Research Article

Preliminary pharmacological investigations on *Lens culinaris* seeds for wound healing potential in rats

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Abstract

Objective: *Lens culinaris* Med. (family: Fabaceae) is commonly known as “lentils”. It is distributed and cultivated throughout the North region of India. Seeds are rich source of minerals such as calcium, iron, vitamin B and an important component of variety of food in many countries. The aim of present study was study preliminary phytochemical screening and pharmacological screening of *Lens culinaris* seeds extract for wound healing activity. **Material and methods:** The phytochemical analysis of ethanol extract was done for the detection of presence of different phytochemical constituents. Preliminary wound healing activity of ethanol extracts was tested by using excision wound model. Healing effect of ethanol extract of *Lens culinaris* seeds was investigated by measurement of percent contraction area, hydroxyproline estimation and epithelialization period. **Results:** The phytochemical analysis reveals the presence of steroids, fixed oils, flavonoids, glycosides, polysaccharides and proteins. Ethanol extract showed significant wound healing effect by increasing wound contraction and significantly increase in hydroxyproline content of test group. The healing effect was compared with Povidone iodine ointment treated group of animals. **Conclusion:** In conclusion, the observation and results obtained in present study indicated that ethanol extract of *Lens culinaris* seeds was found improved skin healing may be due to presence of semi polar components like flavonoids.

Keywords: *Lens culinaris*, wound healing, povidone iodine, hydroxyproline, excision wound, flavonoids

Introduction

Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation are some of the many phases, which are independent to each other. The process of wound healing occurs in four phases: (i) degranulation, which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition. Any agent which accelerates the above process is a promoter of wound healing.

Lens is suggestive of the lens-like shape of the lentil seed. It is usually cultivated throughout North India, South-East Europe

and in temperate Western Asia. *Lens culinaris* is an annual bushy herb that grows to 75 cm in height. It has slightly hairy compound leaves with 10 to 16 leaflets arranged in pairs. Lentils are considered to be a good source of proteins. This high protein content in lentils and other pulses makes them a significant food source for developing countries and lowincome people (Hoover et al., 2010).

Total carbohydrates represent the major component of lentil seeds with starches occupying most of the carbohydrate mass. Among 23 pulse grains, starch yield percent from lentils is the second highest, up to 47.1 %. Furthermore, lentils are a valuable source of total dietary fibers, with insoluble dietary fiber of approximately 93–99.7 % (Bednar et al., 2001).

The total a-galactosides or raffinose family oligosaccharides account for 53.0 % of the total sugars and oligosaccharides content in lentils. In these oligosaccharides, stachyose represents the major

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oligosaccharide, followed by ciceritol and raffinose (Vidal-Valverde et al., 2002). Iron (Fe) is also present in significant quantity in lentils. Because bioavailability of iron from lentils could be adversely affected by natural chelating agents present in pulses, this adverse effect could be minimized by cooking, germination and fermentation of lentils prior to ingestion. In addition, lentils contain Zn, which ranges between 3.2 and 6.3 mg/100 g. In addition, other trace minerals including Cu, Mn, Mo, and B (Ryan et al., 2007).

Lentils are a significant dietary source of a plethora of vitamins including folate, thiamin (B1) and riboflavin (B2). Other water-soluble vitamins have also been reported in lentils as follows: niacin; pantothenic acid and pyridoxine.

In addition, vitamin E (a, b and c tocopherols) was measured in lentils by Ryan et al. (2007). The a-tocopherol and b and c-tocopherols contents were 1.6 and 4.5 mg/100 g, respectively. This, however, is far from the RDA value of a-tocopherol (15 mg/day).

Lentils are low fat and high in protein (~25%). They are a good source of B-complex vitamins, especially folate, and provide significant amounts of iron and potassium. They are also lower than most legumes in lectins and trypsin inhibitors, compounds that can interfere with digestion. Lentils are an extremely good source of dietary fibre, especially if they are unhulled. Polyphenol-rich lentils have potential health benefits as complementary and alternative medicines, which are exerted in the form of antioxidant, antibacterial, anti-fungal, antiviral, cardioprotective, anti-inflammatory, nephroprotective, antidiabetic, anticancer, anti-obesity, hypolipidemic and chemopreventive activities. Furthermore, lentils are useful as a prognostic marker for various cancers including thyroid and hepatic carcinoma (Faris et al., 2013). Present work aimed to study preliminary screening of Lentils seeds extract for wound healing effect by using incision and dead space wound models.

Material and methods

Collection and authentication of plant material

The seeds of *Lens culinaris* were purchased from local market, Bhopal (M.P.) India. A herbarium sheet was prepared and authenticated the plant species in the department of Botany, Dr. H. S. Gour University, Sagar (M.P.). Plant material was coarsely powdered and stored for further use.

