International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 2, Issue 2, July-Sep. 2009

Research Article

PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND WOUND HEALING ACTIVITY OF *ALLIUM CEPA* LINN (LILIACEAE)

CHITRA SHENOY*[1], M B PATIL[1], RAVI KUMAR[2] AND SWATI PATIL[3]
[1]Department of Pharmacognosy and Phytochemistry, K.L.E.S's College of Pharmacy, Belgaum,
[2]Department of Pharmaceutics, K.L.E.S's College of Pharmacy, Ankola-581314, Karnataka, India.
[3]Department of Pharmacognosy, K M Kundnani College of Pharmacy, Mumbai
E mail: umeshchitra@rediffmail.com
Received –12th June, 2009, Revised and Accepted – 7h August 2009

ABSTRACT

To screen the wound healing activity of different extracts of *Allium cepa* L. in excision, incision and dead space wound models in albino rats. The tubers of *Allium cepa* L. (Family Liliaceae) were exhaustively defatted with petroleum ether (40-60^{0}C) and the marc was subjected to continuous extraction with solvent ether, chloroform, alcohol and chloroform water I.P. All the extracts were tested for various preliminary phytoconstituents and screened for wound healing activity in excision, incision and dead space wound models in albino rats at a dose of 300 mg/k.g. B.W. by oral route. All the five extracts were also subjected to antibacterial screening by using the cup plate method. Alcoholic extract of Tubers of *Allium cepa* has shown better wound healing activity in excision, incision and dead space wound models as compared to chloroform and chloroform water extracts. From the results obtained it can be concluded that alcoholic extract of tubers of *Allium cepa* has significant wound healing activity. The enhanced wound healing activity of alcoholic extract may be due to free radical scavenging action and the antibacterial property of the phytoconstituents(viz; tannins and flavonoids) present in it which either due to their individual or additive effect fastens the process of wound healing. Presence of flavonoids and tannins in alcohol extract was also confirmed by preliminary phytochemical investigation, TLC and HPTLC methods.

Keywords: *Allium cepa*, Wound healing activity, Antibacterial activity, Alcohol extract.

INTRODUCTION

A wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Wound healing can be defined as a complex dynamic process that results in the results in the restoration of anatomic continuity and function. It is a finely orchestrated and overlapping sequence of events involving – control of infection, resolution of functional connective matrix, contraction, resurfacing, differentiation and remodelling. Wounds are generally classified as, wounds without tissue loss (e.g. in surgery), and wounds with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments eg: venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and derma abrasions[1]. Wound healing involves complex series of interactions between different cell types, Cytokine mediators and the extracellular matrix. The phases of normal wound healing include hemostasis, inflammation, proliferation, and remodeling[2].

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 healing process can be physically monitored by assessing the rate of contraction of the wound.

Allium cepa Linn. is a member of the Liliaceae, which consists of over 250 genera and 3700 species. Because of their bulbs, tubers and rhizomes, these plants are able to survive under harsh conditions, e.g. winter or dryness.

The plant *Allium cepa* Linn. (Liliaceae) are proved to shown the antidiabetic[3],

antioxidant[4], antihypertensive5[6], antithrombotic[6], hypoglycemic[7],antihyperlipidemic[8]. The bulb of *Allium cepa* contains Kampferol, β-sitosterol, ferulic acid, myritic acid, prostaglandins[4]. Bulb extract shown to have ecobolic effect in rats[9]. Traditionally plant containing these constituents used as abortifaciant, the bulb extract of *Allium cepa* had showed ecobolic effect in mice and rats[10]. A survey of literature revealed that no systematic approach has been made to study wound healing activity of this plant.

To validate the ethnotherapeutic claim of the plant in skin diseases, wound healing activity was studied. In this communication we report the preliminary phytochemical investigations of the various extracts, the acute toxicity studies, antibacterial activity and wound healing activity.

MATERIALS AND METHODS

Plant material

Bulbs of *Allium cepa* Linn were collected from local areas of Ankola during January 2009 dried and were authenticated by Dr. Harsha Hegde, Chief Botanist, Indian Council of Medical Research (RMRC), Belgaum branch.

Preparation of extracts

Bulbs of *Allium cepa* (2kg) were powdered to coarse form. The powdered materials was loaded in Soxhlet's extractor and defatted with petroleum ether (40-60^{0}C) in 10 batches (30 cycles each batch). The marc was dried and extracted with solvent ether, chloroform, alcohol and chloroform water I.P.(aqueous) in a Soxhlet's apparatus in 10 batches (30 cycles each batch). Finally the extracts were concentrated to semi-solid mass using rotary flash evaporator under vacuum. The traces of the solvents were removed by keeping the dried extracts in to a desiccator.

