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Original Article

Evaluation of Wound Healing Activity of "*Sbutilon Indicum*" Linn, In Wister Albino Rats.

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ABSTRACT

A study was conducted to evaluate the wound healing activity of *Abutilon indicum Linn* in Wister Albino Rats using two different models Viz., excision and incision. There was a significant increase in wound closure rate. The plants of Abutilon indicum was dried in a shade individually. Then the shade-dried plants were powdered to get coarse powder separately. The extraction was carried out by using solvents of increasing polarity starting from petroleum ether and ethanol respectively. All the extracts were obtained by above methods and were subjected to phytochemical studies. For the evaluation of wound healing activity, Wister albino rats were selected for this experiment. The progressive changes in the wound area were monitored by tracing the wound margin everyday. From the result, it is concluded that the petroleum ether extract of "Abutilon indicum" Linn had greater wound healing activity than the Ethanolic extract.

1. Introduction

Plants are integral part of nature. Nature reflects the creative power of living god. Plants have an almost endless variety of uses to human beings. India is birth place of indigeneous medicine such as siddha, Ayurvedha and unani. It is enriched with flora and fauna and therefore the plants have been used since ancient times for treatment of human ailments. In recent years there is an increasing awareness along the masses about the use of herbal drugs which are believed to be safe and does not produce undesirable effect which most of the modern synthetic drugs do.

The World Health Organization (W.H.O) estimated that 80 % of population of developing countries relies on traditional medicine mostly plant drug for their primary health care needs. Siddha systems of medicine is one of the oldest medical systems of india existed separately early periods. The system has well established in india for many centuries. The advantage is side effect is less. So now a days, people are highly accelerated towards the siddha system.

The ayurvedic system is said to below, to the Aryans, who introduced into india, from the central asian plains. The siddha system is to be made universally acceptable and useful particularly in developing and under developed countries. In siddha drug *"ABUTILON INDICUM"* is one of the most important drug. It is distributed all over india and ceylon. It comes under the family *"MALVACEAE"*. In this plant the roots, bark, leaves and seeds are having medicinal properties.

Wound healing is a process that is fundamentally a connective tissue response. The initial stage of this process involves an acute inflammatory phase followed by the synthesis of collalgen and other extracellular macromolelcules, which are later remodeled to form a scar, (Anonymous, 1993). Wound healing studies mainly aim to detect various means and factors influencing the healing process, so that they could be either used or avoided in clinical practice to alter the healing process favourably. The process of wound healing occur in four phases: (i) Coagulation, which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition. Any agent that accelerates the above process is a promoter of wound healing. (Nithya and Balasubramanian, 2008).

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The plant under study, namely *Abutilon inducum* Linn contains Alkaloids, Flavanoids, Phenols, Saponins, Phyto steroids and Terpenoids, Glycosides, protein and amino acids. The present investigation is to determine the wound healing activity of "*Abutilon indicum*" Linn extract in experimental models of wounds in rats.

2. Materials And Methods

Preparation of Crude extracts

A large number of plants of *"Abutilon indicum"* Linn were collected, botanically identified, confirmed and washed with water and then dried in shade for two weeks. Divided drugs was powdered and used for extract.

Preparation of Extracts

Preparations of extracts using different solvents were made to perform the further analysis.

Solvent Extraction (Hot percolation method)

The plants of *Abutilon indicum* was dried in a shade individually. Then the shade-dried plants were powdered to get coarse powder separately. The following process is done for plants of Abutilon indicum individually. About 200gms of shade dried coarse powder was extracted first with petroleum ether at 40 – 60 C by continuous hot percolation using soxhlet's apparatus for 72 hours. The extraction was carried out by using solvents of increasing polarity starting from petroleum ether and ethanol respectively. T h e extraction was continued for 72 hours. The petroleum ether extract was filtered and concentrated to dry mass by using vaccum distillation. A blackish green residue was obtained. The crude extract were dissolved in respective solvents and following chemical experiments were carried out to establish presence of compound.

Screening of Phytochemical Compound

The plant extract was subjected to qualitative tests adopting standard procedures for the identification of the phyto constituents present in it viz., alkaloids, Flavanoids, Phenols, Saponins, Phytosteroids, Terpenoids, Tannins, Sugars, Glycosides, Fixed oil and Fats, Proteins and aminoacids.(Vogel, 1971).

