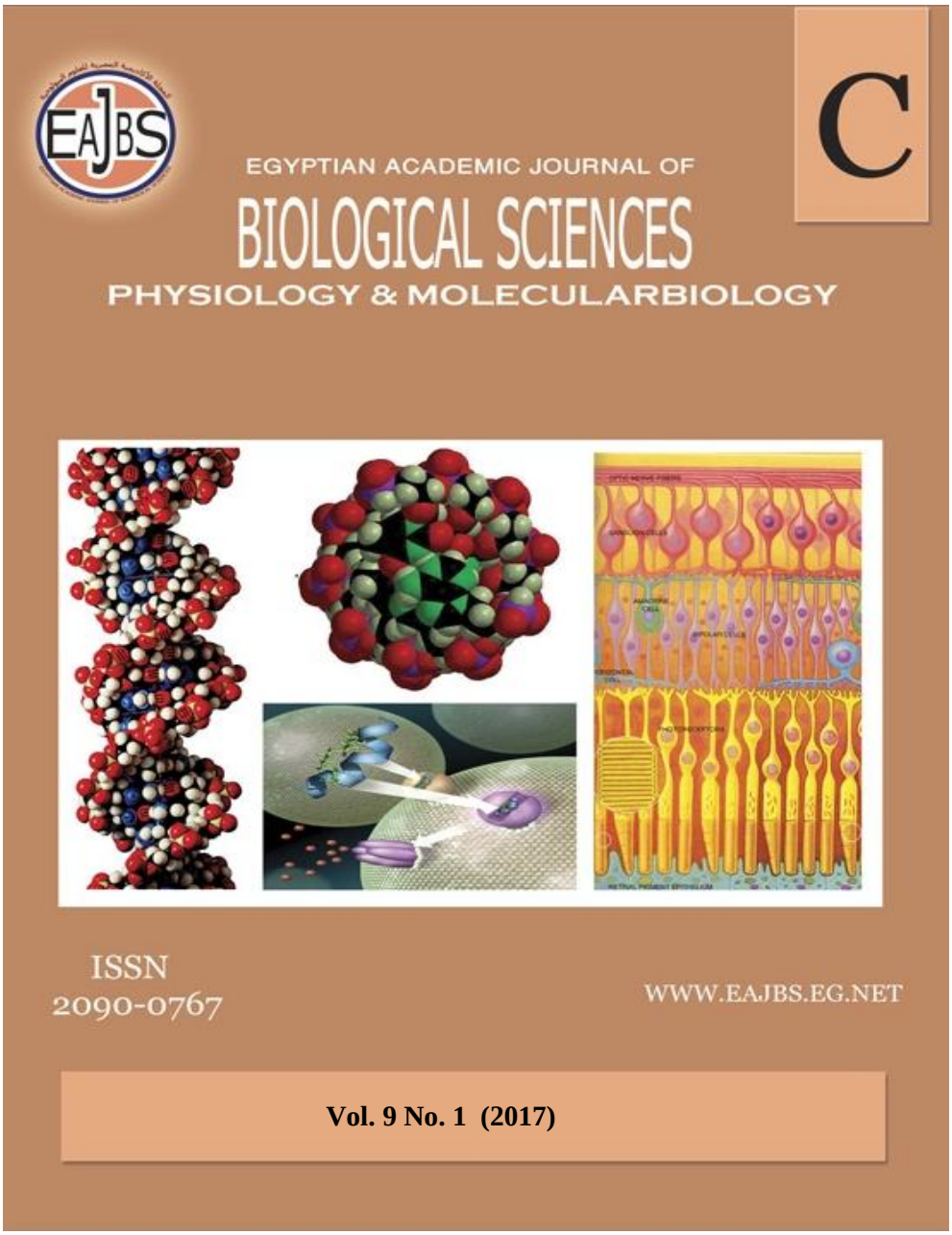

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Analgesic, Antipyretic, and Anti-Inflammatory Activities of *Conus vexillum* Venom

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ABSTRACT

Cone snail venom contains a diverse mixture of biologically active peptides used for predation and defense purposes, by targeting varies classes of ion channel and receptors at sensitive cells with a high level of affinity and selectivity. Some studies showed that some fractions of scorpion and snake venoms have remarkable neuropharmacological effects on various animal models. However, the studies on cone snail venoms in this field are rare, and accordingly; the current study was designed to evaluate the analgesic, antipyretic, and anti-inflammatory activities of the *Conus vexillum* venom. Two doses of crude venom (1/10 and 1/5 LD_{50}, 2.42 and 4.84 mg/kg, respectively) were intraperitoneally injected into the experimental animals. In mice, the peripheral and central analgesic effects were examined using acetic acid-induced abdominal writhing and tail immersion test. Brewer's yeast-induced pyrexia in rats was carried out to determine the antipyretic activity of the same doses. Finally, the anti-inflammatory activity was tested using carrageenan-induced paw edema in rats. The results showed that *C. vexillum* venom produced a significant analgesic activity in acetic acid-induced abdominal constriction response and thermal nociception in mice. Moreover, the venom revealed a significant antipyretic effect on yeast-induced pyrexia. In paw edema, venom showed a significant activity with the highest percentage of inhibition reaching 34.07% and 51.36% on 1/10 and 1/5 LD_{50} doses, respectively after 5-hour treatment, the latter dose almost equal the effect of the standard drug; diclofenac sodium (56.3%). These results revealed potential analgesic, antipyretic, and anti-inflammatory effects of both *C. vexillum* venom doses in various animal models.

