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Anti-inflammatory properties of *Centaurea calolepis* Boiss. and cnicin against *Macrovipera lebetina* obtusa (Dwigubsky, 1832) and *Montivipera xanthina* (Gray, 1849) venoms in rat

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ABSTRACT

Macrovipera lebetina obtusa (Dwigubsky, 1832) and *Montivipera xanthina* (Gray, 1849) (Ottoman Viper) are viper snakes from Viperidae family and found in various locations in Anatolia. Both snakes are responsible for major snake bite cases in Turkey Their venoms cause necrosis, hemorrhage, pain and local edema.

Centaurea L. (Asteraceae) species draw attention as potential anti-inflammatory sources due to their traditional uses and accomplished studies on this field. *C. calolepis* Boiss. is an endemic taxon distributed in Aegean and Antalya regions in Turkey. Chloroform extract of *C. calolepis* and its major compound cnicin, a sesquiterpene lactone, are reported to have strong anti-inflammatory activities *in-vitro*, by previous studies.

In the present study, *in-vivo* anti-inflammatory activities of *C. calolepis* chloroform extract and the sesquiterpenoid cnicin against edema induced by *Macrovipera lebetina obtusa* and *Montivipera xanthina* venoms were evaluated in the rat model. Protein contents and induction doses of the venoms were determined. Carrageenan and snake venoms were used as inducing agents in paw edema tests.

Extract demonstrated strong inhibition on edema at all doses and hours against *M. xanthina* venom and carrageenan. Inhibition ratio of extract at 25 mg/kg dose (84.13% inhibition) after 0.5 h M. *xanthina* venom injection was more than indomethacin's value (45.4% inhibition). The extract also showed significant effect also on inflammation caused by *M. lebetina obtusa* venom at all doses. However, 2.5 mg/kg cnicin was more effective than total extract of *C. calolepis* against rat paw edema induced by (27.31%) *M. lebetina obtusa* venom.

This is the first study reported therapeutic potential of *C. calolepis*, an endemic plant of Turkey, in case of snake-bites cause inflammation by venomous species in natural fauna of Anatolia.

1. Introduction

Envenoming by snake bites are a major health problem and according to WHO (World health organization) reports; most severe cases result from snake bites by members of the families Viperidae and Elapidae. Viperid snake venoms cause local extravasation of plasma and blood into the bitten limb, inflammation and tissue damage, due to the action of toxins on muscle, skin and blood vessels, resulting in pain, edema, blistering, bleeding and necrosis of skin, subcutaneous tissues and muscle (Warrell and Gutiérrez, 2007). Severe local tissue damages lead to permanent tissue loss and disability. Although the most recommended treatment for snakebites are antivenom therapies, they have limitations for local pathological effects induced by most viperid venoms (Gutierrez et al., 2007).

Viperidae family members are the main responsible of the most venomous bites also in Anatolia. *Macrovipera lebetina obtusa* Dwigubsky, 1832 and *Montivipera xanthina* Gray, 1849 are two of the most dangerous species in Turkey (Budak and Göçmen B, 2005; Cesaretli and Ozkan, 2010). Ottoman viper, *Montivipera xanthina* is an semi endemic species which is distributed in Central, Southern and Western Anatolia and some Greece islands and is known as “seritli engerek” (Budak and Göçmen, 2005). Topyildiz and Hayretdağ (2012) have investigated histopathological effects concerning the damage caused by venom on rat tissues and detected edema, hemorrhage, local bleeding, rare inflammatory cells, strong cell infiltration, inflammation around veins and fat necrosis on dermis. Skin edema, cell infiltration and

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inflammatory reactions are suggested as target symptoms for reducing the damaging effects (Topyildiz and Hayretdağ, 2012). For the purpose of finding an anti-inflammatory natural agent against *M. xanthina* venom, our team reported the significant effect of the *Artemisia absinthium* extract at 50 mg/kg on rats, previously. Characterization and inflammation challenge dose of this venom (37.5 μg/paw) was also determined by the same study. The other dangerous snake *Macrovipera lebetina obtusa* (Dwigubsky, 1832) is known from Southeastern, Southern and Northeastern Anatolia (Igci and Demiralp, 2012). Although various studies on molecular and chemical characters of its venom are reported in previous studies(Ayvazyan et al., 2012; Igci and Demiralp, 2012; Sanz et al., 2008). studies on effects of the venom are limited.

