

Full Length Research Paper

Evaluation of wound healing activity of the polyherbal and *Euphorbia hirta* formulations

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Evaluation of wound healing activity of polyherbal and *Euphorbia hirta* formulations was done. The study aimed at preparing a polyherbal formulation containing equal proportions of ethanolic extract of *Tridax procumbens*, *Euphorbia hirta*, *Eclipta alba*, dried rhizome of *Curcuma longa* and gel of *Aloe barbadensis*. Excision wound, incision wound and granuloama wound models were studied. In excision wound model it was observed that 100% wound healing was found on 12th day of the experiment on application of 10% polyherbal ointment and 100% wound contraction was found on 16th day of application of 10% ointment of *E. hirta*. The results were statistically compared with control and found significant. Similarly, period of epithelization, biochemical parameters and weight of granuloma tissues showed better results in the treated groups when compared with control in the studied wound models. Thus it is concluded that topical and oral formulations were found to contain significant wound healing activity.

Key words: Excision wound, incision wound, granuloma.

INTRODUCTION

More than 80% of the world's population still depends upon traditional medicines for various skin diseases (Babu et al., 2002). Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process (Purna and Babu, 2000). Healing is the body response to injury in an attempt to restore normal structure and function. The process of healing involves two distinct processes: regeneration when healing takes place by proliferation of parenchymal cells and usually results in complete restoration of the original cells, and repair when that healing takes place by proliferation of connective tissue elements resulting in fibrosis and scarring (Harshmohan, 2002). Wound healing involves a

complex series of interactions between different cell types, cytokine mediators, and the extracellular matrix. The phases of normal wound healing include hemostasis, inflammation, proliferation, and remodeling. Each phase of wound healing is distinct, although the wound healing process is continuous, with each phase overlapping the next. Open wounds are colonized by bacteria and systemic antibiotics are only indicated if invasive infections is present (Lorenz et al., 2003). The formulation which claims to possess wound healing activity shall be studied with the features like tensile strength of newly formed tissue, biochemical parameters like serum and tissue levels of hydroxyproline, lysyl oxidase, methionine, ascorbic acid etc. which contribute to the all round healing of wound.

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MATERIAL AND METHODS

Plants used

Gel of *Aloe barbadensis*, family Liliaceae containing glucomannan (Rastogi and Mehrotra, 1999), rhizomes of *Curcuma longa* family Zingiberaceae containing yellow matter curcumins (API, 1998), whole plant of *Eclipta alba* family Compositae containing coumestan derivatives, wedelolactones and demethyl-wedelolactone (Govindachari et al., 1956; Wagner et al., 1986), whole plant of *Euphorbia hirta* containing leucocyanidol, quercitol, camphol, quercitin and quercitol derivatives containing rhamnose and chlorophenolic acid (Rastogi and Mehrotra, 1999) and whole plant of *Tridax procumbens* containing β -amyirin, fucosterol and sitosterol, arachidic, behenic, lauric, linoleic, linolenic, myristic, palmitic and stearic acids (Rastogi and Mehrotra, 1999).

Extraction

Dried ethanolic extract of the dried rhizome of *Curcuma longa* and dried whole plants of *T. procumbens*, *Eclipta alba*, *E. hirta* was taken and gel of *Aloe barbadensis* was collected.

Formulation of topical and oral formulations

Topical formulation (ointment)

Two ointments were prepared by fusion method that is, one containing ethanolic extract of whole plant of *Euphorbia hirta* (10% w/w), and other containing equal proportions (2 g each) of ethanolic extract of whole plant of *T. procumbens*, ethanolic extract of whole plant of *E. alba*, ethanolic extract of whole plant of *E. hirta*, ethanolic extract of dried rhizomes of *Curcuma longa* and gel of *Aloe barbadensis* (10% w/w) in hydrophilic ointment USP base.

Oral formulation (suspension)

Two suspensions were formulated that is, one containing ethanolic extract of whole plant of *E. hirta* (10% w/v) and other containing equal proportions (2.5 g each) of ethanolic extract of whole plant of *T. procumbens*, ethanolic extract of whole plant of *E. alba*, ethanolic extract of whole plant of *E. hirta* and ethanolic extract of dried rhizomes of *C. longa* to make 10% w/v of suspensions.