INTRODUCTION

All venomous animals can produce venoms consisting of a highly complex mixture of biologically active compounds (Fry *et al.*, 2009). Snake, scorpion, and cone snail venoms became the topic of new studies for understanding biochemical composition, and the mechanisms by which their venoms cause harmful effects, in order to apply their pharmacological activities in the clinical benefits (Lewis and Garcia, 2003).

Unfortunately, despite the large number of venomous animals and the complexity of their venoms, and the recent development of the venomic technological strategies (Favreau and Stöcklin, 2009), only a tiny proportions (estimated to represent less than 0.1%) of venom components have been identified and characterized, and less than 1% of genetic information is available (Ménez *et al.*, 2006; Lewis *et al.*, 2012).

Along with previous studies approved that bee, spider, scorpion, and snake venoms possess fractions with a high degree of pharmacological activities in different animal models (Altawil *et al.*, 2015; Fernandes-Pedrosa *et al.*, 2013; Yoon *et al.*, 2008; Hoang *et al.*, 2014), some other studies demonstrated that *Conus* peptides could exhibit neuroprotective/cardioprotective activities, suggesting that marine snail venoms are a potentially rich source of drug with diverse mechanisms (Twede *et al.*, 2009). There are about 700 species in *Conus* genus, each could produce a unique venom consisting of a rich cocktail of biologically active components; almost all of them are peptides, colloquially known as conopeptides (Norton and Olivera, 2006).

The diversity of that enormous conopeptides number, in addition to their potent biological activities, recently has withdrawn the attention of neuropharmacologists who are seeking after natural products with pharmacological activities that could be used as novel therapeutic agents for human disorders and diseases (Newman *et al.*, 2003). One of these conopeptides was already emerged to medicine market to treat chronic pain (Ziconotide, ω-MVIIA from *Conus magus*) that is a non-addictive pain reliever, 1000 times as powerful as, and possibly a replacement for, morphine (ANI., 2007). Nowadays, dozens of conopeptides are broadly used in the pharmacological research. For example, AVC1 one of many peptides isolated from Australian species *Conus victoriae* has established very effective in treating post-surgical and neuropathic pain and even accelerating recovery from nerve injury (Baby *et al.*, 2011).

Accordingly, the present study was designed to investigate the potential analgesic, antipyretic, and anti-inflammatory effect of crude *C. vexillum* venom on experimental animal models.

MATERIALS AND METHODS

Venom Collection and Preparation

Live specimens of *Conus vexillum* were collected from a depth of 1-2 m from different locations of Marsa Alam, Red Sea, using trawl net, frozen and transported to Zoology Department, Faculty of science, Suez Canal University where they were stored at -20^{o} C. These cone snails were identified (Oliver and Nicholls, 1980) by marine invertebrate specialists at Marine Biology Department, Faculty of science, Suez Canal University. Each specimen was dissected, and a crude extract was prepared from the venom apparatus (venom duct, bulb, and proboscis) as described by (Cruz *et al.*, 1992). Using liquid nitrogen, venom apparatus was grounded to a very fine powder and suspended in 0.1% formic acid after vortex stirring (5000 rpm / 3 min). The sample was centrifuged at 14,000 rpm at 4^{o}C for 10 min, and the supernatant was collected separately. The precipitate was re-suspended and stirred in the same buffer for 10 min, then centrifuged again. Finally, all supernatants were combined, lyophilized, and stored at -20^{o}C.

Experimental Animals

All animals used in the present study and experimental protocol were approved by the Research Ethics Committee of Faculty of Veterinary Medicine, Suez Canal University, and were carried out according to the Guide

for the Laboratory Animals Care. Adult male albino mice (20-25 g) and albino rats (80-100 g) were purchased from the breeding unit of breeding professional Company (Giza, Egypt). Mice and rats were left for one week to adapt to laboratory conditions. They were kept in plastic cages with wire mesh covers. The animals were kept under standard temperature and humidity and fed with standard diet and water *ad libitum*.