Plants have been used for anti-snake envenoming traditionally in many countries especially where the high-level medical treatments are challenging (Butt et al., 2015; Price, 2016; Ruppelt et al., 1991; Sulochana et al., 2015). It is easily available in the countryside for treatment of snakebite lethal and also local effects. In last years there is an increasing attention for investigating the plant extracts and their compounds for anti-snake venom therapy by researchers (Bhole and Bhavsar, 2017; Félix-Silva et al., 2017; Fernandes et al., 2014; Magalhães et al., 2011a; Nalbantsoy et al., 2013).

The genus *Centaurea* L. (Asteraceae) is represented by 158 species in Turkish Flora, 94 of which are endemic (Erdağ et al., 2014; Erel et al., 2013). Generally *Centaurea* species are used for some inflammatory related diseases like fever, wounds, abscess and rheumatism in traditional medicine. (Baytop, 1999; Ridvan Polat et al., 2013; Sezik et al., 2001). Besides, the use of *C. iberica* leaves in snake bite cases in Hakkari-Turkey is reported (Kaval et al., 2014). *C. calolepis* Boiss. is an endemic species located in Aegean and Antalya regions (Güner et al., 2012) in Turkey. Chloroform extract of *C. calolepis* and its major compound cnicin, a sesquiterpene lactone, had strong anti-inflammatory activity by *in-vitro* NF-κB and iNOS inhibition assays (Erel et al., 2011; Karamenderes et al., 2007). In a previous study, cnicin reduced carrageenan-induced inflammation in rat model (Schneider and Lachner, 1987).

The aim of this study was to determine potential use of *C. calolepis* and cnicin in snake bite treatment as anti-inflammatory agents. For this purpose, anti-inflammatory activities of C. calolepis and cnicin against *Macrovipera lebetina obtusa*, *Montivipera xanthina* venoms and carrageenan-induced inflammation in rat were evaluated comparatively.

2. Material and methods

2.1. Plant material and extraction

C. calolepis was collected from Denizli, nearby Karacasu-Kuyucak, 311 m, in June 2004. Voucher specimens were identified by botanist Prof. Dr. Ozcan Secmen, Faculty of Science, Ege University and deposited in Herbarium of Ege University, Faculty of Pharmacy (IZEF No. 5672 and 5668).

Dried aerial parts of plant (1270 g) were powdered and extracted with n-hexane, chloroform and methanol, respectively at room temperature in ultrasonic bath (3x 4 L, 48 h for each) (Ultrasonic LC-30, Elma) Material was filtered and solvents were evaporated to dryness at 40 °C (Erel et al., 2011). All the extracts were stored in desiccator and stability of chemical compounds was controlled by TLC (thin layer chromatography) before anti-inflammatory assay. Chloroform extract was chosen for assay because of the results obtained in previous studies and also TLC profiles.

Cnicin was purchased from Cfm Oskar Tropitzsch GmbH, Germany (purity grade: 98%).

2.2. Venom and protein content determination

Snake venoms were obtained from our previous study as lyophilized form. *M. l. obtusa* and *M. xanthina* venoms were reconstituted in saline and protein content of crude venom (2 mg/L) was determined by using BCA kit (ThermoScientific) at 562 nm (Bradford, 1976). BSA (Bovine serum albumin) was chosen as standard protein and the assay was performed triplicate.

