

In vivo effect of whole grain flour of finger millet (*Eleusine coracana*) and kodo millet (*Paspalum scrobiculatum*) on rat dermal wound healing

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Influence of finger millet and kodo millet on rat dermal wound healing was assessed by making a 4 cm² (2 × 2 cm) excision wound on the shaven back of rats under ether anesthesia. Finger millet or kodo millet flour (300 mg) as aqueous paste was applied topically once daily for 16 days. The granulation tissue formed on day 4, 8 and 12 was used to estimate some biochemical parameters like protein, DNA, collagen and lipid peroxides. There was significant increase in protein and collagen contents and decrease in lipid peroxides. Biophysical parameters like rate of contraction and number of days for epithelialization were also studied. Rate of contraction was 88-90% in kodo millet and finger millet treated rats in comparison to 75% in untreated rats. The number of days for complete closure of wounds was lower for finger millet (13 days) and kodo millet (14 days) treated rats in comparison to untreated (16 days) rats. The results implicate a possible therapeutical role for finger millet and kodo millet in accelerating the process of wound healing.

Keywords: Antioxidant, Collagen, *Eleusine coracana*, Finger millet, Free radicals, Kodo millet, *Paspalum scrobiculatum*, Wound healing,

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Wound healing and tissue repair are complex processes that involve a series of biochemical and cellular reactions beginning with inflammation and followed by repair and remodelling of the injured tissue¹. The formation of crosslinks and development of tensile strength is mainly contributed by type I collagen in the wound matrix. However, type III collagen is the earliest form of collagen detected in the wound². It forms scaffolding in the early wound healing process and subsequently the accumulation of type III collagen is overcome by the rapid accumulation of type I collagen (which provides mechanical integrity), until the normal ratio of type I to type III is re-established³. The early type III collagen has, in the healing process, important functions such as establishing initial wound structure, guiding inflammatory cells and fibroblasts into the wound site, providing a matrix for re-establishment of blood supply. It also acts to regulate collagen fibre diameter and organization.

Earlier studies on positive influence of *Aloe vera*, a tropical cactus, on the healing of wounds in diabetic rats indicated that it may enhance the process by in-

fluencing phases such as inflammation, fibroplasia, collagen synthesis and maturation, and wound contraction. These effects may be due to the reported hypoglycemic effects of the *Aloe gel*⁴.

Similar studies on wounds treated with curcumin showed earlier re-epithelialization, improved neovascularization, increased migration of various cells including dermal myofibroblasts, fibroblasts, and macrophages into the wound bed, and a higher collagen content⁵. Plant products have been shown to have a good therapeutic potential as anti-inflammatory agents and promoters of wound healing, due to the presence of active alkaloids, terpenes and flavonoids⁶⁻⁸.

Finger millet and kodo millet are rich source of polyphenols and exhibit significant antioxidant activity^{9, 10}. Owing to known role of antioxidants in wound healing and reports that phenolics like curcumin exhibit anti-inflammatory activity⁵, in the present study the finger millet and kodo millet flour were analyzed for wound healing properties on topical application.

Materials and Methods

Hydroxyproline, chloramine T, thiobarbituric acid, 1,1,3,3, tetra methoxy propane, bovine serum albumin

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and calf thymus DNA were obtained from Sigma-Aldrich Chemical Company, St Louis, USA. All other chemicals were of analytical grade commercially available.

Experimental set up— A standard full thickness wound (4 cm²) was created on the shaven back of male albino Wistar rats, 50-200 g, 3 months old, obtained from Tamilnadu Animal and Veterinary Science University, India under light anesthesia. Untreated rats served as control (Group I); Group II rats were treated by topical application of 300 mg of aqueous paste of finger millet (FM) CO-13 flour and Group III rats were treated by topical application of 300 mg of aqueous paste of kodo millet (KM) Vamban 1 flour. In each group rats were taken in replications (n=6). The granulation tissues formed were removed on day 4, 8 and 12 after wound creation and used to estimate the biochemical and biophysical parameters.

Extraction of total protein and DNA— The wet granulation tissue samples were extracted in Trichloroacetic acid (5%)¹¹. TCA (5%; 10 ml) was added to the tissue samples (100 mg wet weight) and kept at 90° C for 30 min in a water bath to extract protein and DNA. The solution was centrifuged at 5000 rpm for 10 minutes and the supernatant was used for the estimations.