Estimation of Median Lethal Dose (LD_{50})

The LD_{50} of *C. vexillum* venom from intraperitoneal injection of mice was calculated according to the method described by (Meier and Theakston, 1986). According to this method, different concentrations (D) of crude venom were injected intraperitoneally into eight weighted mice and then supervising them to record the mortality time (T) for each. The regression line was plotted by using the values of D/T versus D.

Analgesic Activities

Fractions of LD_{50} of *C. vexillum* venom were applied intraperitoneally to mice in order to evaluate analgesic activity using acetic acid-induced writhing test and tail immersion test.

Acetic Acid-Induced Writhing Test

According to the model of (Koster *et al.*, 1959) for peripheral analgesic activity evaluation, 24 healthy male mice (20-25gm weights) were randomly divided into four equal groups, each of six. The 1st control group was intraperitoneally injected with physiological saline (10 mg/kg). The 2nd group received 1/10 LD_{50} (2.42 mg/kg) of *C. vexillum* venom. 3rd group received 1/5 LD_{50} (4.84 mg/kg) of the venom, and 4th group received the commercial standard drug; Aspirin (100 mg/kg IP). After 30 min of previous treatments, abdominal Writhing was induced by intraperitoneal injection of glacial acetic acid (0.6% solution in normal saline, 10ml/kg body weight, Nasr Pharm. Company, Egypt) as described by (Millan, 1994). The number of abdominal constrictions was counted after 5 minutes of acetic acid administration for 15 minutes. As evidence of reduction of writhing percent inhibition of writhing was calculated according to (Koster *et al.*, 1959) in the following formula:

$$\text{Writhing percent \%} = \frac{\text{Control mean - Treated mean}}{\text{Control mean}} \times 100$$

Tail Immersion Test

The lower two-thirds of the tail was immersed in a water bath kept at (55 ± 0.5) °C (Janssen *et al.*, 1963). The time in seconds until tail withdrawal from the water was considered as the reaction time. Mice which had a reaction time less than 4 s were selected. Twenty four albino mice were randomly divided into four groups of six mice each. The reaction time was then measured 1, 2, 3, 4, and 5 hours after i.p. administration of 2 ml/kg saline for group 1 (negative control), 1/10, 1/5 LD_{50} venom for groups 2, 3, respectively, and morphine (3mg/kg) for group 4 (positive control). The mice were exposed to hot water for no longer than 12 s to avoid tissue injury (de Sousa Lira *et al.*, 2002).

Brewer's Yeast-Induced Pyrexia in Rats

To investigate the antipyretic effect of *Conus vexillum* venom, the method described by (Alpermann, 1972) was carried out. 24 adult male albino rats weighting (80-100 gm) were randomly divided into four equal groups, each of six. Fever was induced by injection 10 ml/kg body weight of 20% aqueous suspension of dried Brewer's yeast (*Saccaromyces*

cerevisiae) in physiological saline below the nape of the neck of the rat. The animals then fasted for the duration of the experiment. The initial body temperature was measured rectally with a lubricated digital thermometer after 17 hours of yeast injection to determine the pyretic response to yeast. Different intraperitoneal post-treatment to each group was administered. The 1st group received normal saline (10 ml/kg), to be considered as the control group. The 2nd and 3rd groups were intraperitoneally injected with the venom (1/10 LD_{50} and 1/5 LD_{50} and mg/kg, respectively). The 4th group received the standard drug; metamizole (5 mg/kg).

Carrageenan-Induced Paw Edema in Rats

Anti-inflammatory activity of *C. vexillum* was evaluated using carrageenan-induced rat paw edema (Eldahshan and Abdel-Daim, 2015). In this method, 30 rats (80-100 g b.wt.) were randomly divided into five groups (6 rats each). The 1st group was used as a control and received 10 ml/kg saline. The 2nd, 3rd, 4th, and 5th groups were intraperitoneally injected with saline (10 ml/kg), (4.84 and 2.42 mg/ kg of the venom) and diclofenac sodium (1 mg/kg), respectively. Then, the paw thickness was measured (zero time). One hour later, approximately 50 µL of 1% carrageenan suspension (freshly prepared before the experiment by dissolving 50 mg of carrageenan powder in 5 ml of 0.9% physiological NaCl) was injected subcutaneously into the plantar surface of the right hind paw of each rat. Spontaneously, paw thickness was measured post carrageenan injection, and at one-hour intervals for five hours, using a skin caliber. The anti-inflammatory activity was calculated as percent inhibition of carrageenan–induced paw edema using the (Girard *et al.*, 2008) formula: Inhibition percent = (control mean-treated mean) /control mean ×100 At the end of the experiment.