Preliminary phytochemical studies

The individual extracts were subjected to qualitative chemical investigation for the identification of the phytoconstituents; sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins.

Powder analysis

The powdered crude drug was subjected to determination of pH, extractive value, total ash ash, water soluble ash, acid insoluble ash and loss on drying as per Indian Pharmacopoeia.

Microorganisms

The test microorganisms used for the antimicrobial activity screening were 4 bacteria (2 Gram positive) – *Bacillus subtilis* and *Staphylococcus aereus* (2 Gram negative) – *Pseudomonas aeruginosa* and *Escherichia coli*.

These organisms were identified and procured from National Chemical Laboratory (NCL), Pune, India.

Antibacterial activity

The agar diffusion method[11] was used to evaluate the antibacterial activity. Bacteria were cultured overnight at 37^{o}C in Mueller Hinton 10 µl Broth (MHB, Oxoid) and used as inoculum. A final inoculum, using 100 µl of suspension containing 10 ^{8} CFV/ml of bacteria spread on Mueller Hinton Agar (MHA).

The disc (6 mm in diameter) was impregnated with 10 µl of 200 µl/ml , 150 µl/ml , 75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml of each extracts and for each organism placed on seeded agar. Streptomycin (200 µl/ml, 150 µl/ml, 75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml) were used as positive control for bacteria. The test plates were incubated at 37 ^{o} C for 24h for bacteria depending on the incubation time required for a visible growth.

EXPERIMENTAL

Experimental animals

Healthy adult Wister albino rats of either sex weighing (150-200g) were procured from Venkateshwara Enterprises, Bangalore. They were housed individually in polypropylene cages at 23±1ºC in 12:12h dark: light cycle, with free access to standard pellet feed (Chakan Oil Mill, India) and water *ad libitum*. This project was cleared by Institutional Animal Ethical committee.

Acute toxicity study

Swiss albino mice of either sex weighing (18-22g) and of 90 days age were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD[12]. The animals were starved overnight were divided into six groups (n=3) and were fed with increasing doses (10, 30, 100, 300, 1000, 2000, 3000 mg/kgB.W.) of the petroleum ether, solvent ether, chloroform, alcohol and chloroform water extract. The animals were continuously observed for mortality and behavioural responses for 48 h and thereafter one daily for 14 days after administration. The $1/10^{th}$ of the lethal dose was taken as effective dose ED_{50} (therapeutic dose).

Wound models

The animals were starved for 12h prior to wounding. Studies were carried out using ether-anaesthetized rats. The rats were divided into six groups (n = 6). Animals were depilated at the dorsal thoracic region before wounding. The first, third and fifth group served as control similarly second, fourth and sixth groups received alcoholic, chloroform and aqueous extract by oral route at a dose of 300 mg/kg body weight by oral route daily for 10 consecutive days in incision and dead space wound model and for 20 days in the excision wound model.

Excision wound model

An impression was made on the dorsal thoracic region 1cm away from vertebral column and 5cm away from ear using a round seal of 2.5cm diameter 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 diameters. Hemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Contractions, which contribute for wound closure in the first two weeks, were studied by tracing the wound on a transparency paper initially. Then an impression was taken on a millimeter scale graph paper, scar area after complete epithelization and time for complete epithelization in days was evaluated to calculate the degree of wound healing[13]. The parameters were studied were wound closure, epithelization time and scar features. The observation of the percentage wound closure were recorded on 4^{th} , 8^{th}, 12^{th}, 16^{th} and 20^{th} post wounding day and also for epithelization and size and shape of scar area.

Incision wound model

In the incision model[14], the rats were anesthetized by anesthetic ether and two longitudinal paravertebral incisions of 6cm length were made through the skin and cutaneous muscle at a distance of about 1.5cm from the midline on each side of the depilated back. After the incision, the parted skin was sutured 1cm apart using a surgical thread (No. 000) and curved needle (No. 11). The wounds were left undressed. The extracts were given by oral route once a day, till complete healing. The sutures were removed on eighth post-wound day. The skin-breaking

strength of the 10-day-old wounds was measured by the method of Lee[15].

Dead space wound model

For the dead space wound three groups of six animals each were used. Dead space wound was made by implantation of polypropylene tube (0.5cmX2.5cm), beneath the dorsal Para vertebral skin. On the 10th day the granuloma tissue form on the dead space wound was dissected and tensile strength was determined. The excess tissue was cut into two approximately equal halves. One of the granuloma tissue was dried in an oven at 60° C and the dry weight was noted. The granulation tissue so harvested was subjected to hydroxyproline estimation. Their weights were expressed as mg/100 gms body weight as suggested by Dispaquale and Meli[16].

