World Journal of Pharmaceutical research

Volume 1, Issue 3, 786-795. Research Article ISSN 2277 – 7105

EVALUATION OF *PERSEA AMERICANA L.* LEAF EXTRACT FOR WOUND HEALING ACTIVITY- A PRECLINICAL STUDY IN RATS

B. Shivananda Nayak*[1], Ria Ramadeen[1], S. Sivachandra Raju[2]

[1]Department of Pre Clinical Sciences, Biochemistry Unit, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad.

[2]Department of Pharmacology, RAK Medical and Health Sciences University, Ras Al Khaimah, UAE.

Article Received on
7 May 2012,

Revised on 29May 2012,
Accepted on 17 June 2012

***Correspondence for Author:**

*Dr. Shivananda Nayak
Dept of Pre-Clinical Sciences
Faculty of Medical Sciences
The University of the West Indies, Trinidad and Tobago.
Shivananda.Nayak@sta.uwi.edu

ABSTRACT

We evaluated the wound healing activity of leaf extract of *P. americana* in rats. The wound healing activity of the extract of *P. americana* extract was determined in rats (topical application of 100 mg/kg/day) using excision wounds models. The animals were divided into three groups of 6 each. The experimental group animals were treated with the ethanolic extract of *P. americana*, standard group animals were treated with mupirocin ointment (10%), and the control group animals were applied with petroleum jelly. Healing was assessed by the rate of wound contraction, period of epithelialization and hydoxyproline content. On day 13 the tissue was excised from the healed area for histological and hydroxyproline determination. The extract-treated animals exhibited 86% reduction in the wound area when compared to controls (67%) standard (81%). The extract treated wounds were found to epithelize faster than controls ($P < 0.001$). The hydroxyproline content of the tissue obtained from experimental animals was significantly higher. The histological examination of tissue from experimental group animals showed abundant well organized collagen. Wound contraction together with the hydroxyproline content and histological findings support the use of *P. americana* leaf in the management of cut wounds.

Key words: *Persea americana*, Excision wound, Hydroxyproline, Collagen.

INTRODUCTION

Traditional herbal medicine practitioners have described the healing properties of various wild plants.[1, 2] The past decade has seen considerable change in opinion regarding ethnopharmacological therapeutic applications. The presence of various life sustaining constituents in plants has urged researchers to examine these plants with a view to determine potential wound healing activities.

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin layer) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialization and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts exert collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells.

The *P. americana* commonly known as Avocado belongs to the family *Lauraceae*, a family of mainly tropical trees and shrubs. Other well-known members are laurel, cinnamon, sassafras and greenheart. [3] Avocado is native to tropical America. It is a shallow-rooted evergreen tree that grows up to 20 m tall. Its leaves are simple, ovate and spirally arranged.[4] *P. americana* leaves are characterized by their astringent and carminative properties, and these leaves can be used in the treatment of diarrhea, to reduce abdominal accumulation of excess gas, and in the treatment of abdominal bloating. The leaves of the avocado also have a great capacity of relieving coughs and are considered of great value in this role, at the same time, the herbal remedies made from the leaves of the avocado are also used to clear away internal obstructions in the liver of patients, as well as in clearing away high uric acid levels in the body of individuals susceptible to this condition.

The fruit extract of *P. americana* has shown wound healing promoting activity.[5] The aqueous extract of *P. americana* leaves has shown aniconvulsant[6], hypoglycemic[7], hypocholesterolemic [8], vasorelaxant [9,10], heptoprotective [11] and antibacterial [12], and a dose-dependent analgesic, anti-inflammatory and antioxidant properties.[13]

However, there is no scientifically proved data to support the wound healing activities of *P. americana* leaves in literature. Therefore, we have undertaken the present study to explore the effects of *P. americana* leaf extract on wound healing.

MATERIALS AND METHODS

Plant Material and Extraction

The *P. americana* leaves were collected locally in April 2006. The *P. americana* leaves (200g) were cleaned with water and air dried, following which the leaves were ground into a powder using an electric blender. Then 200 g of the powder was suspended in 500 ml of ethanol for 20 hours at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper. The filtrate was placed in an oven to dry at 40°C. The clear residue (13 g) was used for the study. The extract was subjected to preliminary phytochemical tests.