Detection of Alkaloids

A few drops of diluted HCL were separately treated with 1 ml each of various extracts. Then it was filtered. The filtrates were treated with Mayer's reagent. The formation of cream precipitate confirmed the presence of alkaloids. The filtrates were treated with Dragendroff's reagent. The formation of slight orange brown opalescence confirmed the presence of alkaloids.

Detection of Flavanoids

Five ml of each of the various extracts were separately dissolved with 1 ml of alcohol then 1 ml each of stock alcoholic solution was added with a few drops of neutral ferric chloride solution. The formation of blackish red colour indicated the presence of flavanoids.

Detection of Phenols

1 ml of various extracts were dissolved in 5 ml of alcohol and treated separately with a few drops of neutral ferric chloride solution. The change in colour indicated the presence of phenol.

Detection of saponins

1 ml each of the various extracts were separately mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. The formation of foam indicated the presence of saponins.

Detection of phytosterols and terpenoids

5 ml of various extracts were dissolved in 5 ml each of chloroform separately. Then they were subjected to Lieberman – Burchard test to 1 ml each of the stock solution, a few drops of acetic anhydride and 1 ml of con. H2SO4 were added from the sides of the test tubes and allowed to stand for 5 minutes The formation of brown ring at the junction of the two layers and the upper layer turned green indicated the presence of phytosterols and terpenoidal sapogenins.

Detection of tannins

5ml of each of the various extracts were dissolved in minimum amount of water separately and filtered. The filtrates were taken separately and added a few drops of aqueous basic lead acetate solution. No formation of reddish brown precipitate indicated the absence of tannins.

Detection of sugars

5ml of each of the various extracts were dissolved separately in distilled water, filtered and then subjected to Fehling's test. A small portion of the various filtrates were treated with 1 ml of Fehling's solution A and B and then heated gently. No formation of reddish brown colour indicated the absence of sugar.

Detection of Glycosides

Another portion of the extracts were hydrolysed with hydrochloric acid for an hours on the water bath and the hydrolysate were subjected to Legal's test to detect the presence of different glycosides. To hydrolysate 1 ml of pyridine and few drops of sodium nitro-prusside solution were added and then it was make alkaline with NaOH solution. The appearance of pink to red colour showed the presence of glycosides.

Detection of fixed oils and fats

A small quantity of extracts were pressed between the folds of filter paper. Oil stains were seen indicating the presence of fixed oils (in ethanolic extract) and absence of fats.

Detection of proteins and amino acids

Small quantities of extracts were dissolved in distilled water separately and were subjected to Millon's, Biuret's and Ninhydrin reagents. White precipitaite for Millons's reagent and violet colour for Biuret;s reagents and purple colour for Ninhydrin reagent. The active natural compounds present in the extracts were identified through above mentioned screening tests and the results were tabulated.

Wound Healing activity of *Abutilon indicum* Linn on Excision(Morton, 1972)model and incision model.

Wister albino rats (150-180g) were selected for the experiment four groups with six animals in each group weranaesthetized by the open mask method with anaesthetic ether. The rats were depilated on the back. The wound inflicted by cutting a way a 500mm^2 full thickness of skin from a predetermined area, wound was left undressed to the open environment. Then the drugs, i.e.,

the reference standards (0.2% w/w, nitrofurazone oinment) control (simple oinment B.P) only. Abutilon indicumm ethanolic extract oinment (5% w/w, made with simple ointment) were administered till the wound was completely healed. This modoel was used to monitor contraction and would closure time. Wound contraction was calculated as per reduction in wound area. The progressive changes in wound area were monitored plan metrically by tracing the wound margin on graph paper every alternate day.

Determination of Tensile strength

The tensile strength of a wound represents the degree of wound healing. Usually wound healing agents promote a gain in tensile strength. The instrument used for the measurement is a called tensiometer.

Antimicrobial screening of *Abutilon indicum* Linn

The antimicrobial screening was performed with extract of Abutilon indicum, by disc diffusion method. The extract was moistened in a sterile what man filter paper and were placed in the petri plates, in which were swabbed with different cultures. The zone inhibition was measured with the help of antibiotic zone reader.