2.3. Experimental animals

All animal care was approved by Ege University, Local Ethical Committee of Animal Experiment under number 2014069. Male and female albino mice (20–25 g) and Wistar rats (125–150 g) were used through $LD5_0$ determination and hind paw edema assay, respectively. The experiments were carried out in Ege University, Center for Drug R& D and Pharmacokinetic Applications and Ege University, Central Experimental Animals Laboratory.

2.4. Determination of LD_{50}

Value of LD_{50} (dose of venom required to kill 50% of the animals studied) was calculated to establish dose of venom for inducing inflammation on rat's paw. LD_{50} is calculated only for *M. l. obtusa* venom due to LD_{50} value of *M. xanthina* venom was determined in our previous study (Nalbantsoy et al., 2013). Each group was consisted of five albino mice (20–25 g). Saline (control group) and venom from *M. l. obtusa* at different doses (0.5, 1 and 2.5 mg/kg) was administrated intraperitoneal (i.p) and within 24 h 50% death was determined by Graph pad Prism 5. Determination of LD_{50} was accordance with "up and down" procedure in guideline OECD 425 (OECD, 2008).

2.5. Evaluation of inflammation induced by viper venoms

Previously described method (Nalbantsoy et al., 2013) was used in order to evaluate *M. l. obtusa* venom-induced edema. *M. l. obtusa* venom diluted in isotonic saline was injected sub plantar into the left hind paw at 25, 50 and 75 μg/paw to establish challenge-dose, whereas saline was injected into the right paw as control group (n = 6). The paw volumes were measured by using hydro-plethysmometer at 0.5, 1, 2, 3 and 4 h following venom injection.

Same procedure was applied using 37.5 and 60 μg/paw doses to find the challenge dose of *M. xanthina* venom. Isotonic saline and venoms dissolved in saline were sterilized with 0.22 μm filter.

2.6. Assessment of anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan and

Table 1
Experimental groups and inductive agents used in paw edema assay to evaluate anti-inflammatory activity.

Experimental Groups	Inductive agent
C. calolepis extract (12.5, 25, 50 mg/kg)	a. *M. xanthina* venom (60 μg/paw) b. *M. l. obtusa* venom (75 μg/paw) c. Carrageenan (0.1 ml 1%/paw)
Cnicin (2.5, 5, 10 mg/kg)	a. *M. xanthina* venom (60 μg/paw) b. *M. l. obtusa* venom (75 μg/paw)
İndomethacin (10 mg/kg)	a. *M. xanthina* venom (60 μg/paw) b. *M. l. obtusa* venom (75 μg/paw) c. Carrageenan (0.1 ml 1%/paw)

venom-induced paw edema assays (Erel et al., 2014, 2011). Inductive agents and experimental groups are given in Table 1.

In the present study, anti-inflammatory activity of cnicin was evaluated only against venom-induced edema, since carrageenan-induced paw edema assay was performed previously (Schneider and Lachner, 1987).

Rats were deprived of food overnight. Extracts were dissolved in Tween 20 (5%) and administrated per oral (12.5, 25 and 50 mg/kg). Thirty min. later 0.1 ml of inductive agent dissolved in isotonic saline was injected sub plantar into the left hind paw. Isotonic saline (0.1 ml), used control group was injected same way into the right hind paw (n = 6, for each group). Paw volumes were measured by using hydroplethysmometer at 0.5, 1, 2, 3 and 4 h following venom injection. The ant-inflammatory test was repeated with 10 mg/kg indomethacin (Sigma Chemical Co, St, Louis, USA). The percent of edema increase induced by venom or carrageenan was calculated by using the following equation:

$$\text{Edema increase}(\%) = [(V_{treated} - V_{control})/V_{control}] \times 100$$

2.7. Data analysis

Data was shown as mean ± SEM (n). *n* indicates the number of animals. Statistical differences were analyzed by two-way ANOVA and Bonferroni posttests. LD_{50} was calculated by Graph Pad Prism 5. *P* values less than 5% ($p < 0.05$) were considered statistically significant (Graph Pad Prism 5, Graph Pad Software Inc, USA).

