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Research Article
Phytochemical Screening, Anti-Inflammatory and Analgesic Activities Of Formulation Cream of *Silene vulgaris*

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Abstract
The present study evaluates the anti-inflammatory and analgesic activities of cream formulation of *Silene vulgaris* leaves in rats. The anti-inflammatory effect of cream of *Silene vulgaris* at doses of 10 and 20% applied topically and was evaluated in rats during 6 h using the acute anti-inflammatory model of carrageenan-induced paw edema and during 7 days by chronic anti-inflammatory activity of cotton pellet induced granuloma. The analgesic activity using two *in vivo* models: acetic acid-induced writhing test and plantar test (Hargreaves method) was also studied. Furthermore, the acute toxicity of topical cream was evaluated in rats. Finally, a phytochemical analysis of *Silene vulgaris* was performed. The resulted demonstrated a significant reduction of inflammation after carrageenan-induced rat paw edema in treated group with cream of *S. vulgaris* at 20% compared to Diclofenac (85.71 and 58.33%, respectively). Furthermore, a significant reduction of granuloma weight of cotton pellet was noted in treated group with *S. vulgaris* cream compared to control (1.96±0.04, 2.59±0.2, respectively) with an ED50 of 17.2. In analgesic test, the number of writhings induced by acetic acid solution was decreased by 35.5±1.77 in treated group with cream of *Silene vulgaris* at 20%. A potential peripheral analgesia on acetic acid-induced writhing and also in the plantar test was observed. In addition, topical application of cream did not produce any mortality and any visible signs of toxicity and biochemical parameters. The phytochemical profile of *Silene vulgaris* showed some secondary metabolites, mostly flavonoids, tannins and saponins. These data support the traditional uses of *Silene vulgaris* for acute, chronic inflammation and pain. However, more research is needed for its use in clinical studies.

Key words: Anti-inflammatory effect, analgesic activity, *Silene vulgaris*, flavonoids, cream formulation, toxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause an increase risk on the blood clot resulting in heart attacks and strokes and many various sides effects (Kumar *et al*., 2013). Therefore, the developments of potent anti-inflammatory drugs from the natural products are now under considerations. Natural products are rich source for discovery of new drugs because of their chemical diversity.

Pain is mainly a defensive mechanism of the body and is an ill-defined, unpleasant sensation and emotional experience along with acute or chronic tissue damage which is usually induced by an external or internal noxious stimulus (Kanodia and Das, 2008; Michel *et al*., 2003). Medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions (Vikrant and Arya, 2011). The use of herbal medicines is becoming popular due to toxicity and side-effects of allopathic medicines.

In order to evaluate the anti-inflammatory and analgesic effects of *Silene vulgaris* (caryophyllaceae) selected from an ethnobotanical survey of Sefrou region (Middle Atlas of Morocco) (Boukhira *et al*., 2013). The Silene genus belongs to the family of the Caryophyllaceae is distributed mainly in Africa, Asia and South America. Genus of Silene includes more than 700 species (which allocated to 39 sections) of annuals, biennials and perennials with a worldwide distribution and its taxonomy appears very complex. Common names of Silene include campion (shared with the related genus Lychnis) and catchfly. Red campion (*Silene dioica* (L.) Clairv.), white campion (*Silene latifolia* sub sp. alba, *Silene alba* (Miller) Krause) and bladder campion (*Silene vulgaris*) are common wildflowers throughout Europe.

In the present work, the acute and chronic anti-inflammatory activity of cream of *Silene vulgaris* was examined in carrageenan induced rat paw edema and cotton pellet granuloma. Furthermore the peripheral analgesia power was evaluated using two *in vivo* models (the acetic acid and plantar test).

MATERIALS AND METHODS

Plant material: The plant of *Silene vulgaris* (Caryophyllaceae) were collected in March and April 2013 from Sefrou, (Morocco). The voucher specimen was preserved in the herbarium of National Agency of Medicinal and Aromatic Plants Taounate-Morocco and identified by Pr.A. Ouhammou.

Microscopic study

Powder microscopic assessment: In order to study the presence or absence of various types of tissues or structures, the dried leaves is powdered using electric grinder and then subjected for microscopic studies by using the chloral hydrate to clear up microscopic observation.