3. Result and Discussion

Table – 1 Phytochemical analysis for *Abutilon indicum* Linn

Tests	Petroleum ether	Ethanolic extract
Alkaloids	+	+
Flavanoids	+	+
Phenols	+	+
Saponins	+	+
Phyto steroids and Terpenoids	+	+
Tannins	-	-
Sugars	-	-
Glycosides	+	+
Fixed oil and fats	-	+
Protein and amino acids	+	+

Table 2. Effect on topical application of Abutilonn indicum on excision wound model

Post wounding day	Simple ointment	Nitrofurazone ointment (2% w/v)	Extract Ointment (5% w/v)
0	318±2.18	312±2.12	317±2.50
2	285±1.57	282±1.57	279±1.81
4	251±0.95	248±0.33	237±1.04
6	221±0.40	214±0.33	191±0.27
8	189±0.31	178±0.33	149±0.57
10	147±0.95	139±1.04	91±1.63
12	113±1.57	101±1.74	52±2.34
14	89±2.01	62±2.45	10±3.11
16	51±2.71	15±3.31	0±3.3
18	18±3.31	0±3.59	0±3.3

Table 3 Antimicrobial activity of the ethanolic extract of "*Abutilon indicum*" Linn

Organisms used	Zone of inhibition in Ethanolic extract	Zone of inhibition in Petroleum ether
B.subtilis	14mm	22mm
B.stereothermophilus	35mm	42mm
K.aerogenes	15mm	31mm
E.coli	35mm	40mm
P.aeruginosa	18mm	40mm
proteus	21mm	28mm
Candida albicans	22mm	16mm

Photo showing wound healing activity of ethanolic extract of Abutilon indicum on excision model

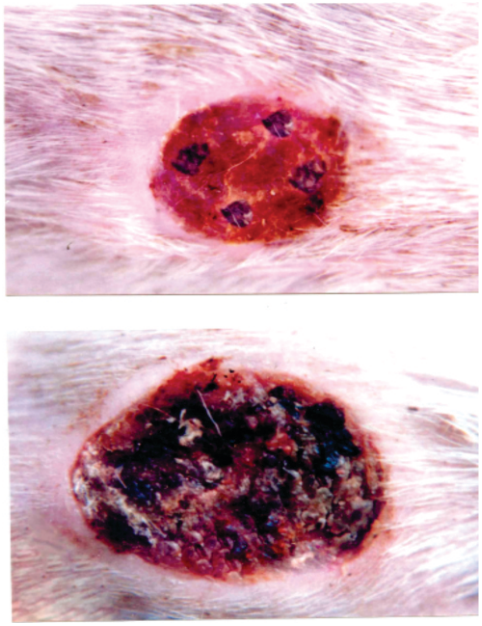
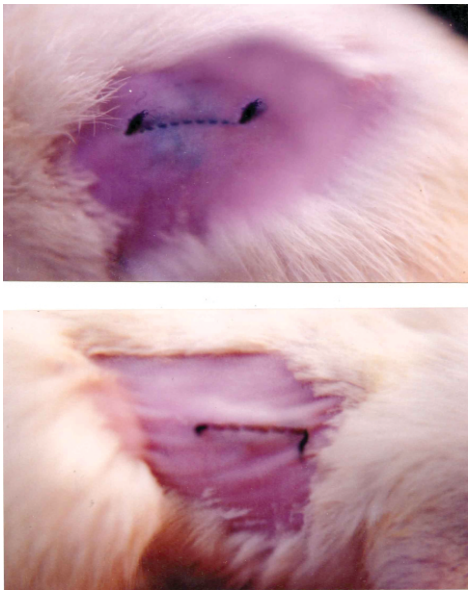


Photo showing wound healing activity of ethanolic extract of Abutilon indicum on incision



4. Discussion

The preliminary phytochemical analysis of the leaf extract of "Abutilon indicum" showed the presence of Alkaloids, Flavanoids, Phenols, Saponins, Phytosteroids, Terpenoids, Glycosides, proteins and amino acids.

The measurement of the progress of the wound healing induced by the Nitrofurazone ointment, and extract ointment as control and simple ointment in the excision wound model shown in the Table. 2. It is observed that the wound contracting ability of the extract ointment was significantly greater than that of the control as well as the reference standard, NFZ ointment.

The wound healing property of the leaf extract of "Abutilon indicum" Linn appears to be due to the presence of its active principles, which accelerate the healing process and confer tensile strength to the healed wound.

The antimicrobial study was conducted using different micro organisms such as *B.subilis, B.stereothermophilus, K. aerogens, E.coli, P. aeruginosa, Proteus, C. albicans.* The extract of petroleum ether showed the higher zone of inhibition than the Ethanolic extract.

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