OPEN ACCESS
Saudi Journal of Medicine
Abbreviated Key Title: Saudi J Med
ISSN 2518-3389 (Print) |ISSN 2518-3397 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsmepub.com/sjm/

Original Research Article

Evaluation of the Local Anesthetic Activity and Formulation of a Topical Local Anesthetic Gel from *Chrysanthemum cinerarifolium* Extract

Shawqi H. Alawdi[1,2*], Abdulkareem Al-Zomor[3], Mohanad Shehab[4]

[1]Assistant Professor, Department of Pharmacology, Faculty of Medicine, Thamar University, Dhamar, Yemen
[2]Assistant Professor, Department of Pharmacy Practice, Faculty of Pharmacy, University of Science and Technology, Ibb, Yemen
[3]Associate Professor; Department of Pharmacy, Faculty of Medicine and Health Sciences, Thamar University, Dhamar, Yemen
[4]Professor, Department of Clinical Pharmacology, Faculty of Medicine, Benha University, Benha, Egypt

DOI:10.21276/sjm.2019.4.7.11 | **Received:** 13.07.2019 | **Accepted:** 22.07.2019 | **Published:** 30.07.2019

***Corresponding author:** Shawqi Alawdi

Abstract

Aims: The present study aimed to investigate the local anesthetic activity of *Chrysanthemum cinerarifolium* extract as well as to formulate a topical local anesthetic gel containing the extract to be used for human. ***Methods:*** The local anesthetic activity was assessed using the intradermal wheal method in eighteen guinea pigs divided into three groups, six in each group. Pain response was elicited by gentle pinprick. Two concentrations (1.0 and 0.3mg/ml) of lidocaine were injected intradermally into the left and right dorsal flank of the animal respectively to form wheals, which were encircled with a marker. Similarly, two concentrations (100 and 50mg/ml) of the plant extract were injected into the left and right dorsal flank respectively of another group. Normal saline was injected into the control group. The encircled areas were each pricked with a pin 6 times at 5 minutes interval for 30 minutes starting with zero-time before the injection. To confirm the animal study, prepared gels of the plant extract (1%) and lidocaine (1%) were applied on two groups each containing 6 human volunteers, and the local anesthetic activity was assessed. ***Results:*** The plant extracts exhibited a local anesthetic activity on guinea pigs. Comparable results were also observed of the local anesthetic activities of both topical gels prepared from the plant extract or lidocaine in human. No adverse effects were observed either in animals or in human. ***Conclusions:*** *Chrysanthemum cinerarifolium* extract possesses a local anesthetic activity in both animals and human.

Keywords: *Chrysanthemum cinerarifolium*; local anesthesia; topical local anesthetic gel.

INTRODUCTION

World Health Organization appreciated the importance of medicinal plants for public health care in developing nations. The use of medicinal plants for therapeutic purposes has been there for thousands of years. Most cultures of the world, both ancient and the recent have depended on plants as a therapeutic agents used in different forms. In addition to their major role in treatment of diseases, plants still remain the foremost safe alternative for a large majority of people worldwide [1].

Chrysanthemum is a genus of several plants that belongs to *Asteraceae* family. They are cultivated as ornamentals for their showy flower heads. *Chrysanthemum cinerarifolium* is economically important as a natural source of insecticide made from the dried flower heads, called pyrethrum, which has been used thousands of years as a mosquito repellent and as a remedy for lice in ancient time. Pyrethrum is a natural mixture of six chemicals called pyrethrins [2]. In folk remedies, *C. cinerarifolium* was used for stimulating salivary glands and found to be useful in treatment of toothache, paralysis of the tongue and muscles of throat as well as neuralgic affections of the teeth. The present study aimed to investigate the local anesthetic activity of *C. cinerarifolium* extract in a live guinea pig animal model, as well as to formulate a topical local anesthetic gel containing the plant extract to be used for human.

MATERIALS AND METHODS

Preparation of Plant Extract

The aerial parts of the plant *Chrysanthemum cinerarifolium* were obtained from agricultural nurseries located in Ibb city, Yemen. The plant specimen was air-dried in a shade, powdered and were subjected to cold extraction method by maceration with

ethanol as a solvent. The weighed powdered was macerated with ethanol 95% for 72 hours (1:4 w/v). After a maceration period for 72 hours in the shaker, the extract was then filtrated and treated in a rotary evaporator at 40°C to separate the alcohol under vacuum. The obtained plant extract was kept in a tight container till further investigations.