Extract preparation: Extraction was carried out in an ultrasonic bath. Flasks containing 20 g of air-dried and crushed plant material and 200 mL of ethanol (70%) were immersed in the ultrasonic bath. Sonication was performed with ultrasound frequency 35 kHz for 45 min. After filtration each mixture was evaporated under vacuum to obtain crude extracts.

Phytochemical screening: Preliminary phytochemical analysis of the extract was performed using the methods described previously (N'guessan *et al*., 2009; Yam *et al*., 2009; Diallo, 2005; Karumi *et al*., 2004; Dahou *et al*., 2003). The polyphenols has been made through the reaction of ferric chloride. Stiasny reaction was used to reveal the presence of catechic and gallic tannins. Furthermore, the flavonoids have been revealed through the reaction to the cyanidin. The saponins have a property to form foam when they are shaken vigorously with water.

Cream formulation: Cream formulation was prepared by the following procedure. The oily phase containing almond oil and 8.5 g bees wax, the aqueous phase was added to the oil phase and mixed. *Silene vulgaris* extract was added to the formulation at doses 10 and 20%.

Evaluation of the anti-inflammatory activity

Experimental animals: Wistar rats, weighing (190-290 g) of either sex were used in these experiments. Rats were kept in well ventilated environment and had a free access to water and food *ad libitum* and were housed in a quiet room under 12 h light: dark cycle for two weeks before experimentations.

Carrageenan induced rat paw edema: Acute inflammation in the rats was produced according to the method described by Winter *et al*. (1962), four groups on rats each containing nine animals per group were used for the study. Carrageenan induced rat paw oedema test was used to determine the anti-inflammatory activity. Cream containing 10 and 20% of

Silene vulgaris hydro-ethanolic extract were applied to the plantar surface of the hind paw. Rats of the control groups received only the cream base and 1% diclofenac applied in the same way as a reference standard. One and half hours after the application of the cream base, topical preparation of *Silene vulgaris* extract and 1% diclofenac; 0.1 mL of 0.5% of carrageenan was injected into plantar surface of right hind paw of rat. The paw size was measured before injection of carrageenan and after injection at 3, 4, 5 and 6 h. Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule. The average increase in paw size of each group was calculated and compared with the control (cream base) and diclofenac groups. The percentage inhibition was then calculated (Ayoola *et al*., 2009):

$$\text{Inhibition } (\%) = \frac{\text{Control } (S_t\text{-}S_0)\text{-Treated } (S_t\text{-}S_0)}{\text{Control}\,(S_t\text{-}S_0)} \times 100$$

where, S_t is the mean paw size for each group after carrageenan treatment and S_0 is the mean paw size obtained for each group before carrageenan injection.

Cotton pellet granuloma method: Sub acute inflammation in the rats was produced according to the method described by Jaijoy *et al*. (2010). Four groups on rats each containing seven animals per group were used for the study. Tissue granulation was induced by cotton pellet implantation in Wistar rats as previously described. Under ether anesthesia, sterile cotton pellets weighting 20±1 mg were implanted an interscapular distance under the skin. Each group of rats was further treated with the cream, reference drugs or control vehicle once daily for seven consecutive days. On the 8th day, the rats were sacrificed and the pellets covered with granulation tissue and thymuses were dissected out and weighed immediately for the wet weight. Both cotton pellets and thymuses were dried at 60°C and their dry weight was determined. The change in body weight from the first and the last day of experiment is recorded. The increase in dry weight of the pellets was taken as the measure of granuloma formation.

Analgesic effect

Acetic acid-induced writhing test: One hour and half after application of cream, base cream and standard reference diclofenac as control groups, each rats was given intraperitoneally 0.7% aqueous solution of acetic acid (10 mL kg^{-1} b.wt.). Immediately after the algic compound injection, each animal was placed in a transparent observation cage and the number of writhes per rat was counted for 30 min. The writhing activity consists of a contraction of the abdominal muscles together with a stretching of the hind limbs (Hernandez-Perez and Rabanal, 2002).

Plantar test method: The analgesic activity was determined by measuring paw withdrawal latency to thermal stimulation system (Hargreaves *et al*., 1988). Before the treatment of sample, animals were individually placed in the chamber and allowed approximately 10 min to acclimate to the testing environment. A radiant heat source mounted on a movable holder below a glass pane was positioned to deliver a thermal stimulus to the mid plantar region of right hind paws. The intensity of the heat stimulus was maintained constant throughout all experiments. When the rat feels pain and withdraws its paw, a photocell detects interruption of a light beam reflection, the I.R. generator is automatically switched off and the timer stops, determining the withdrawal latency.