Estimation of protein— Total protein was determined by using Bovine Serum Albumin (BSA) as standard¹². The total protein contents was expressed as mg/100 mg wet tissue.

Estimation of DNA— Tissue DNA content was determined by using calf thymus DNA as the standard¹³. DNA content of tissue was expressed as mg/100 mg wet weight.

Estimation of total collagen— The total collagen content of the granulation tissue was determined by the estimation of hydroxyproline. The samples were washed with physiological saline, cut into small pieces, defatted with chloroform: methanol (2:1 v/v) and lyophilized. Lyophilized tissue (5 mg) was digested with 6N HCl for 24 hr at 110° C. The amount of hydroxyproline was estimated as per Stegmann and Stalder¹⁴. Collagen content of tissue was expressed as mg/100 mg wet weight.

Estimation of lipid peroxides— Lipid peroxides (as malondialdehyde) of the tissue extracts were determined as per Nagababu and Lakshmaiah¹⁵. The lipid peroxide content was expressed as n mole of MDA/100 mg wet weight.

Rate of contraction— The excision wounds were traced on a paper having a mm scale and the changes in wound size were measured planimetrically. Reduction in wound (reflecting the rate of wound contraction) was calculated as per cent of original wound size (4 cm²).

Period of epithelialization— The wounds were also inspected for complete epithelialization as indicated by shedding of eschar without any raw wound left behind. Days required for this sloughing was taken as the period of epithelialization.

Statistical analysis— All experiments were carried out with 6 rats in each group and results are expressed as mean ± SD. Statistical analysis was done using Single way Anova and Newman-Keuls Multiple Comparison Test. *P* value of less than 0.05 was considered significant.

Results and Discussion

There was a significant increase in protein and collagen contents on the day 4 and 8 of healing in FM and KM treated rats in comparison to control and a decrease by day 12 (Table 1). The levels of collagen and its types contribute significantly to the wound healing process. Collagen is the main protein in the extracellular matrix and plays an important role in the healing process even after the wound is closed¹⁶. Though, the major function of collagen is to provide strength and integrity to the wound, it also plays a role in other functions such as homeostasis, re-epithelialization, cell-cell and cell-matrix interactions¹⁷⁻²⁰.

Increase in total protein contents correlated well with increased collagen content in the granulation tissue in the millets treated group (Table 1). The millets could have stimulated collagen synthesis or increased the proliferation of fibroblasts synthesizing collagen or both. There was also an increase in DNA content on the day 4 and 8 of healing in FM and KM treated rats and a decrease on day 12 (Table 1).

There was a significant reduction in lipid peroxides on the day 4, 8, and 12 of healing in FM and KM treated rats in comparison to control (Table 1). The rate of wound contraction on the day 4, 8 and 12 was also higher in FM and KM treated rats (Table 1). The number of days for complete closure of wound decreased significantly in the treated rats (Table 1).

The antioxidant and anti-inflammatory activities in extracts of leaves of *Hyptis suaveolens*²¹ and macrofungus *Phellinus rimosus* (Berk) Pilat²² and

Table I — Total protein, collagen, DNA, lipid peroxides content, rate of wound contraction of granulation tissues and period of epithelialization in normal and experimental rats

[Values are mean \pm SD of six animals in each group]

