Original Article

Biological Screening of Plants Extract Showing Hypoglycaemic and Wound Healing Properties: *Capparis zeylanica* and *Primula denticulata.*

Sanjay Singh*[1], Sadath Ali[2] and Mamta Singh[3]

[1]Siddhartha Institute of Pharmacy, Uttarakhand, India
[2]Azad Institute Pharmacy & Research, Lucknow, Uttar Pradesh, India
[3]Sardar Bagwan Singh (PGI), Dehradun, Uttarakhand, India

Address for Correspondence

Assistant Professor
Department Pharmaceutical Chemistry,
Siddhartha Institute of Pharmacy, Dobachi,
Near IT Park,
Dehradun-248001,
Uttarakhand, India.
E-mail:
sanjaymph@gmail.com

ABSTRACT

Primula denticulata belonging to family Primulaceae and *Capparis zeylanica* to Capparidaceae. *P. denticulata* is commonly known as drumstick primula and grow in shady places and open slopes. Similarly, *C. zeylanica* is commonly known as Indian caper, a climbing shrub. The literature survey revealed that there is no experimental evidence of antidiabetic and wound healing effect of the plants. The present investigation was planned to evaluate the hypoglycaemic and wound healing properties in plants extract. In the hypoglycaemic activity, the animals were examined up to 21 days, in which Streptozotocin (STZ) was used as diabetes inducer and Glipizide as a standard. For wound healing activity, excision-wound model was used and 1% Silver Sulphadiazine ointment was used as standard. The dose 200 mg/kg body weight was selected from both ethanolic (EtOH) and aqueous extract for hypoglycaemic activity. For hypoglycaemic activity, the result of aqueous extract of *C. zeylanica* ($200.3\pm3.24^{**}$) and EtOH extract of *P. denticulata* ($203.8\pm2.30^{**}$) have shown a good reduction in blood glucose level and compared with normal control. 5% ointment formulation of EtOH extract of *C. zeylanica* and *P. denticulata* have been shown 98.36% and 93.08% wound healing contraction at day18 respectively when compared with normal control. Finally the results concluded that, both the plant extract have probable source of hypoglycaemic and wound healing property from natural origin.

Keywords: *Capparis zeylanica*, *Primula denticulata,* Streptozotocin, Hypoglycaemic, Wound healing.

INTRODUCTION

Diabetes, a chronic metabolism disorder categorized by abnormalities in carbohydrate and lipid metabolism, which leads to postprandial and fasting hyperglycaemia, dyslipidemia and hyperinsulinemia. While many synthetic drugs prove significant therapeutic potential, these drugs have already been restricted due to causing adverse effects such as; hepatotoxicity, cardiomegaly and hematotoxicity and available hypoglycaemic medicines in the market[1].

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated. There are three stages in this process, i.e. Inflammation, proliferation, and remodelling[2]. The use of natural products in primary health care has been an interesting part because of their safety and effectiveness. From published data, numerous plants have been identified for their anti-diabetic and wound healing potential.

This study was principally deal with the biological estimation of *Primula denticulata* (Primulaceae) and *Capparis zeylanica* (Capparidaceae) for hypoglycaemic & wound healing activity. *P. denticulata*, commonly known as drumstick primula, is growing in shady places and open slopes. Many Primula species are also cultivated throughout the world as ornamental plants[3]. *C. zeylanica* is commonly known as Indian caper, a climbing shrub found throughout India and has been used as a 'Rasayana' drug in the traditional medication. It is recommended for the treatment of immune disorders and generally used as a counter-irritant, febrifuge, swellings, boils and piles[4,5]. The various species of genus *Capparis* are useful in the treatment of cough, asthma, inflammation, fevers, cholera and also in gout and rheumatism[6]. Currently phytochemical screening of the plant's leaves is showing the presence of fatty acids and flavonoids[7,8]. Flavonoids have been identified as an antioxidant, antineoplastic, antiulcer, anti-inflammatory and antimicrobial activities[9]. Therefore; aim of the present study was designed for aqueous and EtOH extraction of *C. zeylanica* leave and arial part *P. denticulata* showing hypoglycaemic & wound healing activity.

MATERIALS AND METHODS

The entire experimental animals were approved by the Institutional Animal Ethical Committee Siddhartha Institute of Pharmacy, Dehradun, India Ref. No. SIP/IAEC/10/2011.

