

Evaluation of anti-helminthic and wound healing potential of *Saraca asoca* (Roxb) bark

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ABSTRACT

Introduction: The present work was done in search of traditional cure for live stock diseases and injuries occur to the body. The present study was aimed to evaluate wound healing potential of *Saraca asoca* bark in the form of simple ointment using two types of wound models in rats as incision wound and excision wound models and also evaluated for anti-helminthic activity. **Materials and Methods:** The methanolic extract of *Saraca asoca* Roxb. was evaluated for its anti-helminthic and wound healing activity at doses of 50 mg/ml and 100 mg/ml for anti-helminthic activity and 1% w/w, 4% w/w ointments in the case of wound healing activity. Albendazole 10 mg/ml is used as standard for antihelminthic activity and nitrofurazone 0.2% w/w ointment served as standard in the wound healing model. The parameters studied are time taken for paralysis and death of worms for anti-helminthic activity, tensile strength in incision wound model, percentage wound closure and period of epithelialisation in excision wound model. **Results:** Highest dose of this extract was found to possess good anti-helminthic activity along with significant wound healing activity. This was evident by decrease in time taken for paralysis and death; significant increase in tensile strength and decrease in period of epithelialization; increase in wound contraction when compared to control. **Discussion:** The progressive results are may be due to depletion of glycogen or β -tubulin inhibition in helminthic worms and increase in the viability and strength of collagen fibers in rats by the presence of tannins, flavonoids and alkaloids.

Keywords: *Saraca asoca*, wound healing activity, anti-helminthic activity.

INTRODUCTION

Helminthic infections are one of the common infections affecting a large proportion of world's population. Helminths can live in humans and animals, and are usually transmitted through contaminated food, water, faeces, and unwashed hands or contact with a contaminated object. It is estimated that approximately one-third of the almost three billion people that live on less than two US dollars per day in developing regions of sub-Saharan Africa, Asia and the America are infected with one or more helminth.

^[1] Adding to the global morbidity that results from human helminth infections are the observations that they have both direct and indirect effects on malaria and HIV/AIDS in developing countries. In Sub-Saharan Africa and elsewhere, helminthiases are frequently coendemic with malaria and HIV/AIDS. ^[2] Mostly school aged children and pre school children tend to curb the excessive number of intestinal worms and schistosomes and as a result experience diminished growth, impaired memory.^[3] Despite the remarkable success of mass drug administration, gastro intestinal helminthes quickly develops resistance towards currently available drugs.^[4] Thus, research on new drugs is clearly needed for the treatment of helminthes. Therefore, the present study was carried out for antihelminthic activity. Wound is a break in the normal tissue integrity. It can be caused by either physical, chemical, thermal and microbial agents. Wounds, particularly among the elderly population, can show delayed or disturbed healing; however, delayed or disturbed healing is also evident in patients with comorbidities such as

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diabetes, atherosclerosis, venous/arterial insufficiency, reduced mobility due to chronic infirmity and hypercholesterolemia. To heal a wound, the body undertakes a series of actions collectively known as the wound healing process. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. 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 in the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction. Angiogenesis involves formation of new blood vessels from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts exert collagen and fibronectin to build 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.^[5] A number of drugs ranging from simple non-expensive analgesics to complex and expensive chemotherapeutic agents were available in the management of wound healing either positively or negatively.^[6] Because of draw backs and unwanted effects of current drugs, approaches towards medicinal plants provides promising results in accelerating the wound healing process progressively in a normal manner. As no scientific data has been reported on anti-helminthic and wound healing activity of methanolic extract of *Saraca asoca* Roxb, the present study was done to evaluate the anti-helminthic potential and wound healing activity. *Saraca indica* or *Saraca asoca* is a small evergreen tree having height up to 10 meters. It occurs up to the altitude of 750 meters. Other names are sita-ashok, anganapriya, hemapushpa, madhupushpa, vanjula, vishoka, kankeli, vichitra.^[7] Leaves are narrowly lanceolate, cork like at the base and with a shot pestistipules are intra-petiolar and completely united. The bark is dark brown or grey or almost black with warty surface. Stem bark is rough and uneven due to the presence of rounded or projecting lenticles. It is channeled, smooth with circular lenticles and transversely ridged, sometimes cracked. Flowers are fragrant, yellowish orange turning to scarlet, in short laterally placed corymbose, axillary panicles, bract small, deciduod, calyx petaloid. Seeds are 4–8, ellipsoid-oblong and compressed.^[8] It is effective against a great number of gynecological disorders, ash of the plant is good in rheumatoid arthritis.^[9] It is also used in leucorrhoea and in internal bleeding, haemorrhoids

and haemorrhagic dysentery.^[10] Alcoholic extract of bark shows significant anti-microbial activity.^[11]