Evaluation of local anesthetic activity in animals

The local anesthetic activity was assessed using the intradermal wheal method in guinea pigs and eliciting pain response by gentle pinprick [3]. Two symmetrical circular areas were shaved on the right and left dorsum of the male guinea pigs 24 hours before the commencement of the experiment. Eighteen adult male guinea pigs were used in the study and were divided randomly into three groups. Two concentrations (1.0 and 0.3 mg/ml) of lidocaine were injected intradermally either into the left or right shaved areas to form wheals, which were encircled with a marker. Similarly, two concentrations (100 and 50 mg/ml) of the plant extract were injected either into the left or right shaved areas.

The pain was evoked by pricking the encircled areas with a pin at 5 minutes interval for 30 minutes starting with zero-time before the injection of the drug or extract. Six pinpricks were applied at intervals of about 3-5 seconds. The number of pinpricks when the guinea pig failed to react was recorded. A localized skin twitch, usually associated with squeak, was considered as the normal response of animals to pinprick. A negative response (absence of pain) was recorded when the animal failed to respond either by twitching or squeaking. The total number the pinpricks failed to elicit a pain response during the 30 minutes period was

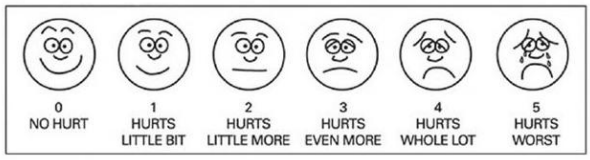

Fig-1: Wong-Baker faces pain rating scale

The obtained gel preparations were tested before application to human volunteers. Parameters included color, consistency, washability, pH, spreadability, extrudability, and irritancy test according to previously described methods [5-7].

RESULTS

The intradermal injection of doses of 50 mg/mL and 100 mg/mL of the *Chrysanthemum cinerarifolium* extract produced 77.8% and 80.6% local anesthesia respectively on guinea pigs. Lidocaine exerted local anesthetic effect of 83.3% and 86.1% at a concentration of 0.3 and 1.0 mg/ml respectively (Figure-2). Both lidocaine and plant extract exerted nearly equal effects during the 30 minutes (Table-2). These results show comparable local anesthetic effects of the plant extract and lidocaine at different concentrations.

added up. The sum out of the possible 42 gave an indication of the degree of anesthesia [4]

Preparation of the Local Anesthetic Gel

Local anesthetic gels containing either lidocaine 0.1% or plant extract 1% were prepared using carbomer as a gelling agent. The composition of formulation is showed in Table-1. Carbomer gel was prepared by dispersing carbomer powder in sufficient quantity of deionized water with the aid of magnetic stirrer (1500 rpm). The drug or the plant extract were added to make 1% concentration, then the pH was adjusted to pH 7 to 7.5 using NaOH 10% with continuous stirring till gel was formed.

Table-1: Formulation of topical gel containing chrysanthemum cinerarifolium extract 1%

Excipient	Quantity
Plant extract	1 g
Carbomer	0.8 g
NaOH 10%	3.2 ml
Deionized water	89.2 ml

Evaluation of Local Anesthetic Activity in Human

To confirm the animal study, prepared gels of the plant extract (1%) and lidocaine (0.1%) were applied on the skin of two groups each containing 6 human volunteers, and the local anesthetic activity was assessed. Sufficient quantities of gel were applied on the inner side of the arm. Local anesthesia was assessed by eliciting pain response by gentle pricking the skin using a pin made of wood at 5 minutes intervals for 30 minutes. The patients in this adopted method were asked to describe the perceived pain using a Wong-Baker faces pain rating scale (WBS) 0–5 (0 = no hurt and 5 = hurts worst) (Figure-1).