Preparation of extracts

The plant seeds were identified and moderately coarse powdered. The powdered plant material was successively extracted with different solvents in increasing polarity order such as petroleum ether, chloroform, ethyl acetate, ethyl alcohol and water. Then all extracts were concentrated under vacuum descicator and weighed.

Phytochemical screening and formulation preparation

All extracts were qualitative screened for detection of various chemical constituents by using different chemical tests (Jain et al., 2014; Jhariya et al., 2015). Ethanolic extract of *Lens culinaris* seeds was selected for 5%w/w ointment preparation using simple ointment base B.P. Ointment prepared by fusion method containing each fatty ingredient e.g. hard Paraffin, cetostearyl alcohol and white soft paraffin were weighed and melted in china-dish in decreasing order of melting point with continuous stirring. In other china dish Wool fat and extract (5%w/w) was melted and added in previously melted fatty substances with continuous stirring until cold (Jain and Sharma, 1998).

Animals

For the incision, dead space, and diabetic wound models, Wistar albino rats (150-200 g) were selected. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and they were kept under standard environmental conditions of laboratory temperature and water *ad libitum*. The animals were maintain alternate cycle of darkness and light at 12 hours. They were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of experiment. The experimental protocols were approved by Institutional Animal Ethics Committee, RKDF University, Bhopal (M.P.). Six animals were taken in each group and three groups were made in each wound model. Group I was denoted as Control group, and was treated with only simple ointment base, while Group II denoted as treated group, was treated with extract ointment (5%w/w) and Group III with standard 5 % w/w Povidone Iodine Ointment USP (Zenith Drugs Pvt Ltd, India) ointment was used.

Incision wound creation

All animals were anaesthetized before wound creation and two Para vertebral long incisions were made through the skin at the distance of about 1.5 cm from the midline on each side of the depilated back of the rat. No local or systemic antimicrobials were used throughout the experiment. The both edges kept together and stitched with black silk surgical thread (No. 000) and a curved needle (No. 11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then simple ointment base, sample drug and standard drug ointment were applied daily up to 9 days; when wounds were cured thoroughly the sutures were removed on the ninth day and tensile strength of cured wound skin was measured using Tensiometer (Lodhi and Vadhre, 2017).

Dead space wound creation

This model was used for the study of granuloma tissue. Animals were anaesthetized by light ether and wound was made by implantation of two polypropylene tubes (2.0 x 0.5), one on either side, in the lumbar region on the dorsal surface in each animal. On the 10th post-wounding day, granuloma tissue formed on implanted tube was dissected out carefully. Granuloma tissue from one tube was dried (60°C) and stored in 10% formalin for estimation of biochemical parameters (Shirwaiker et. al 2003; Lodhi and Vadnere, 2017).

Assessment parameters for healing property

Tensile strength determination

Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the repaired tissue. For this the newly repaired tissue including scar was excised to measure the tensile strength. The instrument used for measurement is called 'Tensiometer' (Rashed et al., 2003).

Protein estimation and granuloma weight

All tissue samples were collected and protein estimation was done (Lowry, 1951). On day 9, granuloma mass samples from dead space wound was taken for the determination of dry granuloma weight.

Hydroxyproline content determination

Wound tissues were analyzed for collagen content by determination of hydroxyproline content, which is a basic constituent of collagen. Tissues were dried in a hot air oven at 60-70°C to constant weight and hydrolysed in 6 N HCl at 130 °C for 4 hour in sealed tubes. The hydrolysate was neutralized to pH 7 then subjected to Chloramine-T oxidation for 20 minutes (Woessner, 1961). The reaction was terminated by addition of 0.4M perchloric acid and developed color with Ehrlich reagent at 60°C was read at 557 nm in UV (Agilent Technology) Spectrophotometer.

Enzymatic and non-enzymatic antioxidant assay

In dead space wound model, one part of granuloma tissue was

used for antioxidant assay. Catalase was estimated following the breakdown of hydrogen peroxide according to the method of Beers and Sizer (1952). Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972) based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) content was determined in granuloma tissue by the method of Moron *et al*, (1979).

Histopathological study

Animals were anaesthetized before taking skin sample using diethyl ether or chloroform. Wound tissue specimens from control, test and standard group were taken after complete healing after sufficient time duration. Skin samples were stored in 10% formalin for histopathological study.

Statistical Analysis

All data were statistical analyses by using One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison of the results in the all observations.