3. Results

3.1. Protein content determination

Two mg/ml of *M. l. obtusa* and *M. xanthina* crude venoms were consist of 1999.5 µg/ml and 1822.3 µg/ml protein, respectively.

3.2. Determination of LD_{50}

Twenty h after *M.l.obtusa* venom injection of single dose at different concentrations (2.5, 1, and 0.5 mg/kg) LD_{50} value was established at 1.057 mg/kg.(See Table 2).

3.3. Venom-induced inflammation

25, 50 and 75 µg/paw of *M. l. obtusa* venom was administrated subplantary into the paw and edema increase was measured. Half an hour after 75 µg injection, venom produced a rapid and significant inflammation (Fig. 1). 75 µg/paw was chosen as challenge-dose to induce inflammation. Venom did not produce any behavioral changes in rats during experiments.

Rats were treated with 37.5 and 60 µg/paw *M. xanthina* venom and 60 µg/paw dose was preferred as challenge dose due to the observed significant inflammation.

Table 2
Lethality of *M. lebetina* venom and determined LD_{50} value.

Concentration mg/kg (n = 5)	Dead	Live	Viability rate (%)	LD_{50} (mg/kg)
2.5	5	0	0	1.057
1	2	3	60	
0.5	1	4	80	

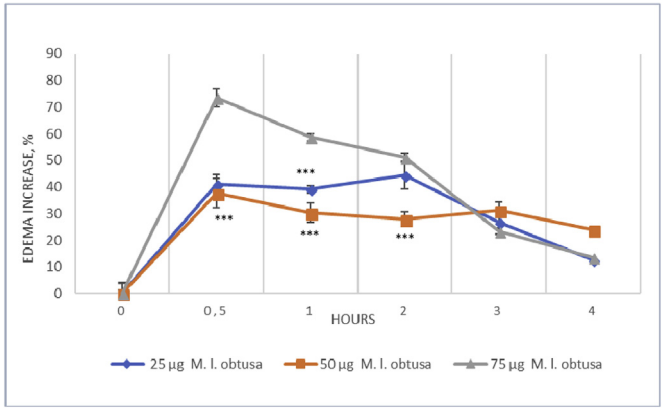

Fig. 1. Dose-response curve of *M. l. obtusa* venom-induced in rat hind paw edema model (25, 50, 75 µg/paw). Data are reported mean ± SEM (n = 6) and ***$p < 0.001$ compared to 75 µg *M. l. obtusa* venom.

3.4. Anti-inflammatory activity of Centaurea calolepis crude extract

C. calolepis extract was administrated p. o. to rats at different doses (12.5, 25 and 50 mg/kg). % edema increases at all doses can be seen at Table 3. 25 mg/kg of extract was established as most effective dose (11.12% edema increase). Half an hour after *M. xanthina* venom injection, the extract reduced inflammation (84.13% inhibition) better than indomethacin (45.4% inhibition). Same concentration of the extract was more effective in comparison with indomethacin at 2, 3 and 4 h. 50 mg/kg of the extract decreased inflammation less than 25 mg/kg dose at 0.5 and 1 h. However, this concentration inhibited edema obviously compare to control group (Fig. 2a).

Considering the effect of extract against *M. l. obtusa* venom; 12.5 mg/kg and 25 mg/kg doses of the extract were effective at 0.5 and 1 h (edema increase: 35.89% and 36.43%, respectively). At the end of 1 h edema increasing was reduced to 31.05%. Inflammation decreased distinctly at 2, 3 and 4 h (Fig. 2b).

Inflammation of carrageenan was observed two h late comparing to venom. Extract inhibited edema responses more than indomethacin with all doses at 0.5 and 1 h. At the end of 1 h, administration of extract with 12.5 mg/kg showed 9.97% edema increase in rats. Time-dependent decrease was observed with all doses of extract. (Fig. 2c).

