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Research Article

WOUND HEALING ACTIVITY OF VARIOUS EXTRACTS OF FRUIT OF *PYRUS COMMUNIS* L. IN NORMAL RATS

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ABSTRACT
To evaluate the fruit of *Pyrus communis* L. for its wound healing property by different wound models using wistar albino rats. The wound healing activity of ethyl acetateandethanol extracts of fruits of *Pyrus communis* (EAEPC and EEPC 200 mg/kg) was investigated by various wound healing model in normal rats such as excision, incision and dead space wound model. Povidone iodine is used as standard. The wound was created according to standard procedure in different model and the wound contraction area, epithelization period, scar area, breaking strength and weight of granulomawere assessed. The phytochemical screening and total phenolic content was estimated in both extracts. Hydroxyproline content of harvested granulation tissue was estimated in all groups of animals. The result suggests various extracts of *Pyrus communis* have significant ($p < 0.01$) wound healing activity in different models. In this study we concluded that fruit of *Pyrus communis* is natural remedy for treating different type of wounds.
Keywords: *Pyrus communis*, Incision wound, Excision wound, hydroxylproline and phenolic content.

INTRODUCTION

Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissue. In general wounds are classified as acute wound and chronic wound[1]. Wound healing can be defined as a complex dynamic process that results in the restoration of anatomic continuity and function. It is a finely orchestrated and overlapping sequence of events involving vascular response phase/hemostasis, inflammation, proliferation, maturation and remodeling[2,3]. Many Ayurvedic herbal plants have a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way. The Pear (*Pyrus communis* L.) is among the most economically important fruit tree crops of the temperate zones[4]. It belongs to family Rosaceae. Ancient Greek poet Homer described Pears as one of the 'gifts of God'. This prehistoric fruit has been under cultivation both in Europe and Asia for long times, also known as European Pear[5] Sand pear (Japanese and Chinese species) has been domesticated as edible fruit and cultivated in Asia for more than 3000 years[6]. It has astringent, sedative activity and act as febrifuge. Its leaves and bark can be used in wound healing. Leaves, buds, and bark of the tree are domestic remedies among the Arabs on account of their astringent action[7]. Pear is a rich source of Vitamin C, ascorbic acid and it is an antioxidant. It acts against reactive Oxygen species[8,9]. Arbutin is commonly used in urinary therapeutics and as a human skin whitening agent. It decreases melanin in the skin. In the past, the presence of arbutin in Pear has been correlated with biochemical processes that operate as defense mechanism against bacterial invasion. Therefore acts as antibacterial too. The flowers of common pear are used in folk medicine as components of analgesic and spasmolytic drugs[10]. Its fruits are good source of pectin, help in maintaining desirable acid balance in the body. As per survey the pear possess antioxidant and microbial activity but there is no report available for wound healing activity on this fruit. Hence, the present study is undertaken for the investigation of wound healing activity of fruits of *Pyrus communis* in wistar albino rats.

MATERIAL AND METHODS

Preparation of Extracts

The collected fruits were shade dried completely. The dried fruit was then coarsely powdered. The powdered materials were defatted with petroleum Ether and successive extracted with solvent ethyl acetate and 80 % ethanol by maceration. Final compound was concentrated by vacuum drying.

Preliminary Phytochemical Screening

The extracts of *Pyrus communis* was screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin, glycosides[11]. The concentration of total phenol in extracts was determined using spectrophotometric method[12].

Experimental Animals

All the experiments were carried out using Swiss Albino mice (25-30 g) and Wister rats (150-200 g). The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2^{0}C and relative humidity of 30–70 %. A 12 hrsday: 12 h night cycle was followed. All animals were allowed free access to water and fed. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) of Sri Krisnachaithanya College of pharmacy, Madanapalle, Andhra Pradesh, India. No: SKCP/IAEC/PGCOL/12-13/01

Acute Oral Toxicity Studies

The acute toxicity study was carried out with extracts of *Pyrus communis* as per OECD 423 Guidelines. Mortality in each group within 24 h was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity. The extracts of *Pyrus communis* had good margin of safety and did not shown any lethal effects on the animals up to the doses of 2000 mg/kg. Hence the LD50 of both extracts

of *Pyrus communis* were considered as 2000 mg/kg. Studies were carried out with 1/10 of the LD50 as effective dose 200 mg/kg.