Evaluation of *E. hirta* and polyherbal formulations for wound healing activity

All the animal studies were performed in Department of pharmacy, Barkatullah university, Bhopal. The department is an Animal Ethical Committee approved institution. The approval was granted by meeting held on 26/12/2006 with ref. No. BUPH/AEC/7865. The department is also approved for animal experimentation (Letter No. 444/01/C/CPCSEA Dated July 2001).

Excision wound model

For the excision wound study, each group containing six animals were selected. A wound of about 1 cm² area was made on the depilated dorsal thoracic region of rats. To make the wound, the rats were anaesthetized under light ether. The aseptic conditions were maintained and observed throughout the study. The wounded rats were individually caged and percentage wound closures were

observed from 2nd post wounding day to 22nd day, alternately (Morton and Malone, 1972).

Topical application

Group I (control)

The hydrophilic ointment base was applied topically, once daily.

Group II (Test 1)

10% ointment, in hydrophilic base, of 95% ethanolic extract of whole plant of *Euphorbia hirta* was topically applied, once daily.

Group III (Test 2)

10% polyherbal formulation was topically applied, once daily.

Oral administration

Group I (control)

2% tragacanth suspension was given orally, once daily, 200 mg/kg BW.

Group II (test 1)

10% w/v suspension of 95% ethanolic extract of whole plant of *E. hirta* in 2% tragacanth was given orally, once daily, 200 mg/kg BW.

Group III (test 2)

10% w/v suspension of polyherbal formulation in 2% tragacanth was given orally, once daily, 200 mg/kg BW. Parameter of percentage wound contraction and period of epithelialization were evaluated. Biochemical parameters like determination of hydroxyl-proline (Newman and Logman, 1950), hexosamine (Dische and Borenfreund, 1950) and protein content (Lowry et al., 1951) were also evaluated on 11th post wounding day. The percentage wound contraction was determined using the formula:

$$\% \text{ wound contraction} = (\text{Healed area} / \text{Total area}) \times 100$$

Wound contraction was measured each day using graph paper planimetrically.

Incision wound model

Thirty six male albino rats of either sex weighing 150 to 200 g were taken. Paravertebral incisions of 3 cm were made on the depilated skin of the rats anaesthetized under light ether with the help of a surgical blade. The incisions were sutured using 4 to 0 silk thread with cutting edges. Sutures were removed on the 8th post wounding day and the tensile strength were determined on 11th post wounding day by continuous constant water flow technique of Lee (Lee, 1968) of the rats of each group. White blood cell (WBC) count was also done to evaluate the extent of protection (Ehrlich and Hunt, 1969).

Table 1. Percentage wound contraction in excision wound model by topical application.

Days	Control	Test 1	Test 2
2	19.23± 0.94	25.41±1.05*	28.25±1.11*
4	22.31±0.87	42.83±0.90*	47.15±0.92*
6	27.7± 1.28	73.61±0.89*	75.72±0.96*
8	33.34±0.84	81.26±0.99*	92.36±1.07*
10	35.62±0.96	88.42±1.20*	97.42±0.95*
12	40.57±1.21	92.33±0.94*	100± 1.03*
14	44.12±1.17	96.14±1.13*	-
16	49.68±1.16	100±1.08*	-
18	53.21±0.91	-	-
20	57.81±1.10	-	-
22	60.33±0.88	-	-

Each group contain six animals, Mean ± SD. *Indicates significant activity at $p < 0.001$. #Indicates post wounding days.

Topical application

Group I (control)

The hydrophilic ointment base was applied topically, once daily.

Group II (Test 1)

10% ointment, in hydrophilic base, of 95% ethanolic extract of whole plant of *E. hirta* was topically applied, once daily.

Group III (Test 2)

10% polyherbal formulation was topically applied, once daily

Oral administration

Group I (Control)

2% tragacanth suspension was given orally, once daily, 200 mg/kg BW.

Group II (Test 1)

10% w/v suspension of 95% ethanolic extract of whole plant of *Euphorbia hirta* in 2% tragacanth was given orally, once daily, 200 mg/kg BW.

Group III (Test 2)

10% w/v suspension of polyherbal formulation in 2% tragacanth was given orally, once daily, 200 mg/kg BW. Measurement of tensile strength and total leucocytes count WBC (Shanbhag et al., 2005).