Acute toxicity study: According to the procedures set by organization for economic, co-operation and development guidelines (OECD., 2001), acute toxicity studies were accomplished for the plant extract of *S. vulgaris*. The rats were divided into two groups of five animals. One group treated by application of cream base (control group), the second is treated by application of formulation at 20%. The animals were observed for 2 h for any behavior changes, neurological and autonomic profiles or death cases after 24, 48 and 72 h and 5 days. The animals were observed for obvious toxic symptoms and mortality in each group during 5 days by studying a single application of dose of 20% of cream formulation, the general behavior of the animal, the weight, the morphological appearance of organs (liver, spleen, heart, kidneys and stomach) and the relative organ weights in comparison with the control group, calculated by the following formula (Ramadan *et al*., 2012):

$$\text{ROW} = \frac{\text{organ weight}}{\text{body weight}} \times 1000$$

Biochemical parameters: Biochemical parameters were assayed on serum, all serum analysis was collected in heparin tubes for the determination of different biochemical parameters like glucose, cholesterol, proteins, triglycerides, creatinine, urea, asparate aminotransferase (AST), alanine aminotransferase (ALT). All parameters were studied by an auto-analyzer "Olympus AU 640".

Statistical analysis: Results were expressed as Mean±SEM. The results were analyzed for statistical significance using student's t-test to compare two groups. In the statistical analysis of the results, $p<0.05$ was considered to be the minimum significance level.

RESULTS

Microscopic study: In order to study the presence or absence of the microscopical characteristics of *Silene vulgaris*, such as hairs, crystals, stomata: The chloral hydrate was used to clear up microscopic observation by using an optic microscope. Microscopic assessment helps mainly in the confirmation of purity of crude drugs and in the detection of adulteration. Powder microscopic study of *Silene vulgaris* leaves shows the presence of diacytic stomata and a large size of calcium oxalate crystals (Fig. 1a, b and c). We noted in this plant the absence of secretory hairs.

Phytochemical screening: Phytochemical screening of extract of *S. vulgaris* revealed the presence of flavonoids, tannins, coumarins, triterpenoids and saponins (Table 1).

Carrageenan induced rat paw oedema model: The anti-inflammatory activity of topical cream of hydro-ethanolic extract of *S. vulgaris* was also performed by comparing its activity with that of diclofenac and cream base contol at 3, 4, 5 and 6 h after the injection of 0.5% carrageenan in the plantar surface of the right hind paw of rate. The results of the anti-inflammatory activity of topical cream are shown in Table 2. The cream at 20% exerted a strong inhibitory effect when compared to diclofenac after 5 and 6 h (80.3, 85.71, 65.52 and 58.33%, respectively).

Cotton pellet induced granuloma formation: The formulation *Silene vulgaris* extract was evaluated by cotton pellet induced granuloma formation to understand its potential in chronic inflammatory phase. The result obtained from this experiment is shown in Table 3. In this experiment, the group treated with topical cream reduced transudative weight and granuloma formation. In addition, cream of *S. vulgaris* and diclofenac increased the body weight gain. Cream at 20% and standard group did not cause significant variations in the thymus weight of animals whereas the cream at 10% reduced the thymus weight (Table 4). Cream at 10%,

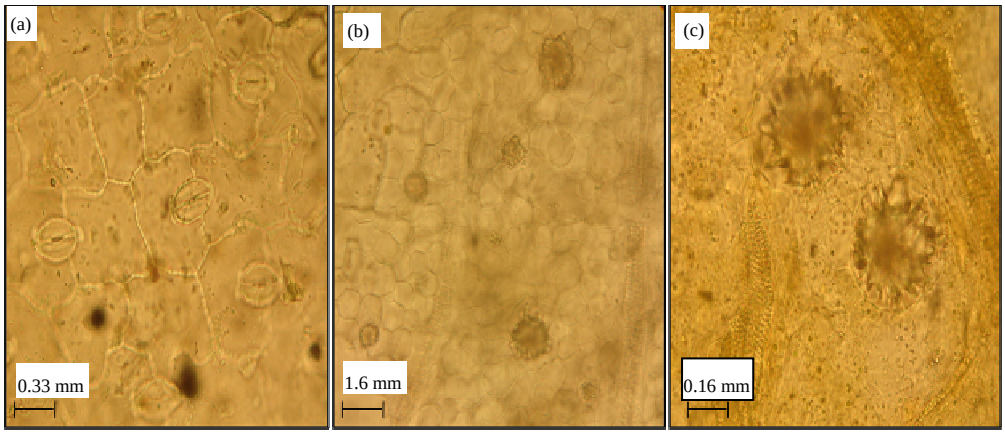

Fig. 1(a-c): Microscopic study of *Silene vulgaris* leaves, (a) Epidermis with diacytic stomata (400×), (b) Twins crystals (100×), (c) Twins in crystals large include vessels (400×)