Experimental Study Design

Wistar albino rats weighed about 150-200 g were divided into four groups of six rats each

Group I: Control, administer 2 ml/kg of 1 % v/v tween 80

Group II: Standard drug 5 % povidone iodine ointment applied topically.

Group III: Receive 200 mg/kg ethyl acetate extracts of *Pyrus communis* (EAEPC) orally.

Group IV: Receive 200 mg/kg of ethanol extracts of *Pyrus communis* (EEPC) orally.

Excision Wound

All the animals were anesthetized by using Thiopentone sodium [50 mg/kg, ip]. An impression was making on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2. Animals were treated daily with drugs as mentioned above under experimental design from 0^{th} day to 11^{th} post-wounding day. Wound area is measured on 0^{th}, 3^{rd}, 6^{th}, 9^{th} and 11^{th} day for determination of wound contraction and percentage wound contraction was calculated. Falling of scar leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization[13].

Incision Wound

Para vertebral straight incision of 6 cm length was making through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete homeostasis, the wound was closed by means of interrupted sutures placed at equidistance points about 1 cm apart. Animals were treated daily with drugs as mentioned above under experimental design from 0^{th} day to 10^{th} post-wounding day. The sutures were removed on eighth day. The wound breaking strength was determined on 10^{th} day by tensiometer.[14,15]

Measurement of tensile strength

Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of the repaired tissue. For this purpose the newly repaired tissue including scar was excised to measure the tensile strength measurement is called a tensiometer. For the quantitation one of the edges of the wound was fixed while applying a measurable force to the other one. The load (weight) in grams required to disrupt the wound is determined after complete healing of the wound, and that was on day 10^{th} day of surgery.

Dead Space Wound Model

This type of wound was created by implanting subcutaneously 10 mg sterilized cotton pellet in the lumber region of dorsal side in anesthetized rats. Animals were treated daily with drugs as mentioned above under experimental design from 0^{th} day to 10^{th} post-wounding day. On the 10^{th} day, granulation tissue harvested on the implanted cotton was carefully dissected out wet weight was noted and dried at 60ºC for 24 h to get a constant weight and weighed. The Protein (measured as hydroxyproline) content of the tissue was estimated using the techniques described by Neuman and Logan[16,17].

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Dunnett's method of multiple comparisons was employed using Graphpad Instat 3.0 software. $p < 0.01$ was considered to be statistically significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of extracts of *Pyrus communis* shows presence of flavonoids, glycosides, tannin, alkaloids, phenolic compound and carbohydrate. (Table 1)

Table 1: Preliminary phytochemical screening of *Pyrus communis* L.

Extracts	Steroids	Alkaloids	Glycosides	Flavonoid	Tannin	Phenolic compound	carbohydrate
Ethyl acetate	-	-	-	+	+	+	-
Ethanol		+	-	+	+	+	-

Where + = present, - = absent

Total phenolic content assay

Total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (mg of GA/g of extract). The total phenolic content in the examined extracts shows 49.33 ± 0.08 and 46.63 ± 0.12 mg GA/g (Table 2).

Table 2: Phenolic content of various extracts of *Pyrus communis* L.