Results and discussion

The process by which tissue repair takes places is called as wound healing and is comprised of a continuous sequence of inflammation and repair, in which epithelial, endothelial, inflammatory cells, platelets and fibroblasts briefly come together outside their normal domains and interact to restore resemblances of their used discipline and having done so as to resume their normal function.

In phytochemical study, results were showed the yield as 3.6, 1.7, 4.2, 6.8 and 4.4 %w/w for petroleum ether, chloroform, ethyl acetate, ethyl alcohol and aqueous extract, respectively. Ethanol extract showed higher percentage of yield. The qualitative chemical analysis was confirmed the presence of sterols in petroleum ether and glycosides, flavonoids and amino acid were present in ethanol extract. Carbohydrates, proteins, and proteins were found positive in aqueous extract.

Collagen is a major protein of the extracellular matrix and is

Table 1. Effect of ethanol extract ointment and reference ointment on various wound parameters of incision wound in rats

Groups	Hydroxyproline (mg/ g tissue)	Protein content (mg/g tissue)	Tensile strength (kg/cm ²)
I	17.65±1.89	57.12±2.85	0.284±0.024
II	48.52±2.34**	82.31±4.26*	0.648±0.031**
III	52.67±2.69**	85.46±2.68*	0.675±0.074**

n=6 albino rats per group, tabular value represents Mean S.D. *P<0.05; **P<0.001; (Comparison of I with II and III)

the component that ultimately contributes to wound strength. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of this hydroxyproline, therefore has been used as an index of collagen turnover. The increased hydroxyproline content of the excision wounds (Table 1) has indicated faster collagen turnover leading to rapid healing with concurrent increase in the tensile strength of the treated wound (Gupta and Gupta, 1985).

Plant products have been shown to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing, due to presence of active terpenes, alkaloids and flavonoids (Udupa, 1994).

The *Lens culinaris* contains flavonoids, tannins and saponins and the ethanolic extract of rhizome have reported antimicrobial activity. A glycosidal mixture extract of *Centella asiatica* has been reported to be responsible for enhanced repair only in

incised wounds and in stimulating collagen in human skin fibroblast cells.

The results of present study showed that ethanolic extract of *Lens culinaris* possesses a definite prohealing action. Significant increase was also observed in skin breaking strength and hydroxyproline content which was a reflection of increased collagen content that was further supported by histopathological evidence and gain in granuloma breaking strength (Table 1 & Figure 1). This indicated improved collagen maturation by increased cross-linking while an increase in dry granuloma weight indicated higher protein content (Shukla et al., 2018).

Phytochemical screening revealed the presence of tannins and flavonoids. Flavonoids have been documented to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important

Table 2. Effect of extract ointment and reference ointment on enzymes and non-enzymatic antioxidants level from dead space wound tissues in rats

Groups	Enzymatic and non-enzymatic assay			Granuloma dry weight (mg)
	SOD($\mu\text{g}/50 \text{ mg tissue}$)	CAT($\mu\text{mol}/50 \text{ mg tissue}$)	GSH($\mu\text{mol}/50 \text{ mg tissue}$)	
I	22.51 $\pm$ 1.75	42.36 $\pm$ 2.34	32.85 $\pm$ 1.85	30.52 $\pm$ 1.54
II	37.82 $\pm$ 1.95*	67.51 $\pm$ 3.02*	54.84 $\pm$ 2.46*	41.75 $\pm$ 1.95*
III	37.46 $\pm$ 1.88*	68.42 $\pm$ 3.2*	55.97 $\pm$ 2.43*	45.76 $\pm$ 1.86*

n = 6 albino rats per group, value represents Mean S.D. *P<0.05, when compared each treated group with control group

Figure 1. Photomicrograph of rat skin on 9th day after post wounding, which received daily: (A) simple ointment base (B) 5% ethanolic extract ointment (C) Reference ointment. Figure shows increased collagen fibres, fibroblasts cells and new blood vessels formation in extract and reference ointment group. Hematoxylin and eosin x 100.

components of wound healing (Shirwakar et al., 2003). Thus the enhanced wound healing may be due to free radical scavenging action of the plant, and enhanced level of antioxidant enzymes in granuloma tissues. Better collagenation seen under the influence of this plant extract may be because of improved antioxidant status.

A reduction in granuloma weight after day 9th was observed in the extract ointment treated animals. This could also be due to the better maturation of collagen which in variably leads to shrinkage of granulation tissue. An alternative explanation for this could be an indirect steroid like antigranulation effect (Udupa et al., 1991).

Initial increased in protein content in the drug treated animals indicates that the drug acts at every cellular level. The increase in specific activity in the drug treated animals in all days after wounding is suggestive of enhanced synthesis of enzyme protein (Udupa et al., 1991). The wound healing property of *Lens culinaris* appears to be due to the presence flavonoids, which accelerates the healing process and confers breaking strength to the healed wound.