3.5. Anti-inflammatory activity of cnicin

% edema increases at all doses of cnicin against *M. xanthina* and *M. lebetina* venoms can be seen at Table 3.

M. xanthina venom-induced inflammation was very high at first half hour (70.1%) and reduced at following hours. All doses of cnicin showed equal effect with indomethacin at 0.5 h. The most effective dose of cnicin was determined as 10 mg/kg (28.31%) (Fig. 3a).

M. l. obtusa venom formed 73.5% of edema in rat paw compare to control group at first half an hour. Edema formation was 27.31% with 2.5 mg/kg of cnicin, whereas it was 12.67% with 10 mg/kg of indomethacine. At all hours 2.5 mg/kg of cnicin was found to be the most effective dose reducing edema by half at the end of 1 h (25.72%) (Fig. 3b, Table 3).

Effect of cnicin against carrageenan-induced hind paw edema was determined previously (Schneider and Lachner, 1987). They reported cnicin inhibited edema as 77%, whereas it was 58% for indomethacin.

Table 3
Effect of *C.calolepis* extract and cnicin against *M.xanthina, M.l. obtusa* venom and carrageenan-induced edema. Significant %edema increases are highlighted.

Group	Inductive agent	Dose (mg/kg)	%edema increase				
			0.5. h.	1.h.	2.h.	3.h.	4.h.
Control	*M.xanthina*	(60μg/100 μl/paw)	70.10 ± 8.11	54.59 ± 10.25	28.72 ± 6.12	17.54 ± 5.41	3.15 ± 5.18
	M.l. obtusa	(75μg/100 μl/paw)	73.5 ± 3.39	58.67 ± 3.39	51.17 ± 1.35	23.5 ± 1.45	13.5 ± 1.11
	Carrageenan	(0.1 ml 1%)	14.43 ± 3.42	23.34 ± 2.57	34.35 ± 3.93	44.2 ± 4.90	49.64 ± 4.33
C. calolepis	*M. xanthina*	12.5	39.78 ± 4.34	34.89 ± 4.28	32.43 ± 6.15	31.12 ± 6.35	27.11 ± 5.31
		25	**11.12 ± 2.54**	**13.29 ± 3.71**	**5.87 ± 1.73**	**3.83 ± 1.89**	**2.98 ± 1.31**
		50	15.62 ± 3.81	18.99 ± 5.19	17.00 ± 4.43	16.24 ± 5.75	12.07 ± 3.38
	M.l. obtusa	12.5	35.89 ± 3.54	31.05 ± 5.92	23.31 ± 4.17	16.17 ± 3.84	13.98 ± 4.51
		25	36.43 ± 11.50	35.88 ± 12.22	27.32 ± 10.58	18.88 ± 4.76	15.23 ± 4.08
		50	44.68 ± 6.86	58.10 ± 4.28	38.88 ± 5.65	27.03 ± 3.38	22.55 ± 4.32
	Carrageenan	12.5	**10.59 ± 0.70**	**9.97 ± 1.08**	**6.59 ± 1.24**	**3.24 ± 0.94**	**1.80 ± 0.69**
		25	11.70 ± 7.54	24.15 ± 5.85	11.88 ± 2.31	10.21 ± 1.31	1.67 ± 0.94
		50	**7.82 ± 3.08**	**15.50 ± 5.50**	**9.15 ± 1.55**	**4.97 ± 2.22**	**1.36 ± 0.65**
Cnicin	*M.xanthina*	2.5	37.18 ± 2.73	37.04 ± 3.21	33.83 ± 1.78	32.26 ± 2.56	31.03 ± 1.99
		5	41.77 ± 7.82	38.46 ± 6.87	36.03 ± 8.16	31.47 ± 7.80	28.39 ± 7.09
		10	28.31 ± 7.28	26.17 ± 7.08	23.50 ± 6.83	20.70 ± 6.25	17.51 ± 5.03
	M.l. obtusa	2.5	**27.31 ± 5.18**	**25.72 ± 6.43**	**22.28 ± 5.49**	**19.57 ± 6.31**	**19.10 ± 5.21**
		5	40.36 ± 7.25	33.64 ± 5.09	30.11 ± 4.33	23.85 ± 4.86	20.32 ± 2.87
		10	53.69 ± 9.08	43.63 ± 6.27	33.21 ± 6.78	28.54 ± 6.53	21.96 ± 4.13
Indomethacin	*M.xanthina*	10	38,27 ± 7.56	37,49 ± 6.51	33,45 ± 7.58	27,85 ± 6.07	20,81 ± 5.87
	M.l. obtusa	10	**12.67 ± 6.78**	**12.49 ± 5.67**	**8.92 ± 5.24**	**8.06 ± 4.37**	**7.96 ± 3.72**
	Carrageenan	10	10.03 ± 3.01	19.87 ± 4.03	18.7 ± 3.45	19.99 ± 2.14	16.01 ± 2.59