Statistical Analysis

The obtained data were represented as mean ± standard errors (SE) of 6 animals. The data were analyzed statistically using analysis of unpaired Student's t-test when comparing two group variance, followed by (one-way ANOVA). Statistical significance was considered at $P<0.05$. Statistical Package for Social Sciences (SPSS, 16 ver. for Windows) was used throughout this analysis.

RESULTS

Acute Toxicity Study

The approximate LD_{50} of *C. vexillum* venom was calculated to be 24.2 mg/kg body weight, and 1/5 and 1/10 LD_{50} (4.84 and 2.42 mg/kg) have been used in the pharmacological treatments.

Analgesic Activities

Acetic Acid-Induced Writhing Test

In Figure 1 and Table 1, results of writhing test revealed significant reduction (*P<0.05*) in the writhing behavior after treatment with both doses of *C. vexillum* venom (1/10 and 1/5 LD_{50}) and aspirin, compared with the acetic acid control group. The subsequent calculations of pain inhibition percentage showed that the analgesic activity of 1/5 LD_{50} dose was more effective than other treatments.

Table 1: Analgesic effect of *C. vexillum* venom using acetic acid-induced abdominal writhing in mice.

Treatment	Number of Writhing per 10 min	Pain Inhibition Percentage (PIP)
Acetic acid control	37.67 ± 1.8 [(a)]	
1/10 LD_{50} *C. vexillum* venom	15 ± 1.75*	60.18%
1/5 LD_{50} *C. vexillum* venom	9 ± 0.68*	76.11%
Aspirin (100mg/Kg)	11.17 ±1*	70.35%

(a) Data are presented as Mean ± SE (6 animals/group).
(*) Represents a significant difference between Acetic acid control and treated groups using Student Unpaired t-test (*P<0.05*).

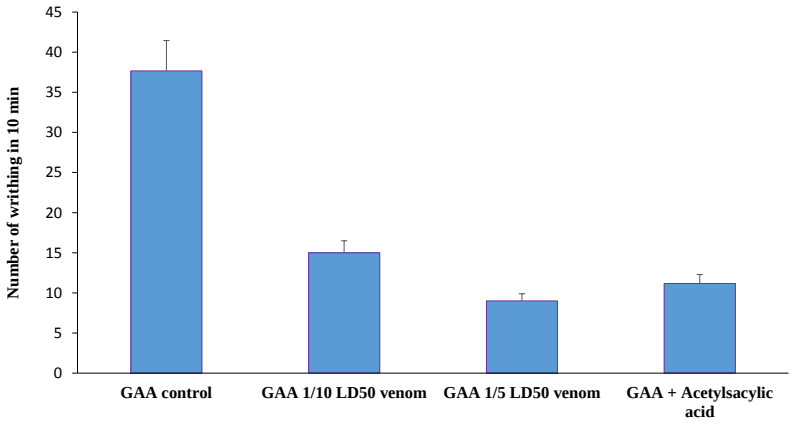

Fig. 1: Analgesic effect of *C. vexillum* venom using acetic acid-induced abdominal writhing in mice. Data are represented as Mean ± SE (6 animals/group).
(*) represent a significant difference between GAA control and treated groups using Student Unpaired t-test (*P<0.05*).

Tail Immersion Test

From Table 2 and Figure 2 of tail immersion test, it is apparent that the tail flick response latency time increased significantly *(P<0.05*) after injection the mice with *C. vexillum* venom (1/10 and 1/5 LD_{50}), regarded to the control group. The most potent effect of these doses was observed two hours post venom treatment. The response time of 1/5 LD_{50} was much closer in all time intervals to commercial morphine values than 1/10 LD_{50} but without clear significance.

Table 2: Analgesic effect of *C. vexillum* venom using tail immersion test in mice

	Response time after drug treatment (seconds)					
Treatment	**0 hr**	**1st hr**	**2nd hr**	**3rd hr**	**4th hr**	**5th hr**
Normal control	1.50±0.34 [(a)]	1.50±0.34	2.13±0.21	1.83±0.17	2.02±0.26	1.83±0.31
1/10 LD_{50} venom	1.67±0.33 (+11.3%)	4.5±0.43 * (+200.0%)	5.83±0.71 * (+173.7%)	3.67±0.33 * (+100.5%)	3.33±0.33 * (+66.5%)	3.00±0.26 * (63.9)
1/5 LD_{50} venom	2.00±0.37 (+33.3%)	5.33±0.33 * (+255.3%)	6.67±0.62 * (+186.3%)	4.67±0.56 * (+155.2%)	3.83±0.17 * (+91.5%)	3.17±0.30 * (+73.2%)
Morphine (3mg/kg)	2.33±0.42 (+55.3%)	7.00±1.03 * (+366.7%)	6.83±1.3 * (+193.1%)	5.67±0.91 * (+209.8%)	4.83±0.75 * (+141.5%)	3.5±0.43 * (+91.2%)

(a) Data are presented as Mean ± SE (6 animals/group). Percentage inhibitions are in brackets.
(*) Represents a significant difference between normal control and treated groups using Student Unpaired t-test (*P<0.05*).

