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Preliminary Pytochemical Screening and Wound Healing Activity of *Andrographis Peniculata*

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ABSTRACT

Andrographis peniculata (Acanthaceae) is a traditional Indian herb used historically as support for a healthy immune system. The following study was carried out using excision wound model in order to establish the wound healing activity of the drug on Swiss Wistar strain rats of either sex. The treatment was continued till the complete healing of the wound. For topical application, 2%w/w ointment of ethanolic extract and petroleum ether extract was prepared in 2% w/w PEG-4000; the control group of animals was left untreated. It was observed that there was complete healing on 12th post wounding day by applying both the extract. The wound contractions on 12th day in petroleum ether extract, ethanolic extract were shown to be 10.17 ± 0.60 and 16.33 ± 0.67 respectively. The healing was assessed by the rate of wound contraction. *Andrographis peniculata* ethanolic extract possesses better wound healing property in comparison to control and also with petroleum ether extract.

Keywords: Petroleum Ether extract, Ethanolic extract, Povidone Iodine, wound contraction.

INTRODUCTION

Herbal medicines have been practiced for many years by ancient man on trial and error basic. The observed effects of various plant extracts on diseases have encouraged scientists to look into the extracts of roots, seeds, leaves, stems and stem barks of many plants with a view to harness their potential constituents for the treatment of men maladies [1].

A wound is a breach in the normal tissue continuum resulting in a variety of cellular and molecular sequences. Wound may be accidental or as a result of planned surgical interventions in the tissue of the body. The term "wound" is generally applied to more superficial form of tissue damage where as "injury" is used for damages of depart structures. Wound has a variety of effects on the tissue including mechanical separation of functional structure such as blood vessels (bleeding) deformation occurs due to tissue tension, biological effects which results immediately leading to inflammatory responses and secondary effects which occurs at a later stage such as infections [2].

Andrographis paniculata belonging to the family Acanthaceae is a traditional Indian herb used historically as support for a healthy immune system. It has often been called "Indian Echinacea". It is an erect shrub found almost all regions of India. The main compound found in *Andrographis* is a diterpene lactone called andrographilide. *Andrographis* has been found to be an effective anti-biotic, anti-viral, anti-parasitic and immune system stimulant. It is used in viral hepatitis, children's bowel complaints, gastric acidity, liver congestion and flatulence. It is being increasingly used as treatment for cold. Reasonably good evidence tells us that it can reduce the severity of cold sympetoms [3-7].

Study of wound healing effect of plant *Andrographis peniculata* (A. P.) was carried out using excision wound model in order to establish the antiseptic activity of the drug. In the present study, sincere effort has been attempted to establish the scientific validity of the wound healing effect of the whole part of plant.

MATERIALS AND METHODS

The whole plant was collected from adjoining area of Barpali and authenticated by Botany Department, Rajendra Autonomous College, Bolangir, Odisha, India by comparing with the specimen in the Harbarium. The total plant was washed and dried under shade. The dried plant was ground to coarse powder with the help of grinding mill and stored for further study.

Andrrographis peniculata was extracted by maceration method using petroleum ether and ethanol. After completion of the extraction the mark was filtered and was allowed to evaporate the solvent. The solvent free extract was used for wound healing study.

Animal:

Healthy adult albino rats of either sex (Wister strains) weighing about 180 to 250g were used for the study. The animals were housed in large spacious hygienic cages during the course of experimental period. Before to start the experiment, all the animals are maintained under constant condition (temperature: 20±2°C, humidity: 40-60%, 12-h light/ 12-h dark cycle) for two weeks and housed in stainless steel cages in a laboratory room. The animals were access to water

ad libitum and standard rodent diet. The commercial pelleted animal feed, marketed by Brooke bond, Lipeton India Limited, Bangalore was supplied to the animal throughout the experimental period. All animal experiment conducted in accordance with Institutional Animal Ethical Committee (IAEC license no1339/AC/10/CPCSEA.).

Chemicals:

Analytical laboratory grade chemicals were used for the studies, which were procured from Merck limited, Mumbai, India.

Preparation of Extract:

The course powder plant material was extracted with petroleum ether (60-80°C) for 72 hours and than using ethanol in soxhlet extractor for 48 hours [6]. Ethanolic extract was concentrated under the vacuum in rotary flash evaporator and successively in hot air oven till solid to semisolid mass. Extracts were stored in an airtight container in refrigerator below 10°C.

Preliminary phytochemical investigations

The petroleum ether extract and ethanolic extract subjected to preliminary qualitative chemical analysis. Standard method were used for preliminary phytochemical screening of the extract was performed to know the phytoconstituents in the extract [7-8], and it was found that extract contains flavonoids, saponin and terpenoids.

Determination of physical constants:

Leaves analysis parameters like total ash, acid insoluble ash, water soluble ash, Sulphated ash, loss on drying were use for the determination of quality and purity of the crude drug and extractive value were used for evaluation and study of wound healing activity [9].

Pharmacological screening:

Ethanolic extract and pet ether extract of whole parts of *Andrographis peniculata* Plant (2% w/w) ointments were prepared in PEG-4000 (2% w/w solution). The animals were divided into four groups of six rats each as follows: Group I rats were treated with solvent control (2 % PEG-4000 solution). Group II rats were treated with a reference standard 0.5%w/w Povidone Iodine ointment. Group III & IV rats were treated with ethanolic extract and pet ether extract of whole parts of *Andrographis peniculata* Plant (2% w/w) ointments were prepared in PEG-4000 (2% w/w solution) respectively. In the excision wound model the excision wound were made on rats. The rats were depilated on the back after being anaesthetized with ether and cutaneous circular wound of 9 mm diameter were made on the pre-shaved sterile dorsal surface of the animal by cutting. After skin excision the wound was left open to the environment. In each group each animal is having one wound. Animals were housed individually in metallic cages [8].