Plant material

The fresh leaves of *C. zeylanica* and arial parts of *P. denticulata* were collected from Dehradun, Uttarakhand and authenticated at Botanical Survey of India, Dehradun, Uttarakhand, India. Reference No. -111501.

Chemicals

All chemicals used for extraction and activity were analytical grade. Streptozotocin, glipizide and Silver Sulphadiazine were purchased from Himgiri Chemicals, Dehradun, Uttarakhand, India.

Extraction

Dried coarse powder of *C. zeylanica* leave (2.0 kg) and arial parts of *P. denticulata* (2.0 kg) were extracted with ethanol (EtOH) at 48 °C under reflux and hot maceration with water. Extracts were concentrated to dryness under reduced pressure to obtain a slurry (210 and 207

gm). Then extracts were kept in well closed air tight container for further use[10,11].

Hypoglycaemic activity

Experimental animal

Healthy adult Wistar rats of either sex weighing 150-180 gm were selected for the study. The study was carried in accordance with the rules and regulations laid by the Institutional Animal Ethics Committee. The animals were housed with free access to food and water. The basal food intake and body weights to the nearest gram were noted. Rats were starved 24 hrs prior to the study[12,13].

Acute toxicity study

The acute oral toxicity study was carried out in mice as per OECD guidelines. At a dose of (2000 mg/kg) 50% mortality was observed. Hence 200 mg/kg body weight of *C. zeylanica* and *P. denticulata* of both EtOH and aqueous extracts were taken as effective dose for an evaluation of hypoglycaemic activity[14].

Preparation of doses

The plant extracts (200 mg/kg body weight orally) were suspended in 5% aqueous acacia solution. The standard drug glipizide (5 mg/kg body weight orally) was also given as a suspension in 5% normal saline. The control group received normal saline orally[15].

Induction of diabetes

STZ has been generally used to induce type-2 diabetes in animal models, particularly rats & mice. The rats with body weight 150-200 gm were selected for the diabetogenic activity. The animals were deprived for food 12 hours prior to administration of STZ. STZ was freshly dissolved in freshly prepared in 0.01 M citrate buffer (pH 4.5). STZ was given Indian Pharmacopoeia (50 mg/kg body weight). The STZ solution was injected within 15 minutes of dissolution in a buffer solution with 1ml if the tuberculin syringe fitted with a 26 No. Gauge needle. Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of STZ. After the seven day stabilization period, the animals which have the blood-glucose level ≥ 250 mg/dl were selected for the studies[16].

Treatment protocol

The test samples were administered in a single dose by gavage. Animals were fasted 24 hrs prior to dosing. Animals were weighed and food was withheld for 3-4 hours after the dose administered. Diabetic animals were divided into six groups of six animals in each and one group of normal nondiabetic animals. The animals were received following treatments for 21 days.

Group I normal animals received normal saline (1 ml/kg, body weight orally).

Group II diabetic animals received normal saline (1 ml/kg, body weight orally).

Group III diabetic animals received the EtOH extracts of *C. zeylanica* (200 mg/kg, body weight orally).

Group IV diabetic animals received the aqueous extracts of *C. zeylanica* (200 mg/kg, body weight orally).

Group V diabetic animals received the EtOH extracts of *P. denticulata* (200 mg/kg, body weight orally).

Group VI diabetic animals received the aqueous extracts of *P. denticulata* (200 mg/kg, body weight orally).

Group VII diabetic animals received standard drug glipizide (5 mg/kg, body weight orally).

The animals were fasted for 18 hrs before the experiment for estimation of blood-glucose level and considered as initial reading. The extract was given orally for 21 days and blood-glucose level was checked at 0, 7, 14 & 21 day period. The blood was

collected from snipping of tail with a sharp razor in rats. The collected blood was centrifuged at 2000 rpm for 15 minutes and its determination was carried out using GOD-POD kit method in semi autoanalyser[17].

Evaluation of wound healing activity of plant extracts

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cell below the dermis begins to increase collagen production. Later, the epithelial tissue is regenerated.

Excision wound model

The rats were inflicted with excision wounds as described by Morton and Malon[18]. The rats were anesthetized prior to the creation of the wounds, the rat under light ether anesthesia under aseptic conditions. The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 2.5 cm in width and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open[19,20]. The animals were separated into four groups 3 in each. The group I animals was left untreated and considered as the control. Group II animals served as reference standard and treated with 1% Silver Sulphadiazine ointment. Animals of groups III and IV were treated with EtOH extract of *C. zeylanica* and *P. denticulata* respectively, for 20 days.