Extract	mg of GA/g of extract
Ethyl acetate	49.33 ± 0.08
Ethanol	46.63 ± 0.12

Excision wound

In an excision wound model the extracts EAEPC and EEPC at a dose of 200 mg/kg showed significant ($p < 0.01$) wound healing activity on 11^{th} day by increased wound contraction and % of wound contraction compare to control and no significant to standard. The extracts and standard showed significant changes in complete epithelization when compared to control (18.83 ± 1.16 days). It's also showed a scar area of 19.06 ± 2.55, 23.96 ± 3.23 and 13.91 ± 2 mm^2 as compared to control 31.61 ± 2.92 mm^2. (Table 3, 4 and Figure 1-9)

Table 3: *Pyrus communis* on wound area in excision wound model

Groups	Treatment	Excision wound				
		Wound area in mm^2				
		0^{th} day	3^{th} day	6^{th} day	9^{th} day	11^{th} day
Control	2 ml of 1 % v/v tween 80	500	475.23 ± 6.51	398 ± 14	229.06 ± 11.20	179.28 ± 7.21
Standard	5 % Povidone ointment	500	313.51 ± 8.68	251.8 ± 11.05*	132.46 ± 7.24*	52.98 ± 4.06*
EAEPC	200 mg/kg	500	362.93 ± 8.90*	260.08 ± 9.53*	146.83 ± 8.41*	59.5 ± 4.64*
EEPC	200 mg/kg	500	380.03 ± 8.71*	294.41 ± 12.48*	158.8 ± 11.45*	70.51 ± 6.47*

Values are expressed as Mean ± SEM. Significant (*P < 0.01) compared with treated groups Vs control.

Table 4: *Pyrus communis* on excision wound model in Scar area and epithelization period

Groups	Treatment	Mean size of scar area in mm^2	Period of epithelization (days)
Control	2 ml of 1 % v/v tween 80	31.61 ± 2.92	18.83 ± 1.16
Standard	5 % Povidone ointment	13.91 ± 2*	12.33 ± 0.51*
EAEPC	200 mg/kg	19.06 ± 2.55*	13.16 ± 0.75*
EEPC	200 mg/kg	23.96 ± 3.23*	13.83 ± 0.75*

Values are expressed as Mean ± SEM. Significant (*P < 0.01) compared with treated groups Vs control.