Table 1: Phytochemical screening of hydro-ethanolic extract of *Silene vulgaris*

Chemical compounds	Extract of *Silene vulgaris*
Flavonoids (flavone)	+
Tannins	+
Gallic tannin	+
Catechin tannin	+
Coumarins	+
Quinones	-
Saponins	+
Triterpenoids	+

+: Presence, -: Absent

Table 2: Effect of topical administration of cream formulated from *Silene vulgaris* on carrageenan induced paw edema in rats

			Time after carrageenan injection (h)							
			3		4		5		6	
Groups	Dose (%)	S_0	S_{3h}	I (%)	S_{4h}	I (%)	S_{5h}	I (%)	S_{6h}	I (%)
Control		2.62±0.06	2.86±0.05	-	2.88±0.05	-	2.91±0.06	-	2.92±0.05	-
Cream	10	2.83±0.06*	3.14±0.02***	29.17	2.98±0.02	42.31	2.94±0.02	62.07	2.98±0.04	50
Cream	20	2.44±0.07	2.66±0.08	10.71	2.56±0.06***	56,04	2.50±0.08***	80.3	2.49±0.10***	85.71
Diclofenac	1	2.68±0.06	2.95±0.05	14.58	2.79±0.07	56.73	2.78±0.08	65.52	2.8±0.07	58.33

Values are expressed as Mean±SEM, n = 9, ***Significantly different from control, p<0.001, S: Edema circumference (cm) at time, % I: Percent edema inhibition of test substance at time

Table 3: Effects of cream of *Silene vulgaris* on granuloma formation and transudation on cotton pellet-induced granuloma in rats

Groups	Dose (%)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight	Granuloma weight (mg/mg)	GI (%)
Control	-	265.5±10.17	71.75±3.91	193.75±6.93	2.59±0.2	-
Cream	10	240.4±21.93	67.60±2.69	172.80±19.53	2.38±0.13	8.11
	20	218.5±7.7*	59.25±0.84**	159.25±7.75*	1.96±0.04**	24.23
Diclofenac	1	223,6±7.82**	60.20±4.73	163.40±5.06**	2.01±0.24	22.39

Values are expressed as Mean±SEM, n = 7, Significantly different from control, **p<0.01, ***p<0.001, GI: Granuloma inhibition

Table 4: Effects of cream formulation of *Silene vulgaris* on body weight and dry thymus weight on cotton pellet-induced granuloma in rats

		Body weight (g)			
Groups	Dose (%)	Initial	Final	Gain	Dry thymus weight (mg/100 g)
Control		287.0±17.39	301.75±10.78	14.75±6.62	51.62±3.07
Cream	10	207.5±9.81	249.25±11.06	41.75±13.98	31.85±3.88
	20	258.6±16.77	279.60±13.27	21.00±5.8	54.49±2.38
Diclofenac	1	204.0±8.98	258.75±1.91	54.75±9.78*	55.83±3.80

Values are expressed as Mean±SEM, n = 7, ***Significantly different from control, p<0.001

Table 5: Effect of cream of *Silene vulgaris* on the relative weight of spleen, liver, heart and kidneys

			Weight (g)			
Groups	Dose (%)	Body weight (g)	Liver	Heart	Kidney	Spleen
Control	-	301.75±10.77	15.93±0.58	1.02±0.04	1.01±0.03	0.57±0.02
Cream	10	249.25±11.06	12.12±0.49	0.88±0.04	0.98±0.04	0.63±0.05
	20	279.60±13.27	14.29±0.67	1.00±0.06	0.97±0.02	0.71±0.05
Diclofenac	1	258.40±6.25	13.50±0.34	0.92±0.03	1.00±0.02	0.66±0.66

Values are expressed as Mean±SEM, n = 7

Table 6: Analgesic effect of cream of *Silene vulgaris* on acetic acid induced writhing in rats

Groups	Dose (%)	Number of writhings	Inhibition (%)
Control	-	68.5±5.04	-
Cream	10	37.0±3.32	46.0
	20	35.5±1.77***	48.2
Diclofenac	1	31.8±5.54**	53.6

Values are Mean±SEM, n = 6, ***p<0.001 when compared to control, Student's t-test

20% and diclofenac increased the spleen weight compared to the control (Table 5) but did not cause variations in the liver, heart and kidneys weights in comparison to the control group.