Statistical analysis

All the results were analyzed by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The level of significance was set at $P<0.05$.

RESULTS

The average percentage yield of various extracts of *Allium cepa* is shown in table 1.

Table 1 : The percentage yield of various extracts of *Allium cepa* L.

Sl. No.	Extracts	Nature of extract	Colour	Yield (%)
1.	Petroleum ether	Semisolid	Dark yellow	3.70
2.	Solvent ether	Semisolid	Dark brown	7.28
3.	Chloroform	Semisolid	Dark brown	2.00
4.	Alcohol	Semisolid	Dark brown	6.00
5.	Chloroform water	Semisolid	Dark yellow	14.27

Powder analysis parameters like pH, extractive value, total ash, water soluble ash, acid insoluble ash and loss on drying were determined on the powder of *allium cepa* bulb. In powder analysis ash values are useful in determining the quality and purity of crude drug, especially in the powder form and the extractive values are useful for their evaluation, especially when the constituents of a drug can not be readily estimated by any other means. Further these values indicate the nature of the constituents present in a crude drug. The results of physicochemical characterization of *allium cepa* are presented in table 2.

Table 2 : Physicochemical characterization of *Allium cepa*

S. No.	Parameters	Result
1.	Total ash **(%)**	4.22
2.	Acid insoluble ash **(%)**	2.0
3.	Water soluble ash **(%)**	1.8
4.	Loss on drying **(%)**	2.25
5.	Extractive value **(%)**	8.5
6.	pH	6.5

The phytochemical tests revealed that the bulbs of the plant possess alkaloids in chloroform, extract. The other constituents like flavonoids, tannins, glycosides, carbohydrates and proteins in ethanolic and aqueous extracts. The results of phytochemical screening are given in table 3.

Table 3 : Phytochemical screening of different extracts of *Allium cepa*

Extracts	Steroids	Alkaloids	Glycosides	Saponin	Flavonoid	Tannin	Carbohydrates
Petroleum Ether	-	-	-	-	-	-	-
Solvent Ether	-	-	-	-	-	-	-
Chloroform	-	+	-	-	-	+	-
Alcohol	-	-	++		+++	+	-
Chloroform water	-	-	+	-	++	+	+

+++**:** high concentration; ++**:** medium concentration; +: low concentration;

-: constituents not detectable

The LD_{50} was found to be more then 3000 mg/ kg BW p.o. in acute toxicity testing. The therapeutic dose 300mg/ kg BW p.o. was calculated as $1/10^{th}$ of the lethal dose for the purpose of wound healing investigation. The results are tabulated in table 4.

Table 4 : Results of Acute oral toxicity studies of various extracts of *Allium cepa*

Sl. No	Extracts	LD_{50} (mg/kg)	ED_{50} (mg/kg)
1.	Petroleum ether	3000	300
2.	Solvent ether	3000	300
3.	Chloroform	3000	300
4.	Alcohol	3000	300
5.	Chloroform water	3000	300

Antibacterial activity was done for all the five, pet ether, solvent ether, chloroform, alcohol, and aqueous extracts. During antibacterial study chloroform, alcohol and aqueous extracts showed maximum zone of inhibition against almost all organisms in cup plate method. So the chloroform, alcohol and aqueous extract were taken for wound healing activity. The results of antibacterial activity of various extracts of *Allium cepa* are shown in table 5.

Table 5 : Results antibacterial activity of various extracts of *Allium cepa*

S. No.	Name of the Extract	Zone of Inhibition in mm at conc. of 200 μg/ 0.1 ml			
		Bacillus subtilis	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***
1.	Alcohol	20	17	19	23
2.	Chloroform	18	15	16	20
3.	Chloroform water	19	18	18	21
4.	Solvent ether	13	12	14	16
5.	Petroleum ether	15	13	17	21
6.	Control (DMF)	R	R	R	8
7	Standard	21	19	22	25

Diameter of cup – 6mm; Standard drug – Streptomycin; DMF - Dimethyl formamide; R - Resistance

The results of excision wound model are given in table 6. In this parameter the mean percentage of wound area were calculated on 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} post wounding days. In an excision wound model, alcohol extract at a dose 300mg/kg BW p.o. of *Allium cepa* showed significant wound healing activity (% wound contraction on 18^{th} day (95.3±1.0, $P<0.05$) compared to control (86.1±1.1). It also showed complete epithelization 18.5± 0.8days $P < 0.05$) when compared to control (21.5±0.4). The alcohol extract showed) showed significant decrease in mean scar area (7.2±1.2, $P < 0.05$) as compared to control (28.2±1.5).