4. Discussion

By this study it is shown that *Centaurea calolepis* extract and its main compound cnicin have significant effects on inflammatory response induced by prevalent agents. Especially the crude extract formed very strong inhibition at different doses against carrageenan and also *M. xanthina* venom. Suggested treatment of snake bite cases is anti-venom therapy in Turkey. *M. lebetina obtusa* and *M. xanthina* are dangerous Viperidiae species and found in various locations in Anatolia. Their venoms cause necrosis, hemorrhage, pain and local edema (Budak and Göçmen, 2005; Gutiérrez et al., 2007; Igci and Demiralp, 2012). In this study Both of venoms showed induction very quickly, contrary to carrageenan, 1/2 h after injected into the paw and then decreased time-dependent. That's why first half an hour is very important to prevent damage related to inflammation in snake bite cases. So repairing of damaged tissue as primary approach in the treatment is important and anti-inflammatory agents special to snake venoms will be solution in this point.

Centaurea species are used for fever, wound, abscess, rheumatism related to inflammation in traditional medicine in Anatolia (Baytop, 1999; Rıdvan Polat et al., 2013; Sezik et al., 2001). Anti-inflammatory effect of these species was supported by previous bioactivity assays (Erel et al., 2014, 2011; Karamenderes et al., 2007). Studies on anti-inflammatory activity of *Centaurea* species have an increasing attention in recent years (Sokovic et al., 2017). First study performed by Negrete et al. (1993) was based on traditional use of *C. chilensis* and isolated 2 elemanolides, as a mix were administrated *in-vivo* by carrageenan-induced paw edema assay. The mixture inhibited edema significantly comparison with naproxen. They reported activity of sesquiterpene mix was related to α-methylene- γ-lactone ring (Negrete et al., 1993). Cnicin was used in present study, is a germacrenolide sesquiterpene, substituted α-methylene- γ-lactone, may be resulted in anti-inflammatory activity. In another previous study, The relationship between molecule structure and activity of 20 sesquiterpenes was investigated and founded that saturation of 11,13-double bound decreased in activity (Hall et al., 1980). Polysaccharides isolated from *C. cyanus* flowers have been reported 69 percentage of inhibition of carrageenan-induced edema at 60 mg/kg (Garbacki et al., 1999). Koca et al. (2009) reported 200 mg/kg methanol extract of *C. iberica*, distributed in Anatolia, had inhibition at 31.6% by acetic acid-induced capillary permeability assay (Koca et al., 2009). In present study was observed 92.18% of inhibition of carrageenan-induced edema by 50 mg/kg chloroform extract of *C. calolepis* Comparing to study of Koca et al. (2009), chloroform extract, which includes apolar compounds, could be found more effective than methanolic extract. In a previous study performed by us were evaluated *in-vitro* NF-κB and iNOS inhibition activities of 5 *Centaurea* species. Chloroform extract of *C. athoa* was chosen as most effective extract (IC_{50}: 6 μg/ml and 16 μg/ml, respectively) and showed strong anti-inflammatory activity compared to indomethacine at dose of 50 mg/kg by carrageenan-induced paw edema assay (Erel et al., 2014). However, lower doses (12.5 and 25 mg/kg) of *C. calolepis* extract had strong inhibition at 1 h in present study. Cnicin is marker compound of section Acrolophus of genus *Centaurea*. In a previous study, we have isolated it as the major compound of *C. calolepis* (Erel et al., 2011). Schneider and Lachner evaluated that cnicin (27 μmol/kg) inhibited 77% of edema, while indomethacine (28 μmol/kg) reduced 52% (Schneider and Lachner, 1987). It's clear that cnicin influences anti-inflammatory effect of crude extract. Moreover, its activity against snake venom-induced edema was evaluated first time by this study. At 10 mg/kg of cnicin, edema induced by *M. xanthina* venom increased up to 28.31% at 0.5 h, whereas edema increased in indomethacin group up to 38.27%. Chloroform extract, in which cnicin is intense because of its polarity, was used in the study and activity of extract may be related to this compound. Furthermore, activity of the extract was stronger than the pure compound. It may be result of synergistic effect of cnicin and other compounds in chloroform extract.