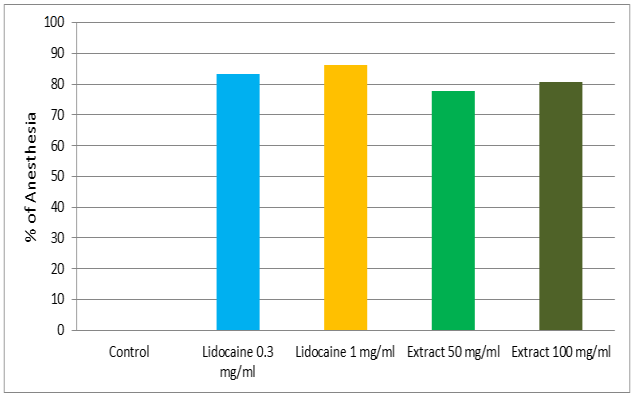

Fig-2: Local anesthetic activity (%) of chrysanthemum cinerarifolium extract as compared to lidocaine

Table-2: Local anesthetic activity (%) of chrysanthemum cinerarifolium extract as compared to lidocaine during 30 minutes in animals

Group/ drug	Concentration	Number of negative responses over time (min)							Total number of negative responses
		0	5	10	15	20	25	30	
Control	Normal saline	0	0	0	0	0	0	0	0
Lidocaine	0.3 mg/ml	0	6	6	6	6	4	3	30
	1 mg/ml	0	6	6	6	5	4	3	31
Extract	50 mg/ml	0	5	6	5	4	4	4	28
	100 mg/ml	0	5	6	5	5	4	4	29

The results of gel evaluation are displayed in table (3). Lidocaine gel was transparent while the extract gel was green in color. Both gels had semisolid consistency, and were found homogenous easily washable. Also, both formulations had slightly alkaline pH which resemble with skin physiology.

Table-3: Evaluation parameters of the prepared topical gel preparations

Gel	Color	Consistency	Washability	pH	Extrudability	Spreadability	Skin irritation
Lidocaine	Transparent	Semisolid	Easily washable	7.4	Good	Good	No sign
Extract	Green	Semisolid	Easily washable	7.2	Good	Good	No sign

Comparable results of local anesthetic activities of both topical gel of the plant extract (1%), and topical gel of lidocaine (0.1%) were observed in the present study in human (Table-4).

Table-4: Local anesthetic activity of the topical gel preparations during 30 minutes in human

Group/ drug	Concentration	Pain sensation score according to WBS over time (min)							Total score
		0	5	10	15	20	25	30	
Lidocaine	0.1 %	3	1	1	2	3	3	3	16
Extract	1%	3	2	1	2	2	3	3	15

DISCUSSION

The present study was conducted to evaluate the local anesthetic activity of the alcoholic extract of *Chrysanthemum cinerarifolium*. Adult male guinea pigs maintained under standard conditions were given intradermal injection of two concentrations of extracts of *C. cinerarifolium* and two lidocaine concentrations at a 5 minutes interval for 30 minutes. *C. cinerarifolium* has been used as analgesic for toothache in the folk medicine. Several chrysanthemum plants have been used as a tonic for digestion, lethargy, constipation, malaria, and chronic rheumatism [8]**.** Alkaloids and other components of *C. cinerarifolium* plant might be the cause of local anesthetic action.

The skin permeability of pig and guinea pig has been previously compared as surrogate for human skin. It has been suggested that both pig and guinea pig are good models for human skin permeability and have less variability as compared to human skin models [9]. The local anesthetic activity test by intradermal injection to form a wheal and pinprick was the standard procedure to check in guinea pigs and had been followed in several studies [4]. For example, it has been demonstrated that 2% aqueous or alcoholic extracts of *A. pyrethrum* showed greater anesthetic activity than lidocaine (0.2%) when tested in guinea pig dermis as well as rabbit cornea. The local anesthetic activity of the extracts lasted 12 hours longer than lidocaine [10]. In this present study, alcoholic extract of *C. cinerarifolium* was used. In general, the solvent used plays an important role in solubility of the active constituents of the plant materials founds and this in turn influences the biological properties of the extracts. It has been shown that sequential extraction of plant material with different solvents of various polarities is useful to extract the biologically active ingredients from plants [11]. In the present study, extraction was carried out with ethanol. The extracts were prepared in 50 mg/ml and 100 mg/ml concentrations, and compared with gold standard 0.3 mg/ml and 1 mg/ml lidocaine (which are used in dental treatments) as positive control. The results in the present study suggest that ethanol extract was effective at both 50 mg/ml and 100 mg/ml concentrations as local anesthetic. Moreover, no adverse effects such as changes in respiration, sunken eyes, and arched back had been seen in the study. Furthermore, the formulated herbal gel did not show any sign of irritation and erythema after 48 hours in human which indicate that the gel has a good compatibility with human skin.