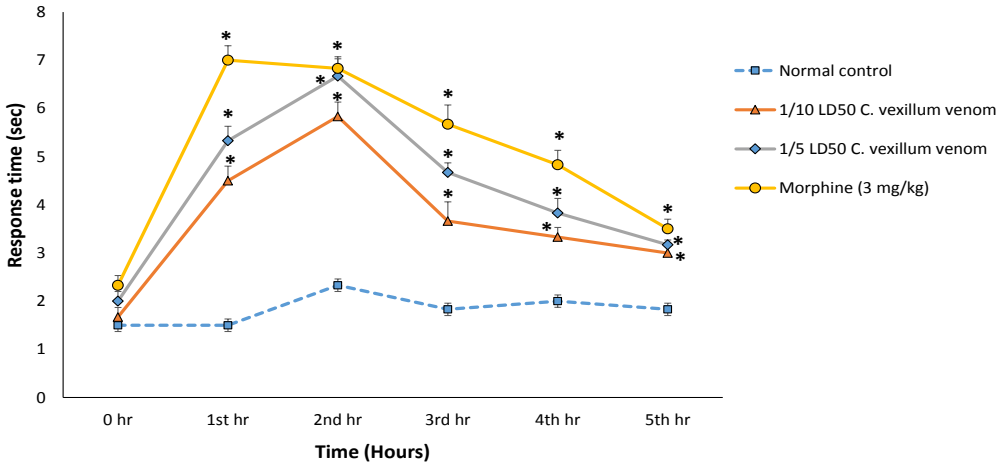

Fig. 2: Analgesic effect of *C. vexillum* venom using tail immersion test in mice.
Data are represented as Mean ± SE (6 animals/group). (*) represent a significant difference between normal control and treated groups using Student Unpaired t-test (*P<0.05*).

Antipyretic Effect of *C. vexillum* Venom

After 18 hours following subcutaneous injection of yeast suspension, marked elevation of the rectal temperature was reported. As illustrated in Figure 3 and Table 3, treatment with *C. vexillum* venom (1/10 and 1/5 LD_{50}) significantly ($P<0.05$) decreased the rectal temperature of the rats, compared to the yeast control group. The beginning of antipyretic effect was observed after the one hour post venom treatment. Interestingly, although both doses showed antipyretic activity without a clear difference to reference drug (metamizol) treated group, but 1/5 LD_{50} was more effective.

Table 3: Antipyretic effect of *C. vexillum* venom using Brewer's yeast-induced pyrexia in rats.

	Average rectal temperature in ^{o}C at time (hr)					
Treatment	**0 hr**	**1st hr**	**2nd hr**	**3rd hr**	**4th hr**	**5th hr**
Yeast control	38.18±0.10 [(a)]	38.48 ± 0.17	38.48±0.12	38.52±0.14	38.53±0.13	38.58±0.13
1/10 LD_{50} venom	38.23±0.076 (0.26%)	37.89±0.049 * (-1.53%)	37.78±0.049 * (-1.82%)	37.95±0.05 * (-1.48%)	38.25±0.076 (-0.73%)	38.45±0.11 (-0.34%)
1/5 LD_{50} venom	38.17±0.067 (-0.026%)	37.77±0.15 * (-1.85%)	37.45±0.16 * (-2.68%)	37.6±0.11 * (-2.39%)	38.02±0.094* (-1.32%)	38.1±0.051 (-1.24%)
metamizol (5mg/kg)	38.05±0.04 (-0.34%)	37.73±0.2 * (-1.95%)	37.26±0.095 * (-3.17%)	37.31±0.15 * (-3.14%)	37.7±0.13* (-2.15%)	37.95±0.022* (-1.63%)

(a) Data are represented as Mean ± SEM (6 animals/group). Percentages of change are in brackets.
(*) represent a significant difference between yeast control and treated groups using Student Unpaired t-test ($P < 0.05$).