Biochemical parameters	Group I	Group II	Group III
Protein			
a	3.57 \pm 0.12	5.06 \pm 0.11 ^{a*}	4.92 \pm 0.09 ^{a*}
b	7.07 \pm 0.08	8.63 \pm 0.10 ^{a*}	8.45 \pm 0.08 ^{a*}
c	6.02 \pm 0.09	7.70 \pm 0.09 ^{a§}	7.59 \pm 0.07 ^{a§}
DNA			
a	1.55 \pm 0.07	1.89 \pm 0.07	1.91 \pm 0.08
b	5.39 \pm 0.10	5.98 \pm 0.08	5.75 \pm 0.16
c	4.26 \pm 0.13	4.93 \pm 0.12	4.66 \pm 0.12
Collagen			
a	2.34 \pm 0.11	3.95 \pm 0.12 ^{a*}	3.78 \pm 0.14 ^{a*}
b	4.82 \pm 0.16	7.04 \pm 0.27 ^{a§}	6.56 \pm 0.10 ^{a§}
c	3.78 \pm 0.16	5.35 \pm 0.27 ^{a*}	5.25 \pm 0.17 ^{a*}
Lipid peroxides			
a	1357 \pm 30.00	573 \pm 13.00 ^{a#}	648 \pm 15.00 ^{a#b#}
b	978 \pm 15.00	374 \pm 16.00 ^{a#}	413 \pm 10.00 ^{a#b#}
c	692 \pm 24.00	201 \pm 7.00 ^{a#}	302 \pm 10.00 ^{a#b#}
Rate of wound contraction			
a	35.00 \pm 1.78	52.33 \pm 0.81 ^{a#}	47.00 \pm 0.89 ^{a#b#}
b	50.00 \pm 1.78	75.66 \pm 0.81 ^{a#}	71.33 \pm 0.81 ^{a#b#}
c	75.16 \pm 1.16	90.83 \pm 0.98 ^{a#}	88.16 \pm 0.75 ^{a#b§}
Period of epithelialization			
	15.83 \pm 0.40	13.50 \pm 0.54 ^{a§}	14.00 \pm 0.63 ^{a§}

a, b, and c represent data on day 4, 8, and 12 of healing.
 Analysis done with One-Way Anova and Newman-Keuls Multiple Comparison Test
 Comparisons are made between- ^aGroup I and Group II, Group III; ^bGroup II and Group III
 Values are significant at * $P < 0.05$, [§] $P < 0.01$, [#] $P < 0.001$
 Values expressed for Protein, DNA and Collagen(mg/100 mg wet wt); Lipid Peroxides(n mole MDA/100 mg wet wt); Rate of wound contraction(% of original wound size); Period of epithelialization(days)

supportive role of antioxidant enzymes in wound healing has been shown. Wound contraction in rats treated with whole plant extract of *Leucas laevis* Rees was better than application of a simple ointment²³. Similar reports on improved wound contraction using methanolic extracts of leaf of *Hypericum patulum* which contains steroids and flavonoids is known²⁴. The healing effects of these extracts are attributable to the synergistic action of the phytochemicals present in them²³.

The millets used in the present study are rich source of phenolics^{9, 10, 25, 26}. We have established the antioxidant activity of finger millet^{9, 27} and kodo millet^{10, 27} phenolic extracts and showed that an intake of millets in diabetic rats improved wound healing²⁸. Hence we attribute the reduction in lipid peroxides on day 4, 8, and 12 to the presence of rich antioxidant phenolics in these millets

Several other factors present in the millets that may have contributed to the accelerated wound healing in the treated rats include calcium and magnesium. They are reported to influence the process of wound healing²⁹. Calcium has an established role in the normal homeostasis of mammalian skin and serves as a modulator in keratinocyte proliferation and differentiation. In wound repair, calcium is predominantly involved as Factor IV in the haemostatic phase, but it is expected to be required in epidermal cell migration and regeneration patterns in later stages of healing³⁰. Finger millet is a rich source of calcium (344 mg/100 g)³¹. Hence, the accelerated wound healing in rats treated with finger millet could be due to presence of high calcium content.

During the early stages of wound tissue regeneration in rats, the methionine content of wound proteins is greater than the cystine content. Later, the content

of cystine becomes much higher than that of methionine. This has been taken to mean that two different types of proteins are synthesized by the regenerating wound tissue. Hence, both the amino acids contribute significantly to increase the rate of regeneration of wound tissue³². Finger and kodo millet contain significant amounts of methionine (210, 180 mg/ g N) and sulfur containing amino acid cysteine (140, 110 mg/ g N)³¹.

Conclusion

This is the first study to show the potential usefulness of finger millet and kodo millet flour in topical application on the process of wound healing. Treatment with finger millet and kodo millet significantly increased levels of biochemical parameters like total protein, collagen and DNA content, and decreased the levels of lipid peroxides when compared to the control. Biophysical parameters like increased rate of contraction and decrease in the number of days for epithelialization were observed. Treatment with finger millet showed better results in comparison to kodo millet. The nutraceuticals in these millets contributing to these beneficial properties need further study.

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