Excel Enterprises, Calcutta, India).

**Total protein, DNA, RNA, and hydroxyproline** Amount of total protein, DNA, RNA and hydroxyproline are considered as the marker of the wound healing<sup>(7)</sup> and these were estimated by conventional methods on equivalent amount of the tissue collected by acu-punch from the healed area.

**Histopathological study** The histopathological changes with different treatments were observed by preparing slides from the tissues collected on d 5 of treatment of each group. Tissues were collected in 10 % formalin solution and staining was done with haematoxylin & eosin.

**Statistical analysis** The result of the different parameters were analyzed statistically using Student's *t*-test method.

## RESULTS

**Acute toxicity (LD<sub>50</sub>)** From this study it was observed that the HPE is safe upto 45 mL/kg body weight in intra peritoneal route on mice.

**Wound healing activity of HPE on wound size, wound index, healing period, and tensile strength** The mean decrease of wound size on d 3, 5, and 7 of each animal in different treatment groups was recorded. It was observed that there was most rapid closure of wounds (3.0 mm,  $P < 0.01$ ) with HPE in topical route. Mean wound size in HPE im route and framycetin were significantly decreased as compared with untreated control group ( $P < 0.05$ , Tab 2).

Tab 2. Effect of human placental extract (HPE) on wound healing in different treatment groups.  $n = 6$  rats.  $\bar{x} \pm s$ .  $^bP < 0.05$ ,  $^cP < 0.01$  vs untreated control.

Groups	Wound size/mm	Wound index	Days required for complete healing	Tensile strength/g
HPE topical	3.0 ± 1.3 <sup>a</sup>	1.9 ± 0.8 <sup>b</sup>	12.5 ± 2.0 <sup>c</sup>	440 ± 84
HPE im	3.6 ± 1.0 <sup>b</sup>	2.1 ± 0.6 <sup>b</sup>	12.2 ± 1.6 <sup>c</sup>	594 ± 66 <sup>c</sup>
Framycetin topical	3.4 ± 1.2 <sup>b</sup>	2.0 ± 0.7 <sup>b</sup>	10.5 ± 1.0 <sup>c</sup>	503 ± 88 <sup>b</sup>
Untreated control	4.6 ± 0.5	2.7 ± 0.3	16.7 ± 1.6	360 ± 176

The mean wound indices of each animal of different treatment groups were measured on d 3, 5, and 7. Better result was obtained in HPE topical treated group with mean wound index 1.9 ( $P < 0.05$ ). Mean wound indices of HPE in im route, framycetin were significantly decreased as compared with untreated control group ( $P < 0.05$ , Tab 2).

Regarding the mean number of days of complete healing, the least number was required in framycetin treated group (10.5 d) and days in HPE by topical and im route were significantly high ( $P < 0.01$ ) with respect

to the value of untreated control group (Tab 2).

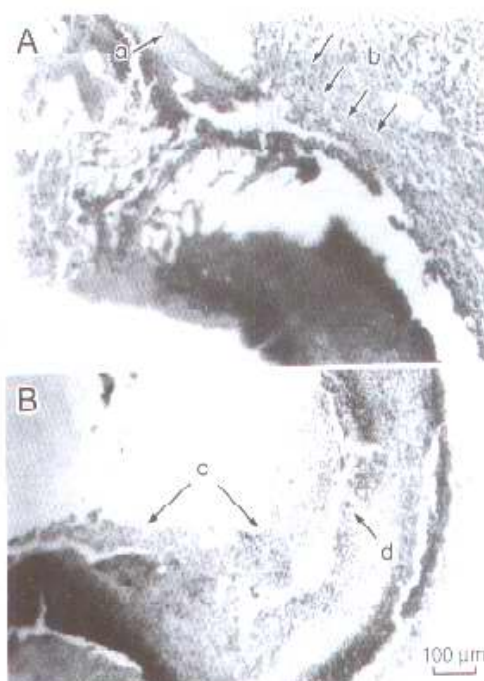
The mean tensile strength was found maximum (594 g) with HPE in im route. Mean tensile strength of framycetin topical group was significantly higher than untreated control group ( $P < 0.05$ , Tab 2).

**Estimation of DNA, RNA, total protein, and hydroxyproline** The amount of mean DNA and RNA was found to be significantly high both in the HPE topical and im treated groups ( $P < 0.01$ ). The value of DNA and RNA in framycetin was also found to be statistically significant ( $P < 0.01$ ). The mean total protein level was maximal in HPE im treated group. There was also substantial increase in HPE topical and framycetin treated groups and all these values were statistically highly significant ( $P < 0.01$ ) with respect to that of untreated control group. The mean hydroxyproline level (mg/g) were found to be statistically highly significant ( $P < 0.01$ ) in all the three treated groups with respect to untreated control group (Tab 3).