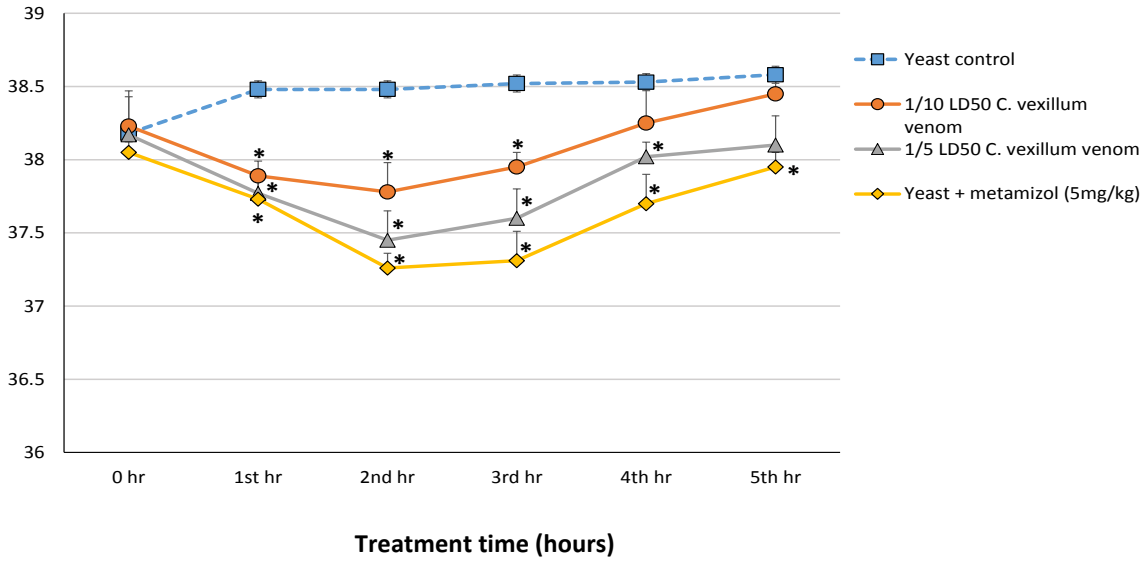

Fig. 3: Antipyretic effect of *C. vexillum* venom using Brewer's yeast-induced pyrexia in rats.
Data are represented as Mean ± SE (6 animals/group).
(*) represent a significant difference between yeast control and treated groups using Student Unpaired t-test ($P<0.05$).

Anti-Inflammatory Effect of *C. vexillum*

In the carrageenan-induced edema test, the paw thicknesses and percentage of inhibition by *C. vexillum* venom (1/10 and 1/5 LD_{50}) and reference drug (diclofenac sodium 20mg/kg) are presented in Table 4 and Figure 4. The dose of 1/5 LD_{50} showed a significant inhibition of paw edema starting from the

first hour, with the percentage of inhibition equals 53.63%, while in the case of 1/10 LD_{50} dose, the significant inhibition of paw edema begun after the 2nd hour post treatment. The anti-inflammatory effect of 1/5LD_{50} was much closer to the effect of diclofenac sodium effect.

Table 4: Anti-inflammatory effect of *C. vexillum* venom using Carrageenan-induced paw edema in rats.

	Thickness of paw (Cm)					
Treatment	**0 hr**	**1st hr**	**2nd hr**	**3rd hr**	**4th hr**	**5th hr**
Normal control	0.29±0.015[(a)]	0.29 ± 0.015	0.29 ± 0.0.15	0.28 ± 0.017	0.29±0.015	0.30 ± 0.018
Carrageenan control	0.28±0.02	0.60±0.018	0.68±0.024	0.72±0.033	0.67±0.04	0.69±0.028
carragnan + 1/10 LD_{50} venom	0.28±0.028 (0%)	0.53±0.044 (-22.71%)	0.56±0.035 * (-30.73%)	0.59±0.027* (-29.52%)	0.56±0.03 * (-27.69%)	0.55±0.032 * (-34.07%)
carragnan + 1/5 LD_{50} venom	0.28±0.025 (0%)	0.43±0.049 * (-53.63%)	0.51±0.039 * (-41.56%)	0.51±0.022 * (-48.05%)	0.47±0.02 * (-44.36%)	0.48±0.024 * (-51.36%)
carragnan + diclofenac sodium (20 mg/kg)	0.29±0.03 (0%)	0.46±0.04 * (-47.0%)	0.48±0.04 * (-53.4%)	0.52±0.048* (-47.37%)	0.49±0.039* (-47.95%)	0.47±0.034* (-56.3%)

(a) Data are represented as Mean ± SEM (6 animals/group). Percentage inhibitions are in brackets.
(*) represent a significant difference between carrageenan control and treated groups using Student Unpaired t-test ($P < 0.05$).

