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Evaluation of sapindaceae species for their wound healing potential

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ABSTRACT

The present study aimed to evaluate the wound healing activity of the alcoholic and aqueous extracts of Cardiospermum halicacabum and Dodonea viscosa, family sapindaceae in various experimental models described as incision and excision wound model. The extracts were subjected for acute toxicity studies and found no mortality upto the dose of 2000mg/kg bodyweight hence 1/5th and 1/10th of the dose tested were used as the test dose and exhibited dose dependant wound healing activity. The phytochemical studies revealed the presence of various flavonoids, tannins, triterpenoids and saponins which might have contributed for the said activities.

Key words: Cardiospermum halicacabum, Dodonea viscosa, wound healing.

INTRODUCTION

It has been found that more than 80% of the world population is dependent on the drugs from natural origin for the treatment of skin related problems. Many of the synthetic drugs are associated with problems like allergy, drug resistance and so on making the scientists to seek alternative drugs [1]. Natural healing process of wound management involves disinfection, debridement and providing a moist environment [2].

The plants *Cardiospermum halicacabum and Dodonea viscosa*, family sapindaceae, categorized under treating rheumatism, stiffness of the limbs, snake bite, nervous diseases, stomach disorders, skin rashes, teeth ache, fever and as astringent in the traditional system of medicines [3-5]. The number of pharmacological properties such as insecticidal [6], anti-filarial [7], and antipyretic [8], anti diarrhoeal [9] activities has been reported with *Cardiospermum halicacabum* and Dodonea viscosa have been reported for its antimicrobial [10], anti-inflammatory [11] and antidiabetic [12] activities.

The crude leaf extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* has been used traditionally by native practitioners to promote wound healing. However, there are no experimental reports on wound healing activities of these two plants in the available literature. In view of this, the present study was aimed to evaluate the wound healing activity of leaves extracts of *Cardiospemum halicacabum* and *Dodonea viscosa*.

MATERIALS AND METHODS

Plant Material
The leaves of *Cardiospermum halicacabum (Linn)* and *Dodonea Viscosa* were collected from in and around Hassan, Karnataka, India.

Extraction
The leaves were washed with water, shade dried, reduced to coarse powder and subjected to successive extraction with, alcohol using soxhlet apparatus and distilled water with maceration and were subjected to preliminary phytochemical analysis [13]

Animals
Albino mice of either sex weighing 18-22 gms and albino rats of either sex weighing 180-220 gms were acclimatized for a period of seven days in laboratory under standard husbandry conditions i.e. room temperature 26 ± 2^0 C, relative humidity 45-55% and light/dark cycle12/12 hours. All the animals were fed with a standard diet (Gold Mohr, Lipton India Ltd., Bangalore) and water was supplied *ad libitum* under strict hygienic conditions. All the experimental protocols were approved by Institutional Animal Ethical Committee of our institute.

Acute toxicity studies
The acute toxicity of alcoholic and aqueous extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* was determined in albino mice weighing 18-22 gms of either sex. After administration with different doses of these extracts, the number of animals survived with each extract was noted for acute (48hours), and chronic (14 days) period of time. The animals were physically active and regularity in consumption of food and water was observed. The dose up to 2000mg/kg body weight did not produce any signs of toxicity or mortality. LD_{50} was calculated according to the **"**Up and Down method" following OECD guidelines No. 425 of CPCSEA.

Wound Healing Activity
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 Malon [14]. The animals were divided into nine groups of 6 animals each. Animals of group 1 (served as control) were provided with plain drinking water. Group 2 animals (served as reference standard) treated with sulphathiazole ointment. The test group rats (group 3-10) were treated with alcoholic and aqueous extracts *Cardiospermum halicacabum* and *Dodonea viscosa* at the dose of 200mg/kg and 400mg/kg body weight respectively for a period of 14 days.

The wound closure rate was assessed by tracing the wound on days 15 post- wounding using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelisation.

Incision Wound Model
The Method followed was HP Ehrlich and TK Hunt [15]. All the animals were anaesthetized and the back hairs of the rats were shaved by using a shaving machine. Five-centimeter long, two linear-paravertebral incisions were made with a sterile surgical blade through the full thickness of the skin at the distance of 1.5 cm from the midline of each side of the vertebral column.

The animals were divided into nine groups of 6 animals each. Animals of group 1 (served as control) were provided with plain drinking water. Group 2 animals served as reference standard and treated with sulphathiazole ointment. The test group rats (group 3-10) were given alcoholic and aqueous extracts *Cardiospermum halicacabum* and *Dodonea viscosa* at the dose of 200mg/kg and 400mg/kg body weight respectively for a period of 9 days.

All the sutures were removed on the 8^{th} post wounding day and the tensile strength was determined on 10^{th} post wounding day by continuous constant water flow technique (tensiometer).