The results of incision wound model are given in table 7. In incision study, the alcohol extract showed significant (200.89±2.62 $P < 0.05$) breaking strength when compared to control (149.35±13.04).

Table 6 : Effect of various extracts of *Allium cepa* on healing of excision wound

Group (N)	Dose (oral)	Excision wound						
		% Wound contraction						
		4^{th} day	**8^{th} day**	**12^{th} day**	**16^{th} day**	**18^{th} day**	**Mean size of scar area mm^2**	**Period of epithelization (days)**
Control	1 ml of 2% Gum acacia	46.6± 2.0	60.0± 1.9	81.1± 1.2	86.1± 1.2	86.1±1.1	28.2±1.5	21.5±0.4
Chloroform	300 mg/kg suspended in 2% acacia	65.5± 2.2	68.2± 2.6	76.0± 3.7	81.9± 2.1	86.6±1.9	22.3±1.7	19.05±0.075
Alcohol	300 mg/kg suspended in 2% acacia	60.1± 1.7*	78.3± 1.4	80.8± 2.3*	91.1± 1.6*	95.3±1.0*	7.2±1.2*	18.5±0.8*
Chloroform water	300 mg/kg suspended in 2% acacia	64.2± 1.7	76.0± 3.7	86.4± 1.7	89.6± 2.1	94.7±1.5	11.2±2.2	19.5±0.6

* indicates significant difference at P<0.05 when compared to control. Values are Mean ± SEM from 6 animals in each group), Data analyzed by One-way ANOVA followed Dunnett's test.

Table 7 : Effect of various extracts of *Allium cepa* on healing of Incision wound

Group (n)	Dose (oral)	Wound breaking strength (g)
Control	1 ml of 2% Gum acacia	149.35±13.04
Chloroform	300 mg/kg suspended in 2% acacia	180.18±3.46
Alcohol	300 mg/kg suspended in 2% acacia	200.89±2.622*
Chloroform water	300 mg/kg suspended in 2% acacia	179.98±14.68

* indicates significant difference at P<0.05 when compared to control. Values are Mean ± SEM from 6 animals in each group), Data analyzed by One-way ANOVA followed Dunnett's test.

The results of dead space wound model are given in table 8. The tensile strengths of the granuloma tissue were determined by the water flow technique of Lee. Alcohol extract showed highly significant increase in breaking strength (213.3±12.9, P<0.05) when compared to control (122.3±14.1) and alcohol extract also showed significant increase in the dry weight of granulation tissue (59.83±0.54, P<0.05) as compared to control (32.67±0.71). Alcohol extract also showed significant increase in the hydroxyproline content (2232.33±0.76, P<0.05) as compared to control (1381.17±0.70).

Table 8 : Effect of various extracts of *Allium cepa* on healing of dead space wound

Group (n)	Dose (oral)	Breaking strength (g)	Granulation tissue dry weight (mg/100g)	Hydroxyproline (µg/100mg)
Control	1 ml of 2% Gum acacia	122.3± 14.1	32.67±0.71	1381.17±0.70
Chloroform	300 mg/kg suspended in 2% acacia	181.1± 12.9	53.89±0.55	1982.00±0.58*
Alcohol	300 mg/kg suspended in 2% acacia	213.3± 12.9*	59.83±0.54*	2232.33±0.76*
Chloroform water	300 mg/kg suspended in 2% acacia	192.9± 5.2	35.50±0.27	1581.17±0.67

* indicates significant difference at P<0.05 when compared to control. Values are Mean ± SEM from 6 animals in each group), Data analyzed by One-way ANOVA followed Dunnett's test.

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[17]. 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, which run concurrently, but independent of each other. Control group wound showed granulation tissue and fibroblast aggregation in the alcoholic extract of *Allium cepa* treated group showed extensive growth of granulation also started along its surface as shown subsequently on 18th day. The treated group of wound showed complete healing of wounds with almost normal architecture of the collagen, reticulin. Increase in tensile strength of treated group wound may be due to increase in collagen concentration, alcoholic extract of *Allium cepa* increase the collagen synthesis.

In excision wound model the increased rate of wound contraction and decrease in period of epithelization in the animals treated with alcohol extract of *Alium cepa* may be attributed to their broad spectrum antibacterial activity.