Acetic acid-induced writhing test: Table 6 demonstrate the results of analgesic effect by acetic acid induced writhing method. Topical administration of cream reduced the number of rat abdominal constriction following acetic acid, indicating analgesic activity for this cream at the doses assayed. Pretreatment with cream of silene (10 and 20%), significantly inhibited by 46 and 48.2% of the writhings, respectively. Pretreatment of the rat with diclofenac significantly (p<0.001) writhing inhibited by 53.6%. The analgesic effect was observed by the group that received 20% produced a comparable result with diclofenac group (Table 6).

Plantar test: As shown in Table 7, the effects of cream of *Silene vulgaris* on the pain induced by the radiant heat (IR). The sample produced a low analgesic activity at 10 and 20%

Table 7: Analgesic effect of cream on plantar test

Groups	Dose (%)	Time of latency (s)
Control	-	14.84±0.81
Cream	10	18.80±0.7
	20	21.78±1.35**
Diclofenac	1	46.82±3.32**

Values are Mean±SEM, n = 5, ***p<0.001 when compared to control, student's t-test

Table 8: Effect of cream at 20% on the relative organ weigh

			Relative Organ Weight (g)				
Group	Dose (%)	Body weight (g)	Liver	Heart	Kidney	Spleen	Stomach
Control	-	206.00±7.23	51.55±3.69	4.19±0.21	4.14±0.14	2.52±0.21	64.7±3.45
Cream	20	184.67±2.4	52.67±2.17	4.46±0.1	4.08±0.13	2.66±0.11	52.4±9.29
Significativity			ns	ns	ns	ns	ns

Values are Mean±SEM, n = 5, ns: Non significant

Table 9: Effect of formulation cream of *Silene vulgaris* on biochemical parameters

Parameters	Control	*Silene vulgaris* cream
Liver profile (U L^{-1})		
AST	269.30±46.1	681.30±2.66^{ns}
ALT	86.33±28.08	66.66±2.33^{ns}
Alkaline phosphatase	157.00±15.50	184.60±43.6^{ns}
Renal profile (U L^{-1})		
Urea	0.40±0.04	0.41±0.01^{ns}
Creatinine	6.33±0.67	6.00±0.00^{ns}
Blood chemistry (g L^{-1})		
Total Proteins	48.67±2.33	55.67±2.67^{ns}
Glucose	1.30±0.18	1.04±0.08^{ns}
Cholesterol	0.52±0.07	0.72±0.01^{ns}
Triglycerides	0.64±0.10	0.41±0.04ns

Values are expressed as Mean±SEM, n = 3, AST: Aspartate transaminase, ALT: Alanine aminotransferase, ns: Not significant

when compared to that of Diclofenac that significantly ($p<0.01$) increased the pain latency.

Acute toxicity: In the acute toxicity study, cream at a dose of 20% caused neither visible signs of toxicity nor mortality. We haven't got any adverse effects on the parameters studied after treatement with cream (Table 8) (weight and general behavior of the animal) compared with the control group.

Biochemical parameters: Table 9 show the results of some biochemical parameters assessed in serum of rats treated with *Silene vulgaris* extract. We noted any significant difference in hepatic functions indicated by AST and ALT values of treated rats of *Silene vulgaris* at different dose of 20% compared to the control. Furthermore, the values of all parameters related to renal and blood functions (urea, creatinine, glucose, triglycerides, cholesterol and total proteins) are not changed when we compared between control and treated animals.

DISCUSSION

Carrageenan-induced edema in the rat paw is a standard model of acute inflammation and hyperalgesia. The inflammatory reactions are biphasic, the initial phase occurs within 2 h of the injection of carrgeenan. It has been reported that during first phase serotonin and histamine are released while bradykinin is released 2 h after the carrageenan injection (Patil *et al*., 2011). In approximately 3 h, the edema volume reaches its maximum and then begins to decline. Over production of prostaglandins are involved in the late phase and may continue until 5 h post carrageenan injection. The secondary phase response is reportedly affected by most of the currently available NSAID (Khuda *et al*., 2013). Based on these reports and our results, it is concluded that the topical cream of *S. vulgaris* showed significant anti-inflammatory effect in late stage of inflammation as compared with the standard NSAID and did not produce any visible signs or symptoms of toxicity when we investigated acute toxicity with a unique application.

