

EFFECT OF HEPATOGARD – AN INDIGENOUS FORMULATION ON DEXAMETHASONE INDUCED ANTIHEALING EFFECTS IN MALE ALBINO RATS

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Abstract : Hepatogard ®, is a multi-ingredient phytopharmaceutical product containing crude powders of eleven plants. Its effect on the different parameters of wound healing was assessed alone and in the presence of dexamethasone. The parameters chosen for the study were the breaking strength of incised wound, breaking strength of granulation tissue and hydroxyproline content. The result showed that Hepatogard increased the breaking strength of granulation tissue but not of incised wound. It reversed the dexamethasone induced decrease in breaking strength in both incised wound and granulation tissue. Even though it had no effect of its own on hydroxyproline concentration, it reversed the dexamethasone induced decrease in the hydroxyproline content of granulation tissue. Thus, Hepatogard has the potential for antagonizing the antihealing effect of steroids in patients receiving steroid therapy.

Key words : hepatogard
wound healing

dexamethasone
hydroxyproline

INTRODUCTION

Hepatogard is a formulation, comprising of the crude powders of *Picrorhiza kurroa* (125 mg), *Andrographis paniculata* (125 mg), *Azadirachta indica* (125 mg), *Terminalia chebula* (41.67 mg), *Terminalia belerica* (41.67 mg), *Phyllanthus emblica* (41.67 mg), *Eclipta alba* (125 mg), *Zingiber officinalis* (125 mg) *Boerhaavia diffusa* (250 mg), *Phyllanthus amarus* (125 mg) and *Piper longum* (65 mg).

Hepatogard is known to be hepatoprotective (1). It is believed to reduce

lipid peroxidation and increase cytochrome P-450 (2) thereby preventing or slowing the onset of necrosis. It also has the capacity to reduce fibrosis which is reflected by the decrease in the level of hydroxyproline (3).

Since the prevention of necrosis and fibrosis may add to the healing of any type of wound or injury, Hepatogard was tried in different wound models in rats. The main aims of this study were to assess the effect of Hepatogard on wound healing. It was also studied for its effect on the dexamethasone induced delay in wound healing.

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The beneficial feature of Hepatogard is its outstanding safety. Experimental and clinical evidence on the ingredients of Hepatogard show the absence of toxic effects. Moreover, ingredients of Hepatogard have been in use for many years as part of traditional forms of medication with exceptional safety ($LD_{50} > 13 \text{ gms/kg}$).

METHODS

Adult male albino Wistar rats (b.wt. 150-200 gm) were individually housed and maintained on animal chow (Hindustan lever rat pellets) and water ad libitum. The rats were fasted overnight with free access to water, before infliction of wounds. The anaesthetic administered was pentobarbitone (30 mg/kg/ip), supplemented with inhaling ether whenever necessary. The surgical materials were sterilized and skin was prepared by clipping the fur and cleaning with 70% alcohol before wounding. The Hepatogard tablets were gifted by Surajmani enterprises. The adult human dose (7200 mg/day) of Hepatogard was converted into rat dose (650 mg/kg orally) by using standard dose converting table (4). A suspension was prepared in 2% gum acacia. Two doses of Hepatogard (650 and 1300 mg/kg) were selected for this study. Dexamethasone was used in the dose of 0.3 mg/kg, i.p. Effect of hepatogard was assessed alone and in the animals pretreated with dexamethasone. Each group had 8-10 animals. There were 6 groups of rats each for incision wound and dead space wound models. The study groups, drugs, dose route of administration and readings are depicted in Table I.

Wound healing studies :

A. Incision wound model :

Two, 6 cms long paravertebral straight incisions were made, 1 cm lateral to the vertebral column on either side through the entire thickness of skin (5) under light anaesthesia. Intermittent sutures were placed 1 cm apart with the help of a black silk thread No.4. The wounds were then mopped with cotton swab soaked in 70% alcohol. The animals received the drugs once everyday throughout the 10 days of study. Sutures were removed on the 7th post-operative day. Wound breaking strength was measured on 10th day by continuous constant water flow technique as described by Lee (6). The anesthetized animal was placed on the operation table and two allis forceps firmly applied 5 mms on either side of the wound margin. One forceps was hooked to a fixed metal rod and another connected to a light plastic graduated bottle, through a string run over a pulley. The bottle was connected to a constant running water stream which had a stop cock to arrest the flow. Tensile strength was measured by allowing the water into the bottle which gradually increased in weight, resulting in disruption of the wound. The flow of water was then stopped and volume of water noted. Four such readings were taken on either side of the wound to give 8 readings. The average of these 8 readings was the tensile strength for that animal. The mean value was then calculated.

B. Dead space wound :

Wounds were created to harvest granulation tissue by implanting

subcutaneously, 2.5 × 0.5 cms polypropylene tubes in the lumbar region through a small (0.5 cms) transverse incision about 4 to 5 cms cephaloid to the site of implantation (7). Drugs were administered daily for 10 days. On the 10th day, granulation tissue harvested on the implanted tube was carefully dissected out along with the tube after anaesthetizing the rats. The tubular granulation was cut along its length and the breaking/tensile strength was measured by the method of Lee (6). Later, these granulation tissues were collected, dried at 60°C for 24 hrs and weighed. The mean dry granulation tissue weight was calculated for each group. The granulation pieces were then utilized to estimate hydroxyproline content by the method of Neuman and Logan (8).