Dead space wound model

Thirty six albino rats of either sex were taken. Under light ether

anesthesia, subcutaneous dead space wound was inflicted in the paravertebral lumbar region by making a pouch through a small nick in the skin. Sterile polypropylene tubes measuring 2.5 cm in length and 0.5 cm in diameter were introduced in the pouch to induce granuloma formation. Each animal received two polypropylene tubes in the groin region. The wounds were sutured and mopped with an alcoholic swab. The harvesting of the granuloma tissue from the surrounding tissue was carried out on the 11th post wounding day. Then it was subjected to measurement of bearing strength of granuloma tissue and histopathological examination. The weight of wet and dry granulation tissue was measured along with the estimation of biochemical parameters like hydroxyproline, hexosamine and protein content (Turner, 1965).

Topical application

Group I (control)

The hydrophilic ointment base was applied topically, once daily.

Group II (test 1)

10% ointment, in hydrophilic base, of 95% ethanolic extract of whole plant of *E. hirta* was topically applied, once daily.

Group III (test 2)

10% polyherbal formulation was topically applied, once daily.

Oral administration

Group I (control)

2% tragacanth suspension was given orally, once daily, 200 mg/kg BW.

Group II (test 1)

10% w/v suspension of 95% ethanolic extract of whole plant of *E. hirta* in 2% tragacanth was given orally, once daily, 200 mg/kg BW.

Group III (test 2)

10% w/v suspension of polyherbal formulation in 2% tragacanth was given orally, once daily, 200 mg/kg BW.

DISCUSSION

The wound healing activity was evaluated by excision wound model, incision wound model, dead space wound model, burn wound model and induced diabetes wound healing model. In excision wound model, it was observed that 100% wound healing was found on 12th day of the experiment on application of 10% polyherbal ointment and 100% wound contraction was found on 16th day of application of 10% ointment of *E. hirta*. The results were statistically compared with control and found significant (Tables 1 and 2). On oral administration of the formulated

Table 2. Percentage wound contraction in excision wound model by oral administration.

Days [#]	Control	Test 1	Test 2
2	16.20 ±0.81	23.42±0.93*	38.11±1.26*
4	20.31±1.15	47.32±1.17*	49.66±0.99*
6	30.61±0.54	55.64±0.96*	58.15±0.93*
8	35.57±0.97	69.91±1.27*	66.62±1.36*
10	37.17±0.56	76.37±0.86*	79.41±1.26*
12	41.88±0.87	81.2±0.76*	85.0±1.42*
14	46.20±0.41	87.5±0.88*	100±0.93*
16	49.42±0.98	94.41±1.20*	-
18	55.90±1.15	100±1.31*	-
20	59.50 ± 0.87	-	-
22	62.76±0.84	-	-

Each group contain six animals, Mean ± SD; [#]Indicates Post Wounding Days *Indicates significant activity at P < 0.001.

Table 3. Period of epithelization (days) in excision wound model.

Groups	Period of epithelization (days)	
	Topical (ointment)	Oral (suspension)
Control	26.17±1.15	28.45±1.21
Test 1	15.12±0.85	17.20±1.22
Test 2	11.61±1.10	13.12±1.34

Each group contain six animals, Mean ± SD. *Indicates significant activity at P < 0.001.

Table 4. Biochemical Parameters of Excision wound model on Topical Application on 11th Post Wounding Day.

Parameter (mg/g tissue wt.)	Control	Test 1	Test 2
Hydroxyproline	19.1±1.3	31.3±2.3*	74.1±2.5*
Hexosamine	3.3±0.4	12.1±1.3*	24.3±2.0*
Protein	19.6±1.3	35.0±1.8*	68.3±1.0*

Values are expressed as Mean±SD * indicates significant activity P < 0.01.

Table 5. Biochemical parameters of excision wound model on oral administration on 11th post wounding day.

Parameter (mg/g tissue wt.)	Control	Test 1	Test 2
Hydroxyproline	16.8±2.1	28.4±1.8*	59.5±2.2*
Hexosamine	5.1±0.2	9.5±1.1*	20.2±0.23*
Protein	17.3±1.2	31.3±1.0*	56.0±1.8*

Values are expressed as Mean±SD; *Indicates significant activity P < 0.01.

suspensions it was found that 100% wound contraction on 14th day was shown by rats treated with polyherbal suspensions whereas complete wound contraction was found on 18th day in *E. hirta* treated group. The results were compared statistically with control and found satisfactory (Table 3). The period of Epithelization was also found to be significantly shorter for the treated groups as compared to control. However the results were more encouraging for the polyherbal ointment (Table 4). Estimation of biochemical parameters- hydroxyproline, hexosamine and protein content was done on the 11th post wounding day and it was found that the respective concentrations of the parameters increased in the treated groups, however the increase was most marked in the topically applied polyherbal formulation followed by polyherbal oral formulation. It was reported that besides collagen C1q of the complement system contains considerable amount of collagen (Nagelschmidt and Struck, 1977). The increase in the biochemical parameters of the topical and oral *E. hirta* formulations was also found to be significant when compared to control (Tables 5 and 6). The period of epithelization was found to be least in the topical polyherbal formulation and that of the oral polyherbal was found to be better than *E. hirta* topical formulation.