Chronic inflammations appeared when the body response is insufficient to eliminate the proinflammatory agents. Thus, the proliferation of fibroblasts and the infiltration of cells (such as neutrophils and exudate) were occurred through the early development of the proliferative cells (Raju *et al*., 2014). These cells can either be in spread or in granuloma formed (Hosseinzadeh *et al*., 2000). The cotton pellet-induced

granuloma model is widely used to assess the proliferative components of chronic inflammation with a transudative phase an exudative phase and a proliferative phase of the inflammatory response (Bagad *et al*., 2013). Reduction in dry pellet weight could be due to a decrease production of fibroblasts and synthesis of collagen and mucopolysaccharides during formation of granuloma tissue and thus signifies the suppression of inflammatory proliferative phase (Recio *et al*., 1995). In this experiment, cream of *S. vulgaris* at higher dose reduced significantly transudative weight and granuloma formation in cotton pellet granuloma method and thus found to be effective in chronic inflammatory conditions.

The increase of the weight of primary lymphoid organs in animals can be related to the influence of drugs on the immune response in particular the increase of the thymus are related to a positive effect on the immune system (Ran *et al*., 2014). In this study thymus weights was increased in the cream of *S. vulgaris* at dose of 20% as compared to control. The body weight gain is considered as an index of anti-inflammatory activity (Vaille *et al*., 1990). In this chronic inflammation model, both cream at two doses and diclofenac increased body weight gain of animals. Other studies noted that topical application of NSAID have been used for decades to relieve the pain of musculoskeletal tissues (Xavier *et al*., 2011).This administration pathway probably reduces adverse effects by maximizing the local effect and minimizing systemic toxicity. The major problem has been the penetration into the target-tissue and therefore, the clinical efficacy. These finding demonstrated that cream of *S. vulgaris* can elicit anti-inflammatory activity when topical application indicates that the anti-inflammatory constituent(s) of *S. vulgaris* which bring this effect can be absorbed percutaneously.

The acetic acid induced writhing test was carried out to confirm the peripheral analgesic activity of cream formulation. Acetic acid induced writhing method is generally used to determine peripherally acting analgesic action and represents pain sensation which acts by triggering localized inflammatory reaction. This method is not only simple, reliable, sensitive but also affords rapid evaluation of peripheral analgesic action (Singh and Majumdar, 1995). In our study, intraperitoneal administration of acetic acid causes the twisting of dorsoabdominal muscles constrictions because of irritation of peritoneal cavity in which acetic acid is thought to discharge of prostaglandins E2 and F2α mediators as well as lipoxygenase mediators that excite pain nerve endings by inflammatory response (Raju *et al*., 2014). These endogenous mediators of inflammatory pain are sensitive to NSAID and opioids (Deraedt *et al*., 1976). It is well known that NSAID and analgesic drugs play role in reduction of the inflammatory pain at the peripheral target sites by inhibiting or blocking the formation of pain mediators whereas bradykinin and prostaglandins are responsible for pain process (Kim *et al*., 2004).

In our study, cream in a dose dependent manner, attenuated significantly the writhing in rats in response to i.p., acetic acid suggests that the analgesic effect may be mediated peripherally via the inhibition of release and synthesis of PGs and other endogenous mediators. Furthermore, in order to corroborate the analgesic activity of *S. vulgaris* extract, plantar test was also used. From the results of two methods, it can be conclude that cream of *S. vulgaris* showed peripheral analgesic action in both acetic acid writhing method and plantar test.

The preliminary phytochemical analysis of *Silene vulgaris* hydro-ethanolic extract showed the presence of flavonoids, tannins, triterpenoids, saponins and phenolic compounds. The work of (Raju *et al*., 2014) reported that flavonoids and terpenoids are responsible for the acute anti-inflammatory effect. Some other studies have already claimed that flavonoids also possessed anti-inflammatory action (Hasan *et al*., 2014). Furthermore, tannins are important compounds known to be potent cyclooxygenase-1 inhibitors and with an important anti-phlogistic activity (Pan *et al*., 2010). In the same context, triterpenes especially saponins reported to have anti-inflammatory activity (Liu, 1995). Therefore, the acute and chronic anti- inflammatory activity of our topical cream might be due to synergy between tannins, flavonoids and saponins. The present study will stimulate further efforts towards the development of new, safe and more effective natural substances with lesser side effects for the treatment of pain and inflammatory diseases.