MATERIALS AND METHODS

The bark of *Saraca asoca* was collected locally and allowed for shade drying. The shade dried bark was crushed in to pieces and powdered. About 100 gm of powdered bark was extracted using soxhlet apparatus for 12 hrs. Alcohol removal was carried out under reduced pressure afforded a solid powder with a yield of 10%.

PHYTOCHEMICAL SCREENING

Methanolic extract was evaluated for the presence of various phytoconstituents by performing different phytochemical tests. The results showed the presence of tannins, flavanoids, saponins, anthraquinone, cardiac glycosides, steroids and carbohydrates which were reported in table 1.

EXPERIMENTAL ANIMALS

Anti-helminthic activity

The anti-helminthic activity was evaluated on adult Indian earthworm, 'Pheretima posthuma' as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings.^[12-13]

Wound healing activity

Wound healing activity was done on male albino rats weighing about 150–200 gm. They were fed with food and water *ad libitum*. They were housed in polypropylene cages and maintained under standard conditions.

Acute dermal toxicity

The acute dermal toxicity study was carried out in adult female albino rats by 'fix dose' method of OECD

Table 1. Chemical tests.

S.No	Chemical test	MESA
1	Alkaloids	+
2	Carbohydrates	+
3	Proteins and aminoacids	–
4	Steroids and Terpenoids	+
5	Saponins	+
6	Glycosides	+
7	Phenolic compounds	+
8	Tannins	+

+ = present; – = absent.

(Organization for Economic Co-operation and Development) guideline No. 434. Extract from the bark of *Saraca asoca* was applied topically at dose level 2000 mg/kg.

DRUG FORMULATIONS

Anti-helmintic activity

50 mg/ml and 100 mg/ml concentration solutions were prepared for methanolic extract. The extract was initially dissolved in dimethyl sulfoxide (DMSO) and the solutions were prepared in normal saline.

Wound healing activity

The methanolic extract of *Saraca asoca* was formulated as 1% w/w and 4% w/w ointments. These ointments were prepared by incorporating 1 g and 4 g of the extract respectively into 100 g of simple ointment base. The standard drug used for wound healing activity was soframycin 1% w/w ointment. Ointments were applied once a day to experimental animals with wounds until they were cured.

STUDY PROTOCOL

Anti-helmintic activity

Each group consists of 6 earth worms.

Group 1 – served as control and released in to normal saline.

Group 2 – served as standard and released in to Albendazole 10 mg/ml.

Group 3 – served as treatment group and released in to 50 mg/ml solution of methanolic extract of *Saraca asoca*.

Group 4 – served as treatment group and released in to 100 mg/ml solution of methanolic extract of *Saraca asoca*.

Wound healing activity

For each model 4 groups were used. Each group consists of 5 animals.

Group 1 – served as control for incision wound model and received only simple ointment base.

Group 2 – served as standard for incision wound model and received 1% w/w Soframycin ointment.

Group 3 – served as treatment group for incision wound model and received 1% w/w ointment of MESA (methanolic extract of *Saraca Asoca*)

Group 4 – served as treatment group for incision wound model and received 4% w/w ointment of MESA.

Group 5 – served as control for excision wound model received only simple ointment base.

Group 6 – served as standard for excision wound model and received 1% w/w Soframycin ointment.

Group 7 – served as treatment group for excision wound model and received 1% w/w ointment of MESA.

Group 8 – served as treatment group for excision wound model and received 4% w/w ointment of MESA.

EXPERIMENTAL PROCEDURE FOR ANTI-HELMINTIC ACTIVITY

The anthelmintic activity was performed according to the method described by T. Ghosh^[14] on adult Indian earthworm *Pheritima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. The earthworms in each group were released into 50 ml of desired formulation. Observations were made for the time taken to paralyze or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color.