In the incision wound model, the tensile strength of the new generated tissue was measured. Collagen, the major protein of the extracellular matrix, is the component that ultimately liberates free hydroxyproline and its peptides (Nayak and Pereira, 2006). The tensile strength of a wound is determined by the rate of collagen synthesis and more so, by the maturation process where there is a covalent binding of collagen fibrils through inter and intra molecular cross linking (Malviya and Jain, 2009). It was found that the tensile strength of new tissue was more in the treated groups with least significant signs of infection. The strength of the tissues was statistically significant in the polyherbal topical group followed by polyherbal oral group (Table 6).

In dead space wound model, it was found that the weight of wet and dry granuloma tissue formed was more in the case of treated groups when compared to control. The increase in the weight of the granuloma was most significant in the topically applied polyherbal formulation groups followed by orally administered polyherbal groups. Similarly, the rise in the biochemical parameters like those of hydroxyproline, hexosamine and protein content of the granuloma tissue was lot more in both topically and orally treated groups. However, the results were most encouraging and statistically significant in the topically treated polyherbal formulations, when compared with control (Table 7 and 8). Histopathological studies showed formation of scanty collagen tissue in the control group (Figures 1 and 2). The test groups treated with polyherbal topical formulations showed formation of dense collagen tissues with minimum presence of macrophages (Figure 2d) and formation of complete epidermis (Figure 2c).

Table 6. Effect of formulations on tensile strength and WBC count in incision wound model.

Group	Tensile strength (g)		WBC count ($\times 10^3$ cell/mm ³)	
	Topical	Oral	0th Day	10th Day
Control	223 \pm 3.5	215 \pm 3.3	13.95 \pm 0.51	30.52 \pm 1.15
Test 1	330 \pm 4.2*	302 \pm 3.3*	14.0 \pm 0.62*	13.98 \pm 1.10*
Test 2	360 \pm 6.3*	347 \pm 4.1*	14.0 \pm 0.46*	13.97 \pm 1.12*

Table 7. Physical and biochemical parameters by topical application in dead space wound model.

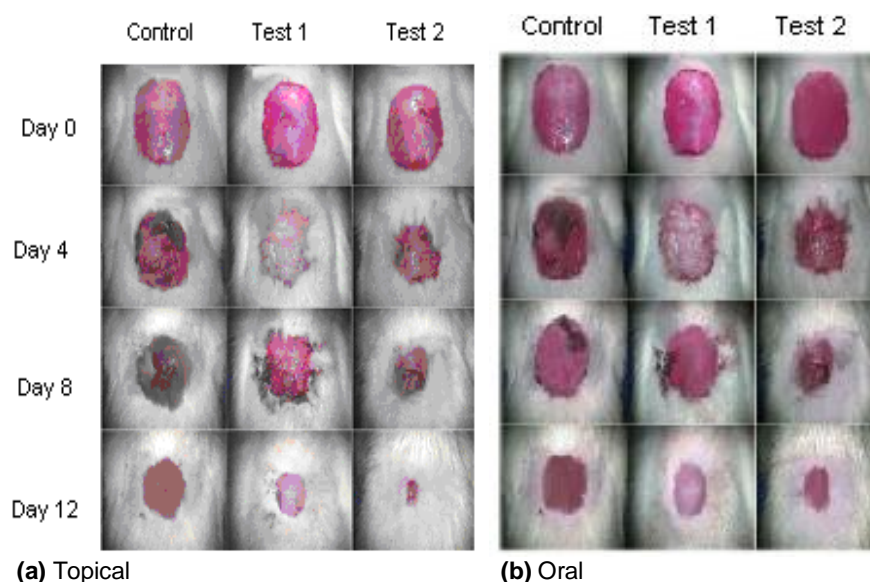
Parameter	Control	Test 1	Test 2
Wet granulation weight (mg/100 g)	229 \pm 11.2	380.41 \pm 13.2*	483.4 \pm 15.3*
Dry granulation weight (mg/100 g)	25.1 \pm 3.1	77.0 \pm 1.4*	94.3 \pm 1.5*
Protein (mg g ⁻¹)	19.71 \pm 1.5	34.1 \pm 2.1*	64.1 \pm 2.1*
Hydroxyproline (mg g ⁻¹)	16.93 \pm 1.2	22.4 \pm 2.6*	30.9 \pm 2.1*
Hexosamine (mg g ⁻¹)	9.74 \pm 0.05	16.4 \pm 1.9*	28.1 \pm 1.4*