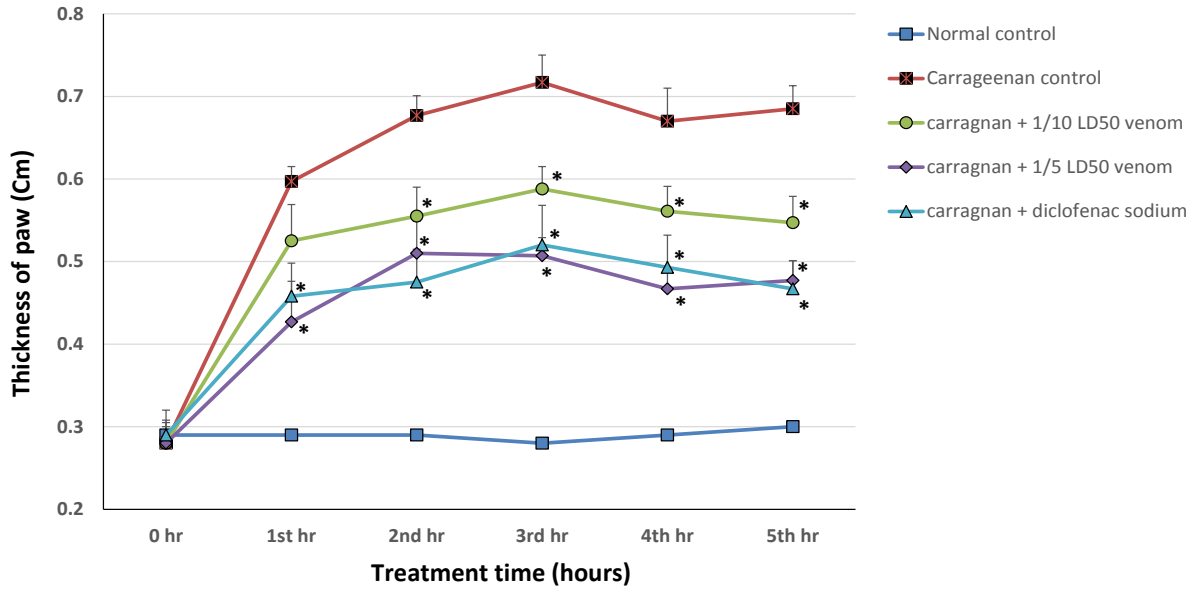

Fig 4: Anti-inflammatory effect of *C. vexillum* venom using Carrageenan-induced paw edema in rats. Data are represented as Mean ± SE (6 animals/group).
(*) represent a significant difference between carrageenan yeast control and treated groups using Student Unpaired t-test ($P<0.05$).

DISCUSSION

Cone snails contain more than 700 species in the single genus *Conus*, in one family, Conidae (Dobson *et al.*, 2012). The marine predator's cone snails use venoms to immobilize their prey. The venoms derived from these mollusks comprise a cocktail of peptides that are called conopeptides and basically target different voltage- and ligand-gated ion channels. Although few of these peptides have been isolated and biochemically identified (Wang and Chi, 2003), they withdrew massive attention during recent years to be pharmacologically examined in order to reveal their neuro/cardio-protective activities, and the findings potently suggest that cone snail venoms

are a rich source of drugs that could be used in diverse mechanisms (Twede *et al.*, 2009). Several studies have indicated that the crude venom from many of *Conus* sp. displayed analgesic activity on treated mice (Balamurgan *et al.*, 2007; Kumar *et al.*, 2014). One peptide (AVC1) isolated from Australian species *Conus victoriae* has received approval in the treatment of post-surgical pain and even accelerating recovery from nervous injury (Baby *et al.*, 2011).

The present study was carried out to evaluate antinociceptive, antipyretic, and anti-inflammatory activities of the crude *C. vexillum* venom on the experimental animal models. Two doses of *C. vexillum* venom were applied to examine the analgesic activity of treated animals in models of peripheral and central pain; writhing and tail immersion test, respectively. The pain induction in the writhing model was achieved by intraperitoneal injection of acetic acid, which cause contraction of the abdominal muscles associated with irritation of peritoneal cavity (Khan *et al.*, 2010a). There are many different pathways for pain generation, one of them occurs via liberating endogenous substances (bradykinin, histamine and serotonin) (Garcıa *et al.*, 2004). Other pain mediators, like arachidonic acid, are released from tissue phospholipids via cyclo-oxygenase (COX), that is activated through prolonged irritation of acetic acid in peritoneal fluids, causing production of prostaglandin, specifically PGE2 and PGF2α (Khan *et al.*, 2010b). Our results of writhing test revealed that there is a significant reduction in the writhing behavior after treatment with *C. vexillum* venom, nearly similar to the analgesic effect of standard drug aspirin. Accordingly, like any analgesic substance (Duarte *et al.*, 1987), the venom could perform a peripheral analgesic activity of killing pain and inhibit the writhing preferably by preventing the prostaglandin synthesis. Similarly, in tail immersion test, it is apparent that the tail flick response latency time increased significantly after injection the mice with *C. vexillum* venom, compared to the control group, indicating that this venom could also induce the central analgesic mechanism.