CONCLUSION

It can be concluded from the present data, that the topical cream of *Silene vulgaris* (Moench) garcke posses a significant acute and chronic anti-inflammatory activity as compared to standard at later phase. In analgesic test, the cream elicited a potential peripheral analgesia on acetic acid-induced writhing and also in the plantar test. In addition, topical application of cream did not produce any mortality and any visible signs of toxicity. The anti-inflammatory and analgesic properties of *S. vulgaris* extract might be due to presence of tannins, flavonoids and saponins. The present finding supports the traditional claims and provides a scientific basis for anti-inflammatory and analgesic studies of cream of *Silene vulgaris* in acute and chronic inflammatory diseases and pain without any adverse effects.

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REFERENCES

Ayoola, G.A., G.A. Akpanika, F.O. Awobajo, M.O. Sofidiya, V.O. Osunkalu, H.A.B. Coker and T.O. Odugbemi, 2009. Anti-inflammatory properties of the fruits of *Allanblanckia floribunda* oliv. (Guttiferae). Bot. Res. Int., 2: 21-26.

Bagad, A.S., J.A. Joseph, N. Bhaskaran and A. Agarwal, 2013. Comparative evaluation of anti-inflammatory activity of curcuminoids, turmerones and aqueous extract of *Curcuma longa*. Adv. Pharmacol. Sci., Vol. 2013. 10.1155/2013/805756.

Boukhira, S., L.E.L. Mansouri and D. Bousta, 2013. Ethnobotanical studies of some medicinal and cosmetic plants used in the province of Sefrou, Middle Atlas of Morocco. J. Ethnobiol. Tradit. Med. Photon, 120: 661-670.

Dahou, N., K. Yamni, S. Tahrouch, L.M. Idrissi Hassani, A. Badoc and N. Gmira, 2003. Screening phytochimique d'une endemique ibero-marocaine *Thymelaea lythroides*. Bull. Societe Pharmacie Bordeaux, 142: 61-78.

Deraedt, R., S. Jouquey, J. Benzoni and M. Peterfalvi, 1976. Inhibition of prostaglandin biosynthesis by non-narcotic analgesic drugs. Arch. Int. Pharmacodyn. Ther., 224: 30-42.

Diallo, A., 2005. Study of phytochemistry and biological activities of *Syzygium guineense* willd. Master's Thesis, University of Lausanne, Switzerland.

Hargreaves, K., R. Dubner, F. Brown, C. Flores and J. Joris, 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain, 32: 77-88.

Hasan, M.M., N. Uddin, M.R. Hasan, A.F.M. Islam and M.M. Hossain *et al*., 2014. Analgesic and anti-inflammatory activities of leaf extract of *Mallotus repandus* (Willd.) Muell. Arg. BioMed Res. Int., Vol. 2014. 10.1155/2014/539807.

Hernandez-Perez, M. and R.M. Rabanal, 2002. Evaluation of the antinflammatory and analgesic activity of *Sideritis canariensis* var. *pannosa* in mice. J. Ethnopharmacol., 81: 43-47.

Hosseinzadeh, H., M. Ramezani and G.A. Salmani, 2000. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* boiss extracts in mice and rats. J. Ethnopharmacol., 73: 379-385.

Jaijoy, K., N. Soonthornchareonnon, A. Panthong and S. Sireeratawong, 2010. Anti-inflammatory and analgesic activities of the water extract from the fruit of *Phyllanthus emblica* Linn. Int. J. Applied Res. Nat. Prod., 3: 28-35.

Kanodia, L. and S. Das, 2008. A comparative study of analgesic property of whole plant and fruit extracts of *Fragaria vesca* in experimental animal models. Bangladesh J. Pharmacol., 4: 35-38.

Karumi, Y., P.A. Onyeyili and V.O. Ogugbuaja, 2004. Identification of active principles of *M. balsamina* (Balsam Apple) leaf extract. J. Medical Sci., 4: 179-182.