A snake venom contains mixture of enzymes which cause systemic effects and local lesions during snake bite. The local lesions are resulted from not only venoms, but also inflammatory mediators induced by envenoming (Gutiérrez et al., 2007; Magalhães et al., 2011b).

For this reason, In the case of snakebite, the general finding is local edema and prevention of inflammation may be potential in the treatment of envenomation. First study in Turkey related to inhibition of local effects induced by *M. xanthina* snake venom was performed by our

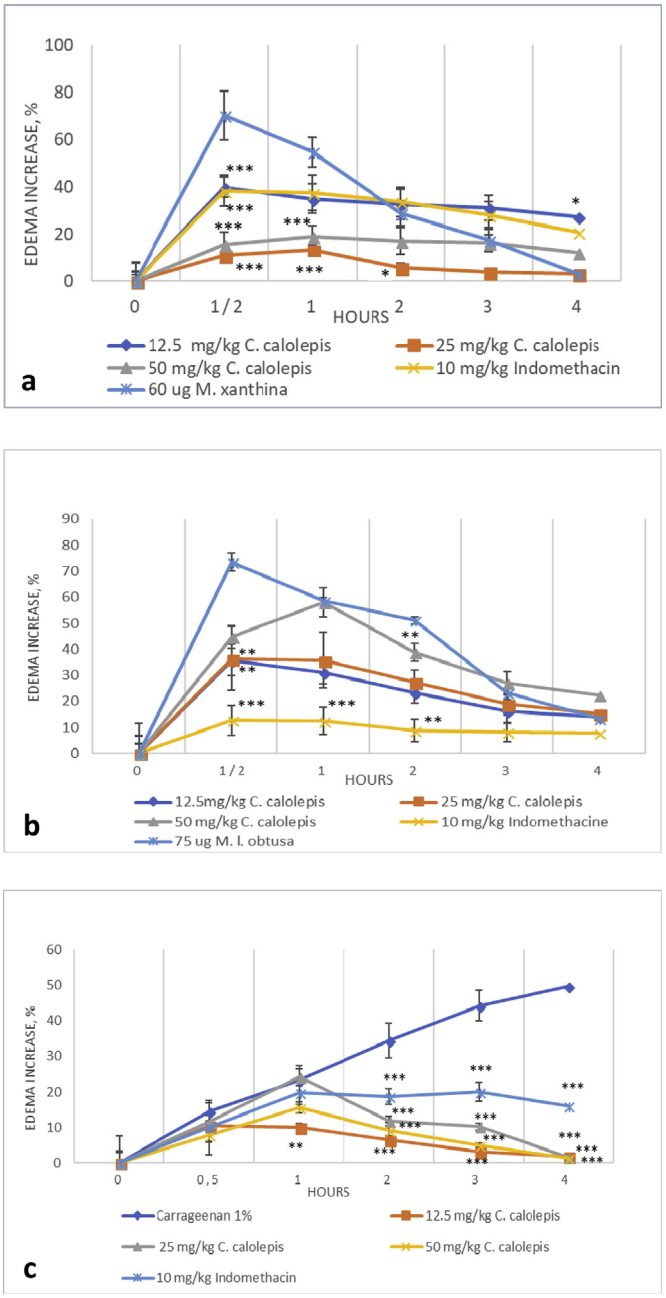

Fig. 2. Effect of *C. calolepis* extract against (*p < 0,05, **p < 0,01 and ***p < 0,001 compared to inducer (*M. l. obtusa, M. xanthina* venoms or carrageenan));*a. M. xanthina* venom-induced edema. *b. M. l. obtusa* venom-induced edema. c. Carrageenan-induced edema.

group by a previous study. 25 and 50 mg/kg of *Artemisia absinthium* methanol extract reduced *M. xanthina*-induced paw edema at 0.5, 1, 2 and 3 h (Nalbantsoy et al., 2013). 