The parameters of the percentage wound closure & epithelialisation time were studied. The measurements of the wound areas of the excision wound model were taken on 1, 4, 8, 12, 14, 16, 18 and 20 days following the initial wound using transparent paper and a permanent marker. The recorded wound areas were measured with graph paper. The period of epithelialisation was calculated. The wound was left open during the study.

Dosage and application

For excision wound studies, all the samples were applied externally.

Group-I: normal control (simple ointment).

Group-II: standard 1% silver sulfadiazine.

Group-III: 5% EtOH extract of *C. zeylanica* ointment.

Group-IV: 5% EtOH extract of *P. denticulata* ointment.

The results of wound contraction studies of all groups were statistically compared and P values were calculated.

The wound contraction was determined using the following formula.

% wound contraction = Total area-Healed area / Total area X 100.

Preparation of ointments

The general method of preparation of various ointments of the extract was as follows: Dried extract was taken in glass mortar and triturated first. Then small parts of PEG-400 were added with triturating to dissolve or to suspend the drugs. Portions of PEG-6000 (melted at 70°C) were added to the above dispersion with triturating to form a homogenous mass of desired consistency[21].

Statistical analysis

The results were expressed as mean ± SEM. The unpaired *t*-test was used for analyzing the data between the two groups. Statistical analysis of data among the groups was performed by using analysis of variance (ANOVA) followed by the Tukey test of significance.

RESULTS AND DISCUSSION

The results exposed that, the hypoglycaemic potential of aqueous extract of *C. zeylanica* (200.3±3.24**), at the same time EtOH and aqueous extracts of *P. denticulata* (203.8±2.30**) have shown good reduction in blood glucose level at 21 days (Table 1) in wistar rats.

The mechanism of the hypoglycaemic effect of the extracts of these plants is not known. However, reports are available to show that anti-diabetic plants may affect circulating insulin level[22]. This action may be due to the presence of saponins and flavonoids[23-26].

In this report, we show that the topical application of the EtOH extract of *C. zeylanica* and *P. denticulata* promoted wound healing activity in excision models in rats. The percentage of Wound Contraction on 18th day of EtOH extract of *C. zeylanica* (93.08/0.17±0.02***) and *P. denticulata* (98.36/0.04±0.01***) have calculated and showed a significant (P<0.001) change in wound healing properties when compare with normal control (Table 2). Several studies signify that plant products are potential agents of wound healing and largely preferred because of the absence of unwanted side effects and their effectiveness[27].

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissues as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, beginning from the fibroblastic stage when the wound area undergoes shrinkage. In the final stage of wound healing, the wound undergoes contraction resulting in a smaller amount of apparent scar tissue.

The EtOH extracts demonstrated a significant increase in wound closure. Any of the phytochemical constituents present in *C. zeylanica* and *P. denticulata* may be responsible for the wound-healing activity. Studies with other plants have shown that phytochemical constituents like tannins or saponins and flavonoids are known to promote wound healing process mainly due to their astringent and antimicrobial properties, which appears to be responsible for wound contraction and increased rate of epithelisation[28].

CONCLUSION

The present study has confirmed that the EtOH and aqueous extracts of *C. zeylanica* and *P. denticulata* have properties that render it capable of promoting accelerated wound healing and hypoglycaemic activity when compared with normal controls. Further investigation into these studies indicated that there is a need to search for usage and biological activities of bioactive compounds isolated from medicinal plants having hypoglycaemic and wound healing properties. Further research is requiring evaluating the biological activities of these herbs or their active constituents that are being used for the treatments.

ACKNOWLEDGEMENT

Authors are thankful to Department of Pharmacology, Pharmaceutical Chemistry & management of Siddhartha Institute of Pharmacy, Dehradun, Uttarakhand, India for the all possible help it has provided for conducting the research.

Conflict of interests

The authors declare that there is no conflict of interests.