Khuda, F., Z. Iqbal, A. Khan, F. Nasir and Y. Shah, 2013. Anti-inflammatory activity of the topical preparation of *Valeriana wallichii* and *Achyranthes aspera* leaves. Pak. J. Pharmaceut. Sci., 26: 451-454.

Kim, H.P., K.H. Son, H.W. Chang and S.S. Kang, 2004. Anti-inflammatory plant flavonoids and cellular action mechanisms. J. Pharmacol. Sci., 96: 229-245.

Kumar, S., B.S. Bajwa, K. Singh and A.N. Kalia, 2013. Anti-inflammatory activity of herbal plants: A review. Int. J. Adv. Pharm. Biol. Chem., 2: 272-281.

Liu, J., 1995. Pharmacology of oleanolic acid and ursolic acid. J. Ethnopharmacol., 49: 57-68.

Michel, Y.B., G.D. Christopher and H.L. Allen, 2003. Chronic Pain Management. In: Wylie Churchill-Davidson's a Practice of Anesthesia, Healy, T.E.J. and P.R. Knight (Eds.). 7th Edn., CRC Press, London, ISBN: 9780340731307, pp: 1139-1235.

N'Guessan, K., B. Kadja, G. Zirihi, D. Traore and L. Ake-Assi, 2009. [Phytochemical screening of some ivorian medicinal plants used by the Krobou people (Agboville, Cote-d'Ivoire)]. Sci. Nat., 6: 1-15, (In French).

OECD, 2001. Guidelines for testing of chemical. Guideline 423, Acute Oral Toxicity-Acute Toxic Class Method. OECD, Paris.

Pan, M.H., C.S. Lai and C.T. Ho, 2010. Anti-inflammatory activity of natural dietary flavonoids. Food Funct., 1: 15-31.

Patil, S., S. Anarthe, R. Jadhav and S. Surana, 2011. Evaluation of anti-inflammatory activity and *in-vitro* antioxidant activity of Indian *Mistletoe*, the Hemiparasite *Dendrophthoe falcate* L. F. (Loranthaceae). Iran. J. Pharmaceut. Res., 10: 253-259.

Raju, G.S., M.M.R. Moghal, M.S. Hossain, M.M. Hassan, M.M. Billah, S.K. Ahamed and S.M. Rana, 2014. Assessment of pharmacological activities of two medicinal plant of Bangladesh: *Launaea sarmentosa* and *Aegialitis rotundifolia* roxb in the management of pain, pyrexia and inflammation. Biol. Res., Vol. 47.

Ramadan, A., G. Soliman, S.S. Mahmoud, S.M. Nofal and R.F. Abdel-Rahman, 2012. Evaluation of the safety and antioxidant activities of *Crocus sativus* and *Propolis ethanolic* extracts. J. Saudi Chem. Soc., 16: 13-21.

Ran, Y., R. Wang, Q. Gao, Q. Jia and M. Hasan *et al*., 2014. Dragon's blood and its extracts attenuate radiation-induced oxidative stress in mice. J. Radiat. Res., 55: 699-706.

Recio, M.C., R.M. Giner, S. Manez and J.L. Rios, 1995. Structural requirements for the anti-inflammatory activity of natural triterpenoids. Planta Medica, 61: 182-185.

Singh, S. and D.K. Majumdar, 1995. Analgesic activity of *Ocimum sanctum* and its possible mechanism of action. Int. J. Pharmacog., 3: 188-192.

Vaille, A., G. Jadot and A. Elizagaray, 1990. Anti-inflammatory activity of various superoxide dismutases on polyarthritis in the Lewis rat. Biochem. Pharmacol., 39: 247-255.

Vikrant, A. and M.L. Arya, 2011. A review on anti-inflammatory plant barks. Int. J. PharmTech Res., 3: 899-908.

Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Exp. Biol. Med., 111: 544-547.

Xavier, W.K.S., B.J. Medeiros, C.S. Lima, H.A. Favacho, E.H.A. Andrade *et al*., 2011. Topical anti-inflammatory action of *Caryocar villosum* oil (Aubl) Pers. J. Applied Pharm. Sci., 1: 62-67.

Yam, M.F., L.F. Ang, O.Z. Ameer, I.M. Salman, H.A. Aziz and M.Z. Asmawi, 2009. Anti-inflammatory and analgesic effects of *Elephantopus tomentosus* ethanolic extract. J. Acupuncture Meridian Stud., 2: 280-287.