**Histopathological attributes** Histopathologically the treatment with the HPE in intra muscular route showed that there were ample infiltration of the collagen fibrils (fibrous cells) and epithelization. In HPE topical treated group there was enough fibrogenesis but not to the same extent of HPE im treated group. Topically framycetin treated group showed good amount of fibrogenesis. In the untreated control group, there was ulcer in the epidermis layer; maximum migration of inflammatory cells and accumulation of oedematous fluids in dermis layer (Fig 1). These indicated immature healing of wounds in this group.

## DISCUSSION

Modern human life is exposed to different kinds of physical injuries leading to varieties of "wound"<sup>(6)</sup>. Basically wound is a surgical problem but it can be



**Fig 1.** Photomicrograph of healed wound tissue on the 5th day. **A:** HPE im treated group showed epithelization (a) and maximum infiltration of collagen fibrils (b). **B:** untreated control group showed presence of ulcer on epidermis layer (c) and maximum infiltration of inflammatory cells on dermis layer (d). HE stain,  $\times 100$ .

manipulated by clinical management. However, no pharmacological work has so far been carried out in the full thickness punch wound in animal model with any extract of human placenta in a systematic way. This necessitated the present study with HPE for its experimental wound healing activity.

The LD<sub>50</sub> of HPE in ip route on mice was found to be  $> 45$  mL/kg body weight, much higher than tolerable dose. The chosen therapeutic dose in rat for wound

**Tab 3.** Biochemical values of wound healing in different treatment groups.  $n = 6$  rats.  $\bar{x} \pm s$ .  $^*P < 0.01$  vs untreated control.

Groups	DNA (mg/g)	RNA (mg/g)	Total protein (mg/g)	Hydroxyproline (mg/g)
HPE topical	$1.52 \pm 0.18^*$	$0.98 \pm 0.08^*$	$14.2 \pm 0.8^*$	$3.51 \pm 0.16^*$
HPE im	$1.53 \pm 0.18^*$	$1.00 \pm 0.03^*$	$14.4 \pm 0.7^*$	$3.90 \pm 0.20^*$
Framycetin topical	$1.16 \pm 0.90^*$	$0.500 \pm 0.018^*$	$13.5 \pm 0.6^*$	$3.10 \pm 0.13^*$
Untreated control	$0.88 \pm 0.04$	$0.380 \pm 0.006$	$6.20 \pm 0.18$	$1.60 \pm 0.04$

healing activity in im route was calculated to be 2.5 mL/kg body weight following the standard calculation of Frederich *et al*<sup>[5]</sup>.

Development of an ideal wound-healing drug is still a challenge to the medical scientists. The ideal drug should fulfil the criteria such as rapid contraction of wound leading to quick healing, reduction of wound index and appreciable gain of tensile strength. Biochemically, the tissue DNA, RNA, total protein, and hydroxyproline will be the marker of good healing property of the drug. The cell proliferation can best be judged on the basis of histological study. Maximum increase in tensile strength and nucleic acids (DNA & RNA) in HPE im treated group indicated the synthesis of collagen fibres in largest amount and good nuclear repairment. The cytoplasmic repairment was revealed from regeneration of protein in appreciable amount. The efficiency of formation of collagen depends mainly on the synthesis of hydroxyproline<sup>[9]</sup>, which was also recorded to be highest in HPE im treated group indicating better healing. This was further substantiated from the picture of histopathological changes showing maximum accumulation of collagen fibrils and epithelization.

In a nutshell, it appears from our experiment that HPE in im route has potent power of inducing high collagenous growth indicating its proficiency in wound healing. The growth promoting role of HPE is manifested probably either by stimulation of the growth factor(s) signals cascade system at the cellular level or itself may act as growth factor(s).

**ACKNOWLEDGEMENTS** Authors are indebted to Dr GK PATNAIK (deceased) and Dr Anil K BALAPURE of Central Drug Research Institute, Lucknow; Prof (Mrs) B MARJIT, Department of Anatomy, Institute of Post Graduate Medical Education and Research, Calcutta; Dr MK MAJUMDAR, Central Drug Laboratory, Calcutta; and Mr Utpal BOSE, Saha Institute of Nuclear Physics, Calcutta for various help.

The laboratory facilities were kindly provided by the Head of the Department of Pharmacology, University College of Medicine, Calcutta. Cordial thanks are extended to Albert David Ltd, Calcutta for funding the research programme.

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## 人胎盘提取物对大鼠伤口愈合的作用

**关键词** 胎盘提取物; 伤口愈合; 动物疾病模型; 半数致死量

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