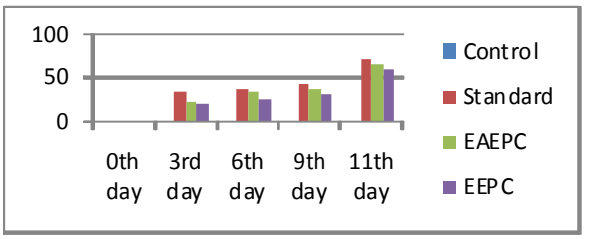

Figure 1: Percentage of wound contraction

Effect of extracts on excision wound model

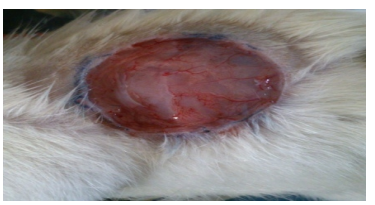

Figure 2: Control 0^{th} day

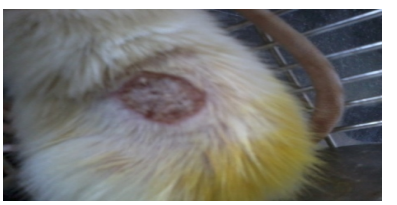

Figure 3: Control 11^{th} day

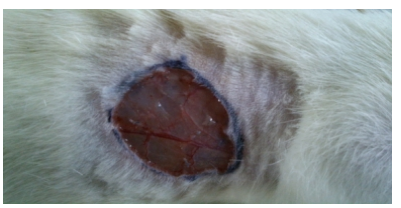

Figure 4: PCEE extract 0^{th} day

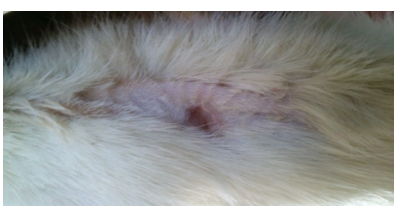

Figure 5: PCEE extract 11^{th} day

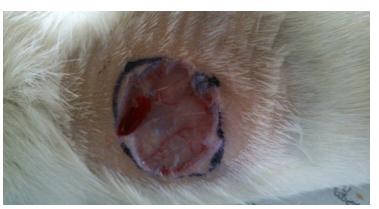

Figure 6: PCE extract 0^{th} day

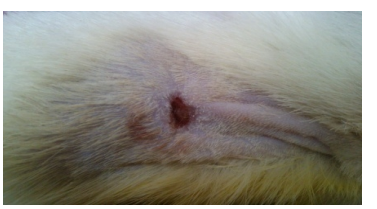

Figure 7: PCE extract 11^{th} day

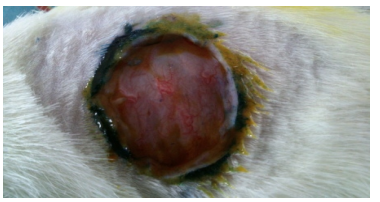

Figure 8: 5 % Povidone iodine 0th day

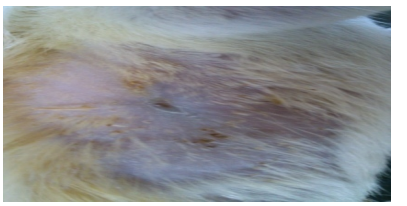

Figure 9: 5 % Povidone iodine 11th day

Incision and Cotton pellet granuloma models

In incision and cotton pellet granuloma models Table 5, 6 and Figure 10-17 shows the extracts and standard showed significant different in breaking strength 392.16 ± 9.75, 355.83 ± 8.88 and 459.5 ± 8.10 when compared to control (180.33 ± 9.21) and also showed significant increase in wet and dry weight of cotton pellet compared to control in cotton pellet model. The hydroxyproline content was found to be significantly increased in Group-II, III and IV ($p < 0.01$) as compared with control.

Table 5: The effect of *Pyrus communis* on incision wound

Groups	Treatment	Tissue breaking strength in g
Control	2 ml of 1 % v/v tween 80	180.33 ± 9.21
Standard	5 % Povidone ointment	459.5 ± 8.10*
EAEPC	200 mg/kg	392.16 ± 9.75*
EEPC	200 mg/kg	355.83 ± 8.88*

Values are expressed as Mean ± SEM. Significant (*P < 0.01) compared with treated groups Vs control.

Table 6: The effect of *Pyrus communis* on dead space model

Groups	Treatment	Tissue wet weight in g	Tissue dry weight in g	Hydroxyproline in µg/g of tissue
Control	2 ml of 1 % v/v tween 80	118.83 ± 3.25	34.66 ± 1.56	1486.33 ± 2.39
Standard	5 % Povidone ointment	186.5 ± 2.04*	51.66 ± 1.44*	2571.16 ± 2.86*
EAEPC	200 mg/kg	178.83 ± 3.22*	49.5 ± 1.50*	2254.66 ± 3*
EEPC	200 mg/kg	169.16 ± 4.38*	46.83 ± 1.85*	1981.5 ± 2.96*

Values are expressed as Mean ± SEM. Significant (*P < 0.01) compared with treated groups Vs control