Statistical evaluation
Statistical analysis was performed by one way analysis of variance followed by Dunnet't' test and $p<0.01$ was considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical studies of revealed that sterols, saponins, carbohydrates, flavonoids tannins, fixed oils and triterpenoids were found with alcoholic extracts and saponins, carbohydrates, flavonoids and tannins with the aqueous leaves extracts of *Cardiospermum halicacabum and Dodonea viscosa.*

The measurement of progression of wound healing induced by the extracts of *Cardiospermum halicacabum and Dodonea viscosa* are shown in table. Remarkable dose dependant wound healing activity was observed with the aqueous and alcoholic extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* as shown in table 1. The dose dependant decrease in epithialisation period was also observed. Likewise, the same dose dependent increase in the tensile strength was demonstrated in incision wound model. The evaluation results of tensile strength are shown in table 2.

Wound healing is a complex process characterized by homeostasis, re-epithialization, granulation tissue formation and remodeling of the extracellular matrix. Though the healing process takes place itself and does not require much help, but risk factors such as infection and delay of healing has bought to promote this process. Wound healing occurs in three stages: inflammation, proliferation and remodeling. The proliferative phase is characterized by angiogenesis, collagen disposition, granulation tissue formation, epithialisation and wound contraction.

In the process of Wound healing the damaged tissue is restored as closely as possible to its normal state and the process of shrinkage of the area of the wound is characterized as wound contraction. It is dependent upon the type and extent of damage, the general state of health and the ability of the tissue to repair. The granulation tissue of the wound is primarily composed of fibroblasts, collagen and new small blood vessels.

Flavonoids have therapeutic uses due to their anti-inflammatory, antifungal, antioxidant and wound healing properties. Moreover, flavonoids and their derivatives are known to decrease lipid peroxidation by improving vascularity and preventing or slowing down the progress of cell necrosis [16-17]. Flavonoids are also known for potent antimicrobial and astringent properties, serve responsible for wound contraction and elevated rate of epithialization [18-19].

Table 1: Evaluation of Sapindaceae species for their wound healing potential by excision wound model

Treatment	Percentage of wound Contraction, Mean ± SEM					Period of Epithialization (Days) Mean ± SEM
	3rd Day	6th Day	9th Day	12th Day	15th Day	
Control	17.47±0.40	28.47±0.49	47.10±0.39	58.87±0.49	70.47±1.91	19.33±0.27
Standard	38.13±0.69**	50.93±0.58**	64.23±0.9**	80.50±0.73**	93.17±0.29**	13.83±0.19**
CH AL200	31.13±0.61**	47.40±0.52**	60.80±0.59**	73.37±0.19**	89.20±0.28**	15.67±0.23**
CH AL400	36.93±0.63**	49.23±0.40**	63.37±0.36**	76.43±0.56**	91.10±0.38**	14.50±0.14**
CH AQ 200	32.97±0.45**	49.94±0.73**	62.43±1.20**	73.47±0.39**	90.30±0.30**	14.33±0.17**
CH AQ 400	36.93±0.63**	49.23±0.40**	65.13±0.51**	73.33±0.96**	92.00±0.74**	14.00±0.11**
DV AL 200	33.67±1.04**	50.77±0.63**	64.90±0.60**	77.70±1.12**	90.17±0.32**	14.33±0.14**
DV AL 400	39.43±0.60**	54.17±0.97**	69.70±0.40**	79.27±0.25**	92.07±0.38**	13.50±0.09**
DV AQ 200	34.63±1.01**	52.03±0.6**	65.73±0.76**	78.33±0.40**	90.87±0.29**	13.33±0.15**
DV AQ 400	46.33±0.68**	56.37±1.08**	71.40±0.54**	86.47±0.17**	92.67±0.33**	13.00±0.11**

*Values are Mean ±SEM, n=6 in each group, *P < 0.05 , ** P < 0.01 as compared to control*

Table 2: Evaluation of Sapindaceae species for their wound healing potential by incision wound model

Treatment	Breaking Strength (gms) Mean ± SEM
Control	265.17±0.78
Reference Standard	423.00±0.99**
CH AL200	314.17±0.25**
CH AL400	359.33±0.94**
CH AQ 200	303.17±0.49**
CH AQ 400	309.67±0.49**
DV AL 200	323.17±0.39**
DV AL 400	383.86±0.19**
DV AQ 200	300.33±0.58**
DV AQ 400	311.00±0.60**

*Values are Mean ±SEM, n=6 in each group, *P < 0.05 , ** P < 0.01 as compared to control*

CONCLUSION

According to the results reported here, the extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* was found to have dose dependant wound healing activity in the experimental models compared to control. Flavonoids of this plant might have contributed to the wound healing process, along with other phytochemical contents of the leaves. However, it needs further evaluation in clinical settings before consideration for the treatment of wounds.

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