Statistical analysis: The data expressed as mean ±SEM were subjected to analysis of variance (ANOVA) followed by studentized range procedure

RESULTS

Wound healing studies:

A. Incision wound studies:

The mean wound breaking strength of a 10 day old incision wound in Group I was 323±20 g. In Groups II and III, the wound breaking strength did not differ significantly from the control. The values were 309±53 g and 316±18 g respectively. There was a significant reduction in wound breaking strength (183±10 g) in Group IV. The wound breaking strengths in Group V and VI (293±11 g and 287±47 g) were significantly more (P<0.01) than Group IV,

but not different from Group I (323±20 g) (Table I).

B. Dead space wound:

The mean granulation tissue breaking strength of a 10 day old wound in Group I was 170±10 g. In Groups II and III, the granulation tissue breaking strength was 321±40 and 380±13 g respectively which was significantly increased (P<0.01) when compared to Group I. There was a significant decrease (P<0.001) in the granulation tissue breaking strength in Group IV (113±37 g) when compared to Groups I, II and III. In Group V, the breaking strength of granulation tissue was 359±23 g, which was significantly more (P<0.01) when compared with Group IV and even Group I. In Group VI, the granulation tissue breaking strength was 304±31 g which was again significantly more (P<0.001) when compared to Groups I and IV, but not with Group III. However, there was no significant difference between Groups V and VI. (Table I).

The mean dry granulation tissue weight in Group I was 50±7 mg. There was significant difference (P<0.05) in the granulation tissue weight (92±7 mg) in Group II when compared to control. The dry granulation tissue weight in Group III (110±14 mg) was again significantly more (P<0.01) when compared to Group I. In Group IV, the dry weight of granulation tissue was 29±13 mg. which was significantly less (P<0.01) when compared to Group I. In Groups V and VI, the mean dry weight of granulation tissue was 105±20 mg and 110±18 mg respectively, which was significantly more (P<0.01) from

TABLE I : Effect of hepatogard on incision and dead space wound models in male albino rats.

Groups	Treatment	Incision wound mean±SE	Dead space wound (Mean±SE)		
		Breaking strength in g	Granulation breaking strength	Granulation dry weight	Hydroxyproline conc. in µ/mg
Group I	Control 2% gum acacia 2.5 ml, oral	323 ± 20	170 ± 10 ^b	50 ± 7	69.02± 16.07
Group II	Hepatogard low dose (H. ld) 650 mg/kg, oral	309 ± 53	321 ± 40 ^{a,b}	92 ± 7 ^a	61.60± 1.65
Group III	Hepatogard low dose (H. ld) 1300 mg/kg, oral	316 ± 18	380 ± 13 ^{a,b}	110 ± 14 ^a	62.14± 2.44
Group IV	Dexamethasone (Dexa) 0.3 mg/kg, ip	183 ± 10 ^a	113 ± 37 ^{a,c,d}	29 ± 13 ^{a,c,d}	24.52± 2.50 ^{a,c,d}
Group V	H. ld + dexa 650 mg/kg + 0.3 mg/kg ip, oral	293 ± 11 ^b	359 ± 23 ^{a,b}	105 ± 23 ^{a,b}	48.15± 7.04 ^b
Group VI	Hd + dexa 1300 mg/kg, oral + 0.3 mg/kg ip	293 ± 11 ^b	304 ± 31 ^{a,b}	110 ± 18 ^{ab}	57.21± 12.71 ^b

Values are Mean ± SE of 8-10 rats in each group

a: Vs control group, b: Vs Dexa group, c: Vs H.ld group, d: Vs H.hd group

All values are significant at P<0.05

Groups I and IV, but not different when compared to Groups II and III, (Table I).

The mean hydroxyproline concentration (µg/mg of dry weight granulation tissue) was 69.02±16.07 in Group I. In Groups II and III, there was no significant change in hydroxyproline concentration (61.60±65 and 62.14±2.44 respectively) when compared to Group I whereas in Group IV, there was a significant reduction (P<0.05) in the concentration (24.52±2.50). But in Groups V and VI, there was no significant reduction in concentration, the values being 48.15±7.04 and 57.21±12.71 respectively. However there was a significant difference (P<0.01) between Groups IV and V and also between Groups IV and VI. (Table I).

DISCUSSION

In our study, Hepatogard as such did not alter the breaking strength of incised

wound when compared to control but it was able to reverse dexamethasone induced decrease in breaking strength of incised wound in both the doses. However, in the dead space wound model, there was a significant increase in breaking strength of granulation tissue as well as the mean dry granulation tissue weight after administration of Hepatogard eventhough it did not affect the hydroxyproline levels. In addition, Hepatogard significantly reversed dexamethasone induced decrease in breaking strength of granulation tissue and hydroxyproline levels.

Since, there was no change in hydroxyproline levels after Hepatogard, it is very unlikely that Hepatogard acted by increasing the collagen content. It is known that dexamethasone reduces collagen synthesis (9). It may be possible that Hepatogard reversed the decrease in wound breaking strength caused by dexamethasone

by altering the maturation process where it might have affected the cross linking or improved the quality of collagen fibrils.

Many constituents of Hepatogard are known to reduce lipid peroxidation thereby not only preventing or slowing the onset of cell necrosis but also improving vascularity (1). Hepatogard by inhibiting lipid peroxidation may increase the viability of collagen fibrils which in turn results in increase in the strength of the collagen fibres. This is suggested by the fact that there was an increase in the wound breaking and granulation breaking strength after Hepatogard. Dexamethasone is known

to inhibit granulation tissue and mucopolysaccharide synthesis by inhibiting DNA synthesis (10). This study has also shown that Hepatogard was not only able to facilitate epithelization process and wound contraction but also reverse dexamethasone induced delay in wound healing. Hence Hepatogard may be useful to prevent the wound healing delaying effects of steroids.

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