Application was done everyday after cleaning with surgical cotton. The measurements of progress of wound healing induced by Povidone Iodine 0.5 % w/w ointment, petroleum ether extract, ethanolic extract and respective control group in excision wound model without infection are shown in the table 1.

Experimental design for wound healing activity:

Group I, Control- treated with PEG-4000 (2%w/w solution) as vehicle.

Group II, Positive control- treated with standard 0.5%w/w Povidone Iodine ointment as reference drug.

Group III & IV, Treated with ethanolic extract and pet ether extract of whole parts of *Andrographis peniculata* Plant (2% w/w) ointments were prepared in PEG-4000 (2%w/w solution) respectively as a test sample.

Statistical analysis:

Values are expressed as mean \pm S.D. statistical significance was determined using the student's *t*-test. Values with $p < 0.01$ were considered significance.

RESULTS AND DISCUSSION

The studies are revealed that the skin wound treated with all extracts to respective groups showed significant activity when compared to the control and the standard on 3rd, 6th, 9th and 12th post wounding days. It was observed that there was complete healing on 12th post wounding day by applying both the extract. The wound contractions on 12th day in petroleum ether extract, ethanolic extract were shown to be 10.17 ± 0.60 and 16.33 ± 0.67 respectively.

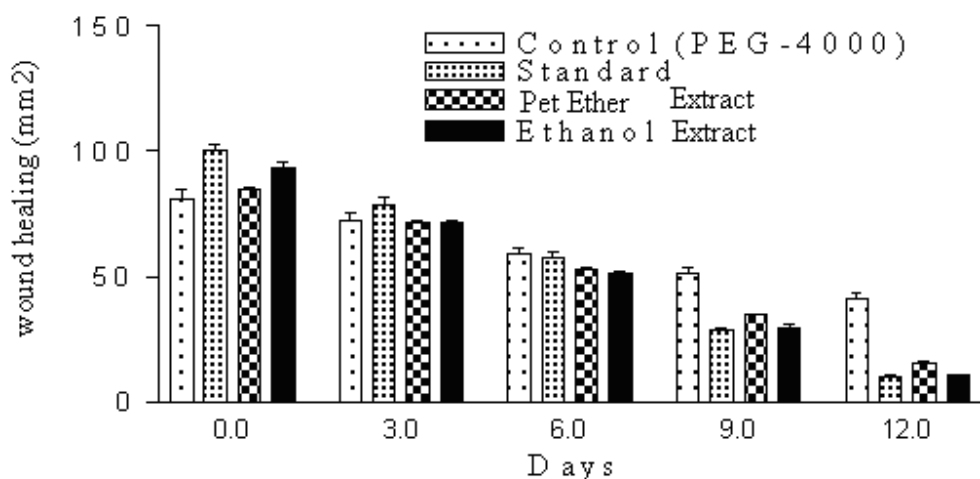


Fig. 1: Excision wound healing (mm²) activities of control, standard, extract with the Pet ether and ethanol

Normal wound healing can be described in three phases –

1. Fibroblastic proliferation and secretion of mucopolysaccharides.
2. Gradual disappearance of mucopolysaccharides and appearance of collagen molecule and collagen fibres.
3. Shrinking of collagen fibres

Table 1: Effect of extract of *A.P* on excision wound healing (mm²) activity on rats

Day(s)	Control (Group-1) (2% w/w PEG-4000)	Standard (Group-2)	Ethanolic Extract (Group-3)	Pet ether Extract (Group-4)
0	81.17 \pm 3.25	100.67 \pm 1.99	83.83 \pm 0.98	100.83 \pm 1.56
3	72.50 \pm 3.08	78.67 \pm 2.92	63.83 \pm 1.08**	76.17 \pm 1.91
6	59.33 \pm 1.97	57.50 \pm 2.66	53.67 \pm 0.84*	59.67 \pm 1.20
9	51.17 \pm 2.56	28.33 \pm 1.33**	36.50 \pm 0.62**	32.33 \pm 1.20**
12	41.33 \pm 2.16	9.83 \pm 0.74**	16.33 \pm 0.67**	10.17 \pm 0.60**

The values represent the -Means \pm SEM, for each group $n=6$, * $P < 0.05$, ** $P < 0.01$ compared to control

These phases run concurrently and independently. Hence drug factors that influence one phase need not affect others. Studies have shown that Vit-A promotes epithelialisation and again in tensile strength, but not wound contraction in animals with steroid depressed wound healing. Similarly topically applied hydrocortisone has been found to dissociate granulation and epithelialisation [9-10].

The phytochemical analysis revealed the presence of flavonoids, saponin and terpenoids in aqueous extract. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis. Flavonoids and terpenoids are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation [11-13].

Studies with all test extracts for its wound healing property on excised wound showed significant activity when compared to the control and the standard.

From the table 1 it is found that in the solvent control group no significant effect was seen up to 12th day, in case of standard drug treated group the significant effect was shown in 9th and 12th day but in case of the animal treated with ethanol extract of *Andrographis peniculata* significant effect was seen from 3rd day and in case of petroleum ether extract the significant effect was seen from 9th day. It was observed that there was complete healing on 12th post wounding day by applying both the extract. The wound contractions on 12th day in petroleum ether extract, ethanolic extract were shown to be 10.17 ± 0.60 and 16.33 ± 0.67 respectively. It is found that the ethanolic extract of A.P shows better activity than the petroleum ether extract. These studies revealed that the excised wound is healed by neovascularisation followed by stimulation of fibroblast resulting in the synthesis of collagen.

On the basis of the result obtained in the present investigation it is possible to conclude that the ointment the extracts of *Andrographis peniculata* have significant wound healing activities.

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