All values are expressed as mean \pm S.D. *P < 0.01 significant compared to control.

Table 8. Physical and biochemical parameters by oral administration in dead space wound model.

Parameter	Control	Test 1	Test 2
Wet granulation Weight (mg/100 g)	220 \pm 10.4	289.5 \pm 14.1*	415.2 \pm 10.3*
Dry granulation weight (mg/100 g)	31.3 \pm 1.6	70.0 \pm 1.2*	89.1 \pm 2.3*
Protein (mg g ⁻¹)	21.5 \pm 1.4	50.5 \pm 2.1*	62.1 \pm 1.5*
Hydroxyproline (mg g ⁻¹)	3.2 \pm 0.08	5.12 \pm 1.5*	24.96 \pm 1.3*
Hexosamine (mg g ⁻¹)	8.5 \pm 0.05	14.8 \pm 1.2*	23.6 \pm 1.5*

All values are expressed as mean \pm SD. *P < 0.01 significant compared to control.

**Figure 1.** Wound contraction in excision wound model.

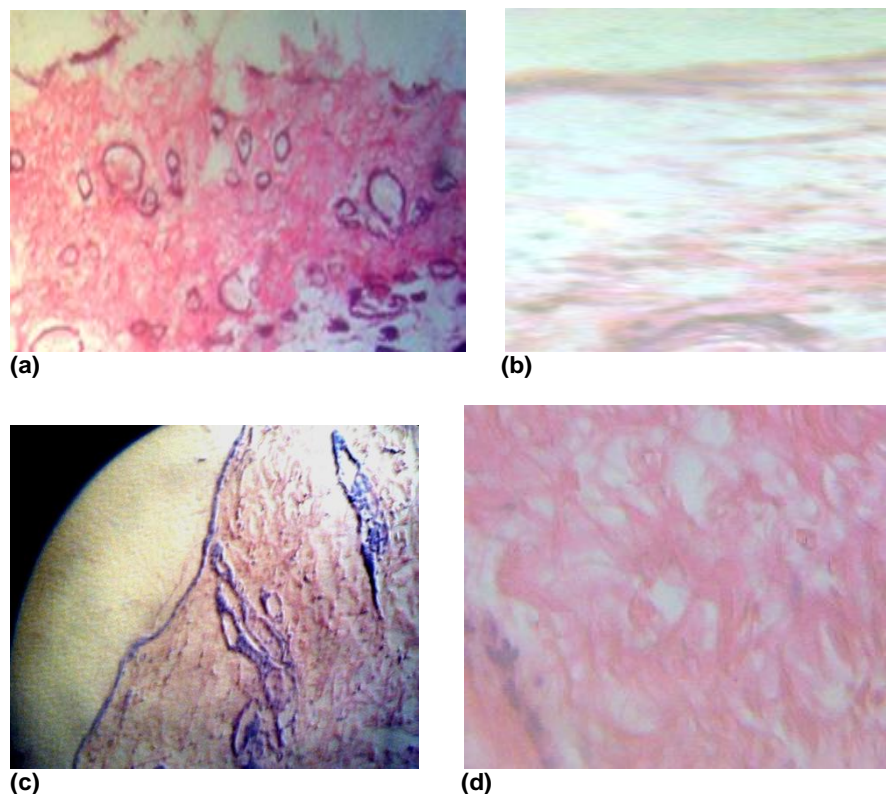


Figure 2. Histopathology of dead space wound model (a) control (oral), (b) control (topical), (c) Test 1 (topical), (d) Test 2 (topical).