Animals

The study was approved by the Ethics Committee for animal experimentation (AHC06/07/1), The Faculty of Medical Sciences, The University of the West Indies, St. Augustine

Healthy inbred gender-matched Sprague Dawley rats weighing 200-250g were used for the study. They were individually housed and maintained on normal food and water *ad libitum*. Animals were periodically weighed before and after the experiment. The rats were anaesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (120 mg/kg body weight) intraperitoneally. Animals were closely observed for any infection and if they showed signs of infection were separated and excluded from the study and replaced.

Wound-healing activity

Excision wound model was used to evaluate the wound- healing activity of *P. americana*

Excision wound model: Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malone. [14] The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area 200 mm^2 and 2 mm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The animals were divided into three groups of 6 each. The experimental group animals were treated with the ethanol extract of *P. americana (*100 mg/kg/day),

standard group animals were treated with mupirocin ointment (10%), and the control group animals were applied with petroleum jelly. The wound closure rate was assessed by tracing the wound on day 1, 3, 5, 7, 9 and 13 post-wounding using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. The day of eschar falling, after wounding, without any residual raw wound was considered as the period of epithelization.

Estimation of Hydroxyproline

Dry granulation tissue from the entire three groups was used for the estimation of hydroxyproline. Hydroxyproline present in the neutralized acid hydrolysate were subsequently oxidized by sodium peroxide in presence of copper sulfate. After that they were complexed with para-dimethylaminobezaldehyde to develop a pink color and that was measured at 540 nm by spectrophotometer.

Histological study

The tissues from the healed area were obtained on day 13 from the experimental, standard and control group animals for the histological study. For the better appreciation of collagen deposition Masson's trichrome stain was used which stain the fibres green.

Statistical analysis

The means of wound area measurements and hydroxyproline content among groups at different time intervals was compared using a one-way ANOVA, followed by Turkey's post-hoc tests. Data were analyzed using the SPSS (Version 12.0, Chicago, USA) and P value was set < 0.05 for all analyses.

RESULTS

The significant reduction in the wound area was observed in the animals treated with the *P. americana* extract compared with those animals applied with petroleum jelly and mupirocin ointment. Figure 1 shows the effects of the *P. americana* leaf extract administered topically on wound healing activity in rats inflicted with excision wound. In this model, *P. americana* treated animals were found to epithelize faster (10.32 ± 0.21) when compared to controls (13.32 ± 0.29) ($p<0.001$). On day 13 the reduction in the wound area of the extract treated animals was 86% when compared to controls (67%) and it was statistically significant ($p<0.001$) (Fig 2).

The histological study of the granulation tissue obtained on 13th day from the experimental animals showed abundant well organized bands of collagen, more fibroblasts and few inflammatory cells (Fig 3c) when compared to section of granulation tissue obtained from controls which showed inflammatory cells, scanty collagen fibres and fibroblasts (Fig 3a).

Table1: Wound healing effect of *P.americana* in Excision wound model

Parameter	Control	Experimental	Standard
Wound area (mm^2)			
Day 1	210.00 ± 2.23	210.00 ± 2.23	210.00 ± 2.23
Day 3	192.50 ± 1.18	184.00 ± 3.57	189.00 ± 3.57
Day 5	176.50 ± 0.67	143.50 ± 0.22	153.50 ± 0.22
Day 7	146.50 ± 2.46	114.00 ± 2.63	124.00 ± 2.63
Day 9	125.00 ± 2.23	89.50 ± 4.29	98.50 ± 4.29
Day 11	96.50 ± 0.67	45.00 ± 0.30	58.00 ± 4.12
Day 13	69.00 ± 0.00 (67%)	30.00 ± 0.00 (86%)**	40.00 ± 3.10 (81%)
Period of epithelialization (days)	13.33 ± 0.29	10.32 ± 0.21**	12.12 ± 0.11
Hydroxyproline (mg/ g tissue)	48.33 ± 6.27	98.31 ± 15.90**	80.33 ± 9.80