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Table 1. Effect of extracts on blood glucose level

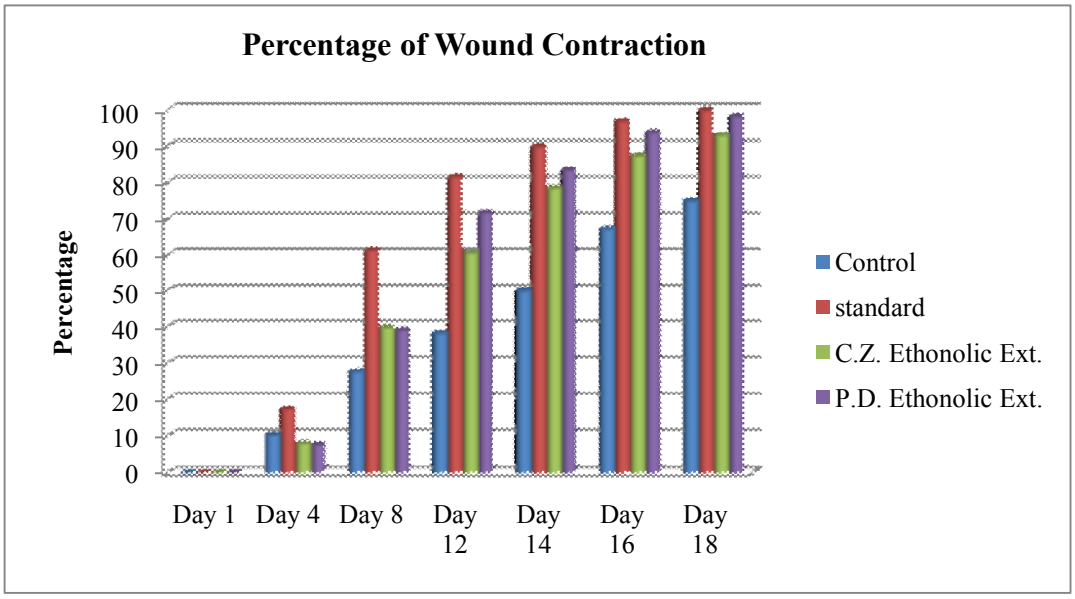
Blood glucose levels					
S. No.	Groups	Day 0 (Mean ± SEM)	Day 7 (Mean ± SEM)	Day 14 (Mean ± SEM)	Day 21 (Mean ± SEM)
1.	Normal Control	89±1.67	88.17±1.49	88.17±1.79	91.83±1.24
2.	Diabetes Control	258.7±1.82	261.2±1.95	259.5±2.23	256±1.91
3.	Standard Drug	259.2±2.18NS	244.2±2.94*	208±2.73**	143.8±5.44***
4.	EtOH extract of C.Z.	258±1.88 NS	252.7±1.56 NS	240.3±1.87 NS	212.7±15.57**
5.	Aqueous extract of C.Z.	258.5±2.40 NS	253.7±1.49 NS	243.2±1.22 NS	200.3±3.24**
6.	EtOH extract of P.D.	257.5±2.68 NS	251.7±1.76 NS	244±1.39 NS	203.8±2.30**
7.	Aqueous extract of P.D.	258.5±2.47 NS	252.7±1.40 NS	244±1.60 NS	204.3±2.94**

Results are expressed as mean ± SEM. ***P<0.001, **P<0.01, *P<0.05, ns= not significant, compare with diabetic control (Index: *C. Z. = C. zeylanica*, *P. D. = P. denticulata*)

Table 2. Wound healing activity of extracts

Treatment	Wound healing activity (% Wound contraction)							
	Day 1	Day 4	Day 8	Day 12	Day 14	Day 16	Day 18	Day 20
Control	2.48±0.02 00.00	2.22±0.02 10.48	1.79±0.01 27.82	1.53±0.02 38.30	1.24±0.02 50.00	0.81±0.01 67.33	0.62±0.01 75.00	0.51±0.01 79.04
Standard	2.39±0.06 00.00	1.97±0.01 17.57	0.92±0.02** * 61.50	0.44±0.01** * 81.58	0.24±0.02** * 89.95	0.07±0.01** * 97.07	-	-
C.Z. EtOH Extract	2.46±0.04 00.00	2.26±0.04 * 8.13	1.48±0.47** 39.83	0.97±0.07** 60.56	0.53±0.04** * 78.45	0.31±0.06** * 87.39	0.17±0.02** * 93.08	-
P.D. EtOH Extract	2.44±0.04 00.00	2.25±0.09 * 7.78	1.58±0.04** * 39.24	0.69±0.02** * 71.72	0.40±0.02** * 83.60	0.15±0.02** * 93.85	0.04±0.01** * 98.36	-

Results are expressed as mean ± SEM. ***P<0.001, **P<0.01, *P<0.05, ns= not significant, compare with the Disease Control (Index: *C.Z. = C. zeylanica*, *P.D. = P. denticulata)*



Graph 1. Percentage of wound contraction