Effect of extracts on incision wound model

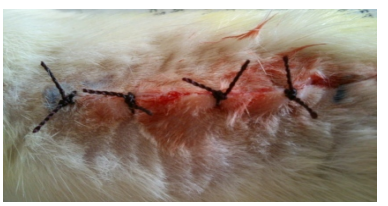

Figure 10: Control 0th day

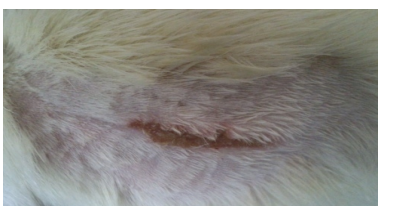

Figure 11: Control 10th day

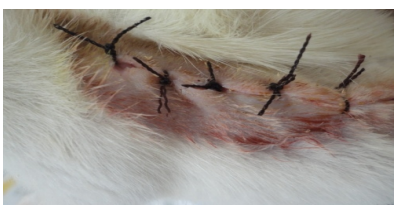

Figure 12: PCEA extract 0th day

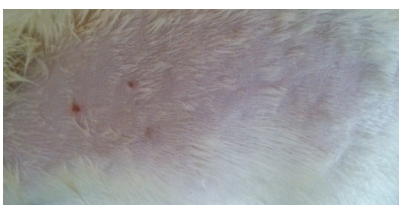

Figure 13: PCEA extract 10th day

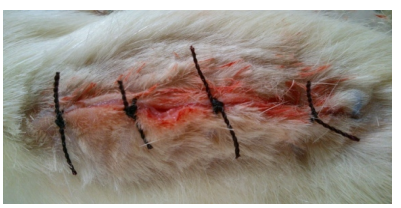

Figure 14: PCE extract 0th day

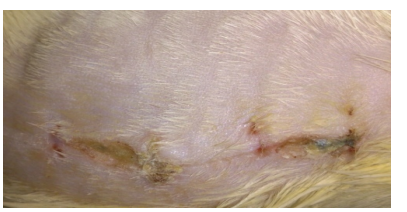

Figure 15: PCE extract 10th day

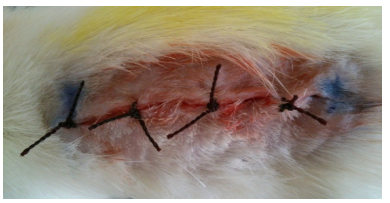

Figure 16: 5 % Povidone iodine 0th day

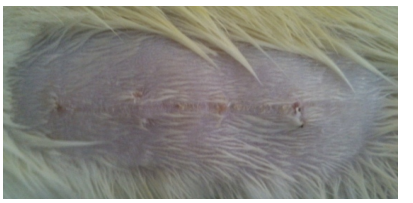

Figure 17: 5 % Povidone iodine 10th day

DISCUSSION

Wound healing involves various phases. Initially involves acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules, which are later removed to form a scar. Drugs, which influence one phase, may not necessarily influence another. Hence different models have been used in our study to assess the effect of various phases. The present studies reflected that ethyl acetate and ethanol extracts of leaves of fruits of *Pyrus communis* (200 mg/kg) was effective in all the models of wound healing activity. The study of the effect on excision model showed that the *Pyrus communis* increased the wound contraction, decreased epithelization and scar area. Significant increase in skin breaking strength which was a reflection of increased collagen levels by increased cross linking of collagen fibers. In addition, increase in wet and dry granulation tissue weight indicated the presence of higher protein (hydroxyproline) content[18]. The above statement was proved by results of hydroxyproline estimation. Earlier studies revealed Flavonoids have therapeutic uses due to their anti-inflammatory, antifungal, antioxidant and wound healing properties[19-22]. Moreover, flavonoids and their derivatives are known to decrease lipid peroxidation by improving vascularity and preventing or slowing down the progress of cell necrosis. Flavonoids are also known to endorse wound healing processes primarily owing to their antimicrobial and astringent properties, which appear to be responsible for wound contraction and elevated rate of epithelization[23]. In other study, Polyphenolic flavonoids and tannins are reported to facilitate wound healing[24]. Once again the earlier report confirmed that Proanthocyanidins or condensed tannins are a group of biologically active Polyphenolic bioflavonoids that are synthesized by many plants. Proanthocyanidins and other tannins are known to facilitate wound healing[25,26]. Tannins promote wound healing through several cellular mechanisms: scavenging of free radicals and reactive oxygen species, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts[27]. The present study, phytochemical screening confirmed that extracts of *Pyrus communis* contains flavonoids, tannins, alkaloids and phenolic compounds. The wound healing potential of the extracts of *Pyrus communis* could be due to the interaction of the mixture of these phyto-constituents with various phases of wound healing.

CONCLUSION

The study indicates the fruits of *Pyrus communis* interact with various phases of wound healing like inflammation, angiogenesis, proliferative, repair and remodeling. So the plant could serve as potent natural drug for wound healing. Further studies need to be conducted to isolate active constituents like flavonoids and tannins respons1ible for wound healing.

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