N = 6, Values are expressed as mean ± SE, *P<.05 and **P<.001vs.control

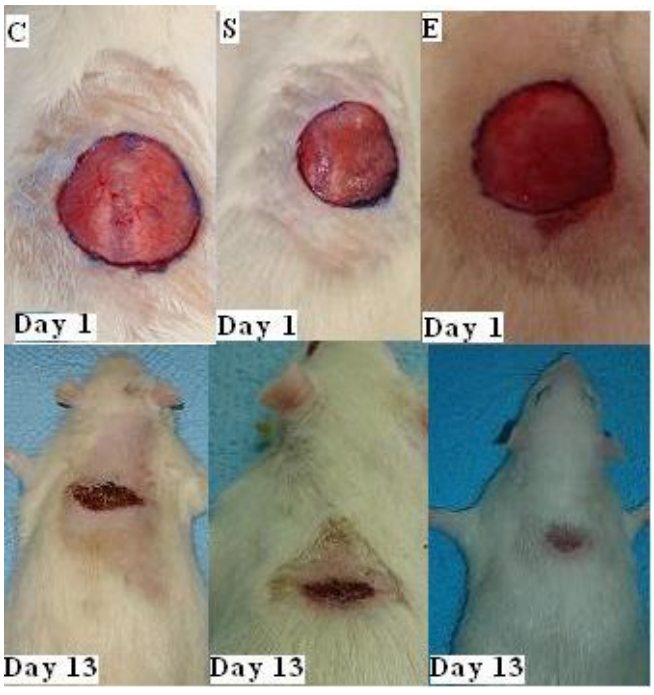

Figure 1: Wound area of Control (C), Standard (S) and Experimental (E) on day 1 and 13.

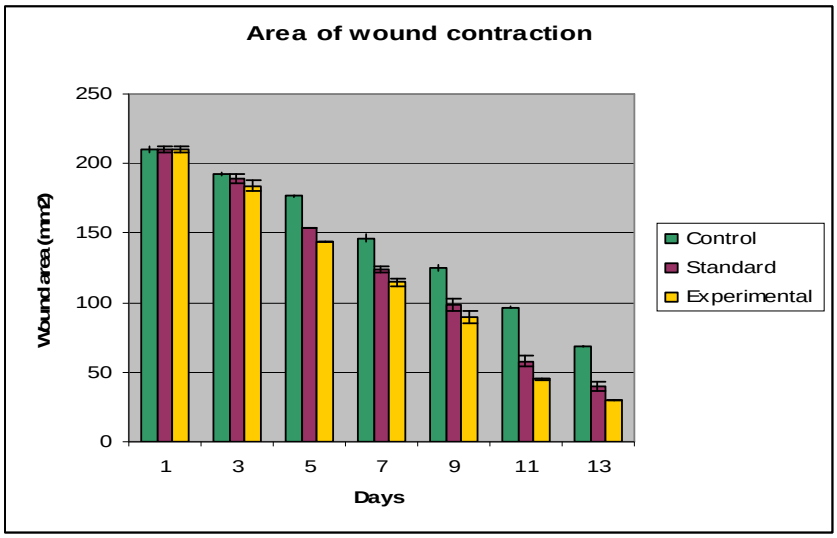

Figure 2: Rate of wound area contraction of control, standard and experimental group of animals on different days.

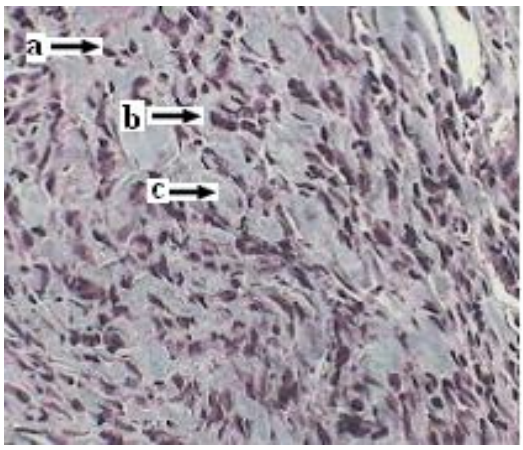

Figure 3a: Control group granulation tissue with less collagen (Masson's trichrome stain) a = inflammatory cells, b= fibroblast, c= collagen fibres.