50 mg/kg of *C. calolepis* chloroform extract used in this study is more effective in comparison with the same dose of *Artemisia absinthium*. These results also confirm that apolar sesquiterpene groups are more effective than polar groups against snake venoms. Also it is clear *C. calolepis* standardized extracts could be a potential treatment agent to reduce local effects of envenomation by *M. xanthina*. However mechanism of action should be investigated by further studies. Viperidae family snake venoms also have systemic

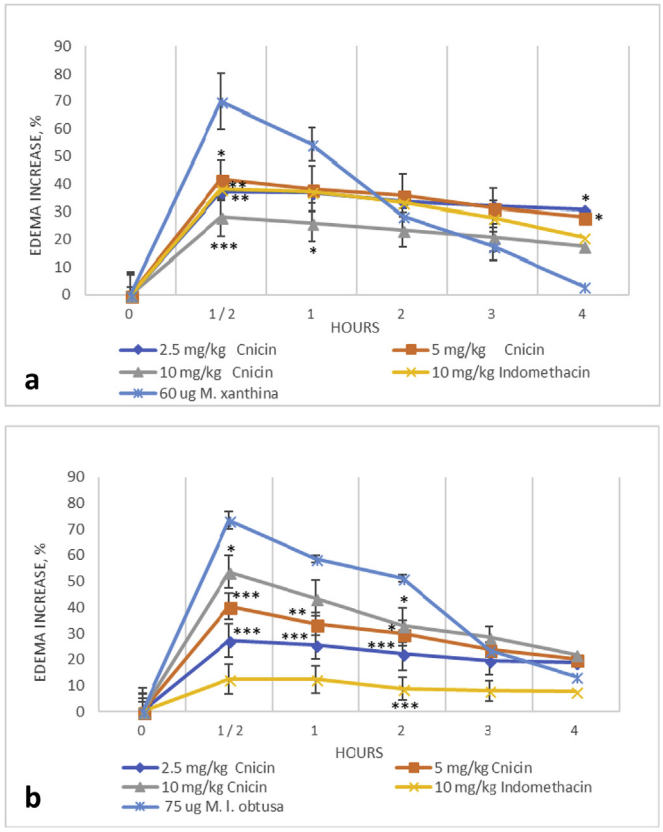

Fig. 3. Effect of cnicin against (*p < 0,05, **p < 0,01 and ***p < 0,001 compared to *M. l. obtusa* and *M. xanthina* venoms); *a. M. xanthina* venom-induced edema. *b. M.l. obtusa* venom-induced edema.

toxicities including haemolytic, proteolytic, neurotoxic and cytotoxic effects that can cause heart stroke, convulsions, shock and death (Okur et al., 2001). Besides the local therapy, *C. calolepis* therapeutic potential on systematic effects caused by M. *xanthina* and *M. lebetina obtusa* venoms could be a topic of further researches.

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Transparency document

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