Significant increase in skin breaking strength and hydroxyproline content which was a reflection of increased collagen levels by increased cross linking of collagen fibres. In addition, increase in dry granulation tissue weight indicated the presence of higher protein content[18]. The breakdown of collagen liberates free hydroxyl proline and its peptides and elevated level of hydroxyl proline is the index of increased collagen turnover.

Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, proteins and other important constituents. Flavonoids have been documented[19] to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing. Phytochemical screening revealed the presence of flavonoids in *Allium cepa*. Thus, the enhanced wound healing may be due to free radical scavenging action and the antibacterial property of the phytoconstituents present in it which either due to their individual or additive effect fastens the process of wound healing. This could be the reason for prohealing activity of *Allium cepa.* This enhanced wound contraction effect *of Allium cepa* and epithelization could possibly be made use of clinically in healing of open wounds. However confirmation of this suggestion will need well designed clinical evaluation.

From the study carried out showed that the alcoholic extract of bulbs of *Allium cepa* possesses a definite proheaing activity, there by justifying its use in the indigenous system of medicine.

ACKNOWLEDGMENT

The authors are thankful to Dr. F.V. Manvi, Principal, K.L.E.S's College of Pharmacy, Belgaum, Karnataka, India for providing all the facilities and support to carry out the research work.

REFERENCES

1. Paul W, Sharma CP Chitosan and alginate wound dressings: A short review. Trends Biomater Artificial Organs 2004;18:18-23.
2. Douglas M, Alan LM, Support for wound healing. Alter Med Rev 2003;8:359-60.

3. Galal EE, Gawad MA Antidiabetic activity of Egyptian onion *Allium cepa* extract; J Egypt Med Assoc 1965; 48: 14-45.
4. Dhanprakash BN, Garima U Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). Food Chemistry 2007; 102: 1389-1393
5. Sakai Y, Murakami T, YamamotoY Antihypertensive effect of onion on NO synthase inhibitor induced hypertensive rats. Biosc Biotecnol Biochem 2003; 67: 1305-11.
6. Yamada K, Namaura A, Sawashita N, Naguchi Y, Yamamoto J An onion variety has Natural antithrombotic effect as assessed by thrombosis/thromolysis models in rodents. Thromb Res 2004; 114: 213-20.
7. Compos KE, Diniz YS, CataneoAC, Faine LA, Alves MJ, Nobeli EL Hypoglycemic and antioxidant effect of onion, *Allium cepa:* diatary onion addition, antioxidant activity and hypoglycic effect on diabetic rats. Int J Food Sci Nutr 2003; 54:241-6.
8. Lata S, Saxena KK, Bhasin V, Saxena RS, Kumar A, Shrivastva VK Beneficial effect of *Allium sativum. Allium cepa* and *Comiphora mukul* on experimental hyperlipidemia and arthrosclerosis- A comparive evaluation. Indian J Pharmacol 1991; 37: 132-5.
9. Chatterjee TK Medicinal plant with antifertility property. Herbal Option1997: 77.
10. Sharaf A Ecobolic effect of *Allium cepa* in mice and rats. Qalit. Plant Mat Veg1969; 17:153.
11. Murray PR, Baron EJ, Pfallar MA, Tenover FC, Yolke RH. Manual of clinical microbiology. 6th ed. Washington, DC: ASM; 1995.
12. Organization for Economic Co-operation and Development, revised draft guidelines 423,"OECD Guidelines for the testing of chemicals" Revised document-October 2000.
13. Werner S, Breededen M, Hubner G, Greenhalgh D G and Longaker M T. Introduction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. *J Investig Dermatol* 1994; 103:469.
14. Ehrlich HP, Hunt TK. The effect of cortisone and anabolic steroids on the tensile strength of healing wounds. Ann Surg 1968;167:324.
15. Lee KH Studies on the mechanism of action of salicylate retardation of wound healing by aspirin. J Pharma Sci 1968;57:1042-3.
16. Dispasquale G, Meli A Effect of body weight changes on the formation of cotton pellet induced granuloma. J Pharm Pharmacol 1965;17:379-82.
17. Chithra P, Sajithalal BG, Gowri C Influence of *Aloe vera* on collagen turnover in healing of dermal wounds in rats. Ind J Exp Biol 1988; 36**:**896-901.
18. Manjunatha B K Wound healing activity of *Solanum violaceum* Ortg. *Indian Drugs* 2006;43: 835.
19. Devipriya S, Shyamladevi C S Protective effect of quercetin in cisplatin induced cell injury in the kidney. *Indian J Pharmacol* 1999;13: 422.