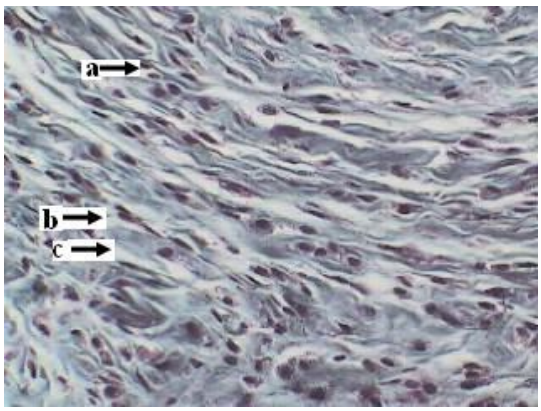

Figure 3b: Standard group granulation tissue with more collagen (Masson's trichrome stain) a = inflammatory cells, b= fibroblast, c= collagen fibres.

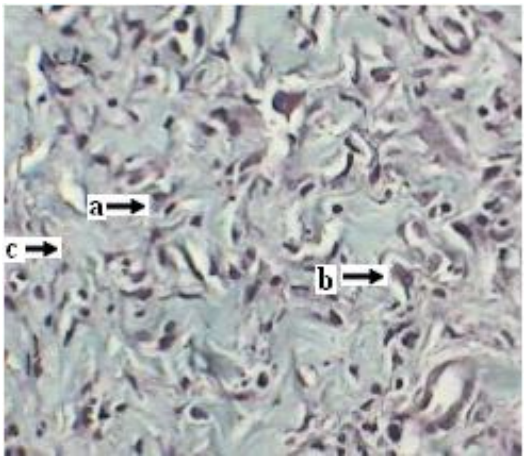

Figure 3c: Experimental group granulation tissue with more collagen (Masson's trichrome stain) a = inflammatory cells, b= fibroblast, c= collagen fibres.

DISCUSSION

These results show that *P. americana* leaf extract significantly increased the rate of wound contraction, epithelialization, and the weight of the wound tissue. The latter is predominantly composed of fibroblasts, collagen, oedema and new small blood vessels. The increased rate of wound contraction and the histological picture of the granulation tissue are indicative of increased collagen synthesis.

Many facets of wound healing under redox control require a delicate balance between oxidative stress and antioxidants. While the normal physiology of wound healing depends on low levels of reactive oxygen species and oxidative stress, an overexposure to oxidative stress leads to impaired wound healing.[15] Antioxidants are postulated to help control wound oxidative stress and thereby accelerate wound healing. [16] Alterations in the antioxidant profile accompanied by elevated levels of MDA, a marker of free radical damage may be contributory to impaired wound healing in immunocompromised rats. *P. americana* leaves contain flavonoids and phenolic acids (Isorhamnetin, luteolin, rutin, quercetin and apigenin) which were powerful antioxidants capable of scavenging free radicals.[17,18]. It is possible that the powerful antioxidant activity of *P. americana* leaf constituents could contribute the wound healing promoting activity.

The vasorelaxant action of *P. americana* leaf extract was endothelium dependent, and was, therefore, possibly dependent on the synthesis and release of nitric oxide (NO) .[9, 10] NO enhances fibroblast collagen synthesis [19] and experimental wound healing in diabetes. [20]. NO plays a key role in wound repair and its beneficial effects on wound repair may be

attributed to its functional influences on angiogenesis, inflammation, cell proliferation, matrix deposition, and remodeling. [21] Vasodilation is an important means by which the wound can be exposed to increased blood flow, accompanied by the necessary inflammatory cells and factors that fight infection and debride the wound of devitalized tissue. NO-releasing nanoparticles accelerate wound healing in NOD-SCID mice [22] by promoting fibroblast migration and collagen deposition. [23]. Increased rate of epithelialization, wound contraction and collagen content in granulation tissue in this study could be due to enhancement of fibroblast collagen synthesis by nitric oxide.

NO releasing nanoparticles are useful in treatment of wound infections. [24] The hypoglycemic [7,8], hepatoprotective [11] and antibacterial [12] effects of leaf extract along with its NO releasing effect could be useful in treatment and/or prevention of wound infections.

The extract has analgesic and anti-inflammatory properties. [13] Generally anti-inflammatory drugs suppress wound healing. However certain natural substances like honey controls inflammation and promotes wound healing by lowering prostaglandin levels and elevating NO levels. [25] Presumably, the leaf extract of *P. americana* could produce analgesic, anti-inflammatory and wound healing promoting effects by lowering prostaglandin levels and raising NO levels.

CONCLUSIONS

Our data suggests the use *P. americana* leaf extract for treating the wound. However, it needs to be studied further to isolate the active ingredients that promote wound healing, before considering it for clinical use.

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