The activity of antioxidants was increased in control group. Due to increased activity of antioxidants, their content was lower during healing process. Slightly increase in SOD, CAT and GSH was found in treatment group of 5 % w/w extract ointment, when compared to control group (Table 2). The level of SOD, CAT and GSH in extract treated group and reference ointment treated animals group were found higher may be due antioxidant activity of flavonoids containing ethanol extract of *Lens culinaris*.

Conclusion

In conclusion, the results showed that ethanolic extract of *Lens culinaris* seeds have effectively stimulate wound healing, increase in hydroxyproline and protein levels in wounded tissue. It also significantly showed antioxidant activity. These finding could justify the *Lens culinaris* seeds can be used to the management of wound healing effect.

Conflict of interest

None

References

- Bednar GE, Patil AR, Murray SM, Grieshop CM, Merchen NR, Fahey GCJ. 2001. Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model. *Journal of Nutrition*, 131:276–286.
- Beers RF, Sizer IW. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, 195:133–140.
- Faris MA, Tahruri HR, Shomaf MS, Bustanji YK. 2009. Chemopreventive effect of raw and cooked lentils (*Lens culinaris* L) and soybeans (*Glycine max*) against azoxymethane-induced aberrant crypt foci. *Nutrition Research*, 29: 355–362.
- Gupta SS. 1994. Prospects and perspectives of natural plants products in medicine. *Indian Journal of Pharmacology*, 26:1-3.
- Hoover JE. 1970. *Remington's Pharmaceutical Sciences*, 14th Edi., Mack Publishing Co., Pennsylvania, PP. 688-689.
- Jain A, Nahata A, Lodhi S, Singhai AK. 2014. Effects of *Tephrosia purpurea* and *Momordica dioica* on streptozotocin-induced diabetic nephropathy in rats. *Biomedicine & Preventive Nutrition*, 4(3):383–9.
- Jain NK, Sharma SN. 1998. *A Textbook of Professional Pharmacy*. 4th edition, Vallabh Prakashan Delhi, pp 261-270.
- Jhariya S, Rai G, Yadav AK, Jain AP, Lodhi S. 2015. Protective Effects of *Tridax procumbens* Linn. Leaves on Experimentally Induced Gastric Ulcers in Rats. *Journal of Herbs, Spices & Medicinal Plants*, 21(3):308–20.
- Kuwano H, Yano K, Ohano S, Lkebe M, Kitamura K, Toh Y, Mori M, Sugimachi K. 1994. Dipyrindamole Inhibits Early Wound Healing in rat skin incisions. *Journal of Surgical Research*, 56: 267-270.
- Lodhi S, Vadrere GP. 2017. Relevance and perspectives of experimental wound models in wound healing research. *Asian Journal of Pharmaceutical and Clinical Research*, 10(7):57.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry*, 193:265-275.
- Moron MA, Depierre JW, Mannervick B. 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta*, 582(1): 67–78.
- Rashed AN, Afifi FU, Disi AM. 2003. Simple evaluation of wound healing activity of a crude extract of *Portulaca oleracea* Linn. (Growing in Jordan) in *Mus musculus* JVI-1. *Journal of Ethnopharmacology*, 88:131-136.
- Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM. 2007. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. *Plant Foods for Human Nutrition*, 62: 85–91.
- Shirwaikar A, Jahagirdar S, Udupa AL. 2003. Wound healing activity of *Desmodium triquetrum* leaves. *Indian Journal of Pharmaceutical Sciences*, 65(5):461-464.

- Shukla R, Gupta G, Kashaw SK, Jain AP, Lodhi S. 2018. Wound healing effect of ethanolic extract from Morning Glory (*Ipomoea carnea* Jacq.) leaves by using different models in rats. *Pakistan Journal of Pharmaceutical Science*, 31(4):1355-1361.
- Udupa SK, Udupa AL, Kulkavi OR. 1994. Antiinflammatory and wound healing properties of Aloe vera. *Fitoterapia*, LXV (2): 141-144.
- Udupa SL, Udupa AL, Kulkarni DR. 1991. Influence of *Tridax procumbens* on Lysyl oxidase activity and wound healing. *Planta Medica*, 57:325-327.
- Vidal-Valverde C, Frias J, Sierra I, Blazquez IF, Lambein F, Kuo YH. 2002. New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology*, 215:472-477.
- Woessner JF, 1961. The determination of hydroxyproline in tissue and protein samples containing small portion of this imino acid. *Archives of Biochemistry and Biophysics*, 193: 440-447.