CONCLUSIONS

The study discloses that the alcoholic extract of *C. cinerarifolium* in both 50 mg/ml and 100 mg/ml concentrations shows significant local anesthetic activity with rapid onset of action in guinea pigs; these results were comparable to lidocaine at 0.3 mg/ml and 1 mg/ml concentrations, respectively. Moreover, nearly similar results were found in human when topical anesthetic gel containing 1% of the extract was compared with topical gel containing 0.1% lidocaine. No toxic effects have been reported in both human and animals.

ACKNOWLEDGMENTS

Sincere thanks are due for pharmacy students at Aljazeera University, Ibb for their willingness to participate as volunteers to apply the local anesthetic topical gel preparations.

Financial support: Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCE

1. Togola, A., Diallo, D., Dembélé, S., Barsett, H., & Paulsen, B. S. (2005). Ethnopharmacological survey of different uses of seven medicinal plants from Mali,(West Africa) in the regions Doila, Kolokani and Siby. *Journal of Ethnobiology and Ethnomedicine*, *1*(1), 1-7.
2. Liu, P. L., Wan, Q., Guo, Y. P., Yang, J., & Rao, G. Y. (2012). Phylogeny of the genus Chrysanthemum L.: evidence from single-copy nuclear gene and chloroplast DNA sequences. *PloS one*, *7*(11), e48970.
3. Tijjani, M. A., Abdurahaman, F. I., Khan, I. Z., & Sandabe, U. K. (2012). The effects of ethanolic extract of Vitex doniana stem bark on peripheral and central nervous system of laborotory animals. *Journal of Applied Pharmaceutical Science*, *2*(3), 74-79.
4. Vogel, G. H. (2008). Effect of peripheral nerve function. In: Gerald-Vogel, H., & Bernward, A., editors. Drug Discovery and Evaluation - Pharmacological Assay. 2nd ed. Heidelberg: Springer, 957.
5. Panigrahi, L., Ghosal, S. K., Pattnaik, S., Maharana, L., & Barik, B. B. (2006). Effect of permeation enhancers on the release and permeation kinetics of lincomycin hydrochloride gel formulations through mouse skin. *Indian journal of pharmaceutical sciences*, *68*(2), 205-211.
6. Aswal, A., Kalra, M., & Rout, A. (2013). Preparation and evaluation of polyherbal cosmetic cream. *Der Pharmacia Lettre*, *5*(1), 83-88.
7. Mendhekar, S. Y., Thorat, P. B., Bodke, N. N., Jadhav S. L., & Gaikwad, D. D. (2017). Formulation and evaluation of gel containing neem, turmeric, aloe vera, green tea and lemon extract with activated charcoal and honey. *Ejpmr*, 4(12): 439-443.
8. Annalakshmi, R., Uma, R., Chandran, G. S., & Muneeswaran, A. (2012). A treasure of medicinal herb-Anacyclus pyrethrum a review. *Indian Journal of Drugs and Diseases*, *1*(3), 59-67.
9. Barbero, A. M., & Frasch, H. F. (2009). Pig and guinea pig skin as surrogates for human in vitro penetration studies: a quantitative review. *Toxicology in vitro*, *23*(1), 1-13.
10. Venkatakrishna-Bhatt, H., Panchal, G. M., Devasankariah, G., Gopalakrishna, G., & Patel, V. K. (2001). Local anaesthetic activity of Anacyclus pyrethrum in animals. *Asian J microbiol Biotechnol Environ Sci*, 3:83-85.
11. Pathmanathan, M. K., Uthayarasa, K., Jeyadevan, J. P., & Jeyaseelan, E. C. (2010). In vitro antibacterial activity and phytochemical analysis of some selected medicinal plants. *Int J Pharm Biol Arch*, *1*(3), 291-299.