EXPERIMENTAL PROCEDURE FOR WOUND HEALING ACTIVITY

Incision wound model: On the depilated backs of the animals, two paravertebral incisions of 6 cm length were made cutting through the full thickness of the skin. Interrupted sutures, 1 cm apart, were placed to approximate the cut edges of the skin.^[15] The sutures were removed on the 7th post wound day and skin breaking strength was measured on the 10th day by continuous water flow technique of Lee.^[16]

Excision wound model: An excision wound was inflicted by cutting away 500 mm² full thickness of a pre-determined area on the depilated back of the rat. Epithelialization period was noted as the number of days after wounding required for the scar to fall off leaving no raw

wound behind. Wound contraction rate was monitored by planimetric measurement of the wound area on alternate days. This was achieved by tracing the wound on a graph paper. Reduction in the wound area was expressed as percentage of the original wound size.^[17]

Statistical analysis

The means of wound area measurement and wound breaking strength between groups at different time intervals were compared using one-way ANOVA, followed by students T-test. In all tests the criterion for statistical significance was $p < 0.05$.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the methanolic extract of *Saraca* showed the presence of triterpenoids, carbohydrates, phenols, saponins, tannins and flavonoids (Table 1). The MESA (100 mg/ml) showed significant ($p < 0.001$) decrease in paralysis time and significant ($p < 0.001$) decrease in death time when compared to albendazole and MESA (50 mg/ml) treated animals (Table 2). In the current study, albendazole that was used as the standard shows its action by blocking glucose uptake and inhibition of polymerization of β -tubulin.^[18] Therefore, antihelmintic activity of methanolic extract of *Saraca asoca* may be associated with the depletion of glycogen stores or by β -tubulin inhibition which prevents vesicular transport. Anti-helmintic activities are well documented by the existence of active compounds such as flavonoids, tanins, triterpenoids.^[19-20] Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers by regenerating dermal and epidermal tissue, in a predictable fashion to repair the damage.^[21] There are three phases in this process such as inflammatory, proliferative and remodeling phases.^[22] In the process of inflammation bacteria are phagocytosed and removed.^[23] The cardinal symptoms of this phase are pain, swelling, redness.^[24] Proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction.^[23] In the

maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis. Infection of wound by microorganisms delays the wound healing. However, it can't be seen with *Saraca asoca* as it has antimicrobial activity.^[25] In Incision wound model the 4% w/w ointment of MESA showed significant ($p < 0.001$) increase in tensile strength when compared to control, standard and MESA 1% w/w treated animals. The 1% w/w ointment of MESA showed significant ($p < 0.01$) increase in tensile strength when compared to control animals (Table 3). Increase in breaking strength of SA – ointment treated animals improved collagen migration by increased cross linking. In excision model of study the MESA 4% w/w showed significant ($p < 0.01$) wound closure activity when compared to the control animals on 4th day. The MESA 4% w/w showed significant ($p < 0.001$) wound closure when compared to the control animals on 8th, 12th and 16th day. The MESA 4% w/w showed significant ($p < 0.001$) activity in wound closure when compared to the Soframycin 1% w/w treated animals on 4th and 16th day. The MESA 4% w/w showed significant ($p < 0.01$), ($p < 0.05$) activity in wound closure when compared to the Soframycin 1% w/w treated animals on 8th and 12th day respectively. The MESA 4% w/w showed significant ($p < 0.05$), ($p < 0.01$) activity in wound closure when compared to the MESA 1% w/w treated animals on 8th and 16th days respectively. The MESA 1% w/w showed significant ($p < 0.001$) activity in wound closure when compared to the control animals on 8th and 12th day. The MESA 1% w/w showed significant ($p < 0.01$) activity in wound closure when compared to the control animals on 16th day (Table 4). The MESA 4% w/w showed significant ($p < 0.001$) wound closure activity in 14 days when compared to the control (21 days) and standard animals (18 days). The MESA 1% w/w showed significant ($p < 0.001$) wound closure activity in 16 days when compared to the control animals. Wound contraction is defined as the centripetal movement of the edges of a full thickness wound in order to promote seal of the defect.^[26] The rate of wound contraction was less in control and standard groups when compared to SA – ointment treated animals. Granulation tissue formed

Table 2. Anthelmintic activity of MESA.