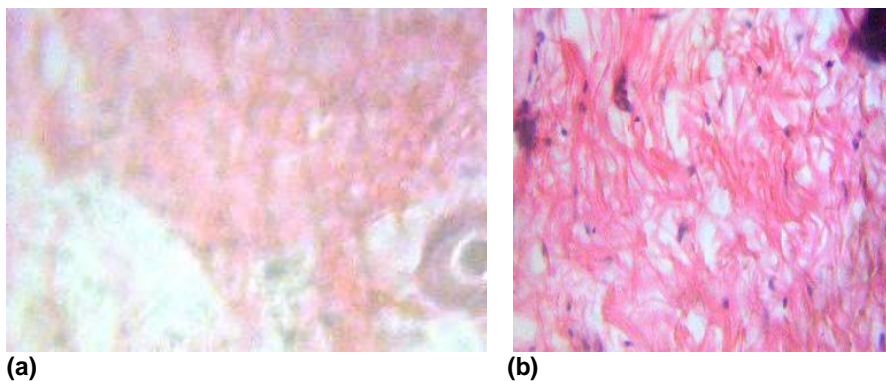


Figure 3. Complete epidermis (a) Test 1 (oral), Test 2 (oral).

The best groups treated with polyherbal oral formulations also showed formation of dense collagen tissues, but slightly less than topically treated groups by polyherbal formulations (Figure 3b). The test group treated with topical formulation of *E. hirta* showed formation of collagen tissues with presence of macrophages (Figure 2c), whereas those treated with oral *E. hirta* formulations showed collagen tissues less than in the topically treated groups of *E. hirta* (Figure 3a).

The wound healing studies revealed that the polyherbal

and *E. hirta* formulations possess significant activity but it may be concluded that the polyherbal topical formulations has got remarkable results in all types of wound healing protocols (Figures 4 and 5). The study has concluded to acclaim *E. hirta* as a prominent wound healer (200 mg/kg BW).

Conclusion

The polyherbal formulations containing equal proportions

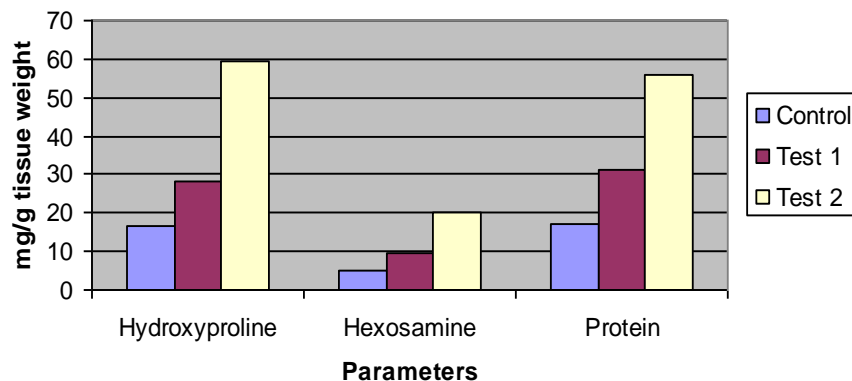


Figure 4. Biochemical parameters of excision wound model on oral administration

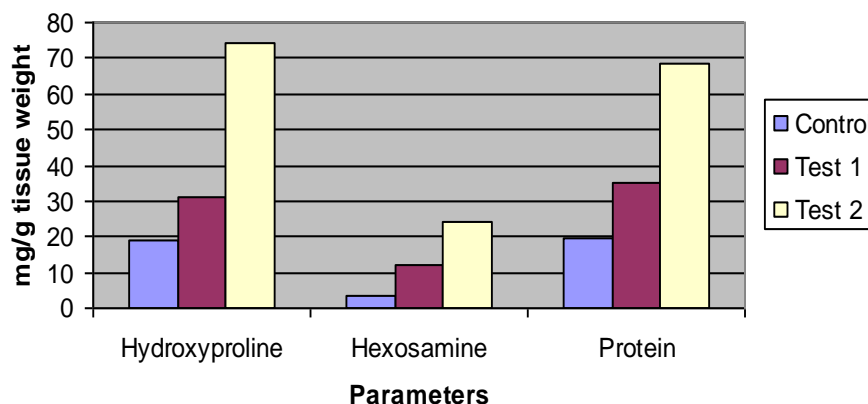


Figure 5. Biochemical parameters of excision wound model on tropical administration.

of the ethanolic extracts of the plants studied can be used both topically and orally for the healing of wounds. Single or multiple factors may play a role in any one or more individual phases, contributing to the overall outcome of the healing process (Guo and Di Pietro, 2010).

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