Serial No	Groups	Dose (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1	Control	–	–	–
2	Albendazole	10	65.66±0.333	77.16±1.046
3	MESA	50	26.16±0.654	29.16±0.307
4	MESA	100	22.5±0.562*+	24.83±0.307*+

Values are expressed as mean ± SEM (n = 6)

*($p < 0.001$) vs standard group, + ($p < 0.001$) vs MESA – 50 mg group

Table 3. Effect of MESA on tensile strength in incision wound model.

Groups	Tensile strength (gm ± SEM)
Control	139.2 ± 0.374
Soframycin 1%	187.4 ± 1.887
MESA 1%	185 ± 2.530**
MESA 4%	204 ± 3.987***+xxx

Values are expressed as mean ± SEM (n = 5)

**($p < 0.001$) vs control group, ** ($p < 0.01$) vs control group, + ($p < 0.001$) vs standard group, xxx ($p < 0.001$) vs MESA – 1% group

Table 4. Effect of MESA on wound closure in excision wound model.

Groups	% of closure of excision wound area				Epithelialization in days
	Day 4	Day 8	Day 12	Day 16	
Control	29.4±2.159	56.8±0.663	73.8±2.354	85.2±1.772	21±0.774
Soframycin 1%	31.8±1.463	66.6±1.435***	84.2±1.393**	89.4±0.509**	18.6±0.244*
MESA1%	33.6±0.600	68±1.378***	86.6±1.288***	90±0.316**	16.6±0.509***
MESA 4%	38.4±1.631**+++	74.4±1.939***+xx	91.8±0.734***+	99.2±1.114***+xx	14.4±0.678***+++

Values are expressed as mean ± SEM (n = 5)

*** (p<0.001) vs control group, ** (p<0.01) vs control group, * (P<0.05) vs control group

+++ (p<0.001) vs standard group, ++ (p<0.01) vs standard group, + (p<0.05) vs standard group

xx (p<0.01) vs MESA – 1% group, x (p<0.05) vs MESA– 1% group

in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, and new small blood vessels. Collagen is a major component that strengthens extracellular tissue and is composed of the amino acid, hydroxyproline, used as a biomarker for tissue collagen.^[27] Oxidative stress is an intrinsic sector of wound healing leading to depletion of enzymatic and nonenzymatic antioxidants. In acute as well as in chronic wounds, the enzymatic antioxidants decreases and leads to depletion of non enzymatic antioxidants due to high oxidative stress.^[28–29] The wound healing activity of this medicinal plant is attributed to the active constituents present in it. Already *in vitro* antioxidant activity of stem bark of *Saraca asoca* was investigated,^[30] by this it is capable of destroying free radicals and prevents extensive tissue damage. The presence of flavonoids in the plant extract may reduce the lipid peroxidation by preventing or slowing the onset of cell necrosis and improves vascularity.^[31] Tannins^[32] and triterpinoids^[33] are known to magnify wound healing process. The plant *Portulaca oleracea* containing the tannins possesses wound healing activity as that of the *R. cordifolia*.^[34] The gel of ethanolic extract of the plant *Vernonia scorpioides* possess wound healing action by promoting regeneration and organization of the new tissue due to the presence of tannins.^[35] The embellin isolated from the ethanol extract of plant *Embliva officinalis* containing condensed tannins when formulated as a gel possess significant wound healing property^[36] as that of gel prepared by *R. cordifolia* ethanol extract. These active constituents promote the process of wound healing by increasing the viability of collagen fibers, by increasing the strength of collagen fibres either by increasing the circulation or by preventing the cell damage or by promoting the DNA synthesis.^[37]

CONCLUSION

The results of the present study revealed that the phyto-constituents tannins, flavonoids, triterpinoids are known

to avail anthelmintic and wound healing activities. These phytoconstituents decreased the life time of earthworms and this might be by the β -tubulin interference. Increased cellular proliferation may be due to the mitogenic activity of the plant extract, which might have significantly contributed to wound healing process. The extract had prominent effects towards cellular proliferation, granulation tissue formation and epithelialisation and this is evident by early dermal and epidermal regeneration. So far, fatality and lethality results in patients with wounds because of infections, these herbal extracts prevent high risk of sepsis and further prevent the prolongation of inflammatory phase. In conclusion, the present study suggests that the methanolic extract of *Saraca asoca* exerts anti-heleminitic and wound healing activity.

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