In a similar study, Kumar (Kumar *et al.*, 2014) reported that crude venom of *Conus lentiginosus* had potent analgesic activity, about as much as three times more than that of paracetamol. While Marwick (Marwick, 1998) reported that *Conus magus* venom had an analgesic effect 1,000 times stronger than morphine. Sakthivel (Sakthivel, 1999) studied the analgesic property of *Conus lentiginosus* and *C. mutabilis,* which was 128 times more than that of paracetamol. The most common example stands as ν-conotoxins that inhibit N-type calcium channels ($Ca_v2.2$) and are commonly used to block these channels in a wide array of physiological preparations. The mechanism of inactivation of N-type calcium channels via G protein-coupled ($GABA_B$) receptor was thought to be the principal mechanism of analgesic action (Callaghan and Adams, 2010; Zheng *et al.*, 2011; Adams and Berecki, 2013). Moreover, recent studies have focused on μ-conotoxins such as μ-KIIIA and μ-SIIIA, which preferentially block neuronal subtypes (NaV1.2) over skeletal (NaV1.4) and cardiac (NaV1.5) muscle subtypes. These peptides have attracted considerable interest because of their potent analgesic activity in animal models of pain (Zhang *et al.*, 2007).

Pathogenic fever or hyperthermia is a directed rise of body temperature over the typical range, and is considered as one of the host protection mechanisms against disease, tissue damage, and inflammation (Pasin *et al.*, 2010). In order to induce hyperthermia, the yeast is injected subcutaneously, leading to increased synthesis of prostaglandin, which finally increases the body temperature (Hess and Milonig, 1972). It

is well known that pyretic regulatory mechanisms are controlled by specific area within the hypothalamus that secret prostaglandins within the central nervous system (CNS) and release them through the blood-brain barrier (BBB) (Zakaria *et al.*, 2008). This study revealed that the treatment with *C. vexillum* venom significantly decreased rats' body temperature, compared with the yeast control group, and this antipyretic effect was detected without a clear difference when compared to the reference drug (metamizole) treated group. The revealed results suggest that the cone snail venom that contains many neurotoxic peptides could penetrate the blood-brain barrier and prevent the prostaglandin synthesis causing the apparent antipyretic activity. The mechanism of antipyretic drugs (Khan *et al.*, 2010b) was explained by blocking the cyclooxygenase enzyme activity leading to decreased levels of PGE2 in the hypothalamic region.

The inflammation is a defensive response that takes place in the living tissues and their microcirculation against a pathogenic injury includes physical, chemical, and infectious insults (Walport and Duff, 1993). There are different stages of inflammation, each is characterized by the synthesis of some mediators, as histamine, 5-hydroxytryptamine, bradykinin, prostaglandins and leukotriense, and movement of fluid and leucocytes from the blood stream to extravascular tissues, which gives rise to the four cardinal signs of inflammation, redness, heat, swelling, and primary hyperalgesia (Levine and Reichling, 1999).

Generally, to evaluate the anti-inflammatory activity of therapeutic agents, or the anti-edematous effect of natural products, carrageenan-induced paw edema is an acceptable frequently-used and well established method for many animal models (Asres *et al.*, 2005) (Tian and Row, 2011), and applied to this study specifically for *C. vexillum* venom. Carrageenan is a sulfated muco-polysaccharide derived from Irish Sea moss, *Chondrus sp.* (Chattopadhyay *et al.*, 2012). Carrageenan-induced rats paw edema is found to be biphasics, the early phase (4.5 times increase in paw volume) is due to release of histamine, 5HT, serotonin and kinin in the first hour after the administration of carrageenan, and after 2-3 hours, a more pronounced late phase (three times increase in paw volume) is attributed to the release of prostaglandin, bradykinin, proteases and lysosome-like substances, followed by an automatic regression of inflammation. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents, that could inhibit and inactivate the late phase mediators either alone or in combination (Chattopadhyay *et al.*, 2012).

C. vexillum venom inhibited mice paw thickness after carrageenan injection compared an anti-edematous drug as diclofenac sodium. The anti-inflammatory effect of *C. vexillum* venom started early (one-hour post carrageenan injection) in the both doses. The results of pre-treatment of *C. vexillum* venom demonstrated that this venom is effective in the early phase of inflammation that reported in control group, and could be regarded to production inhibition of the early-phase mediators like prostaglandin. This finding is further supporting of those of the earlier studies that proved the anti-inflammatory effect of different venoms, as *Tityus serrulatus* scorpion venom (Nascimento *et al.*, 2005), *Heterometrus laoticus* scorpion venom (Hoang *et al.*, 2014), *Naja nubiae* spitting cobra venom (Altawil *et al.*, 2015), *Thalassophryne nattereri* fish venom (Ferreira *et al.*, 2014), and Bee's venom (Yoon *et al.*, 2008).

CONCLUSION

This study showed a potential analgesic, antipyretic, and anti-

inflammatory effect of *C. vexillum* venom in various animal models. Further studies are required for the venom active compounds isolation and characterization in order to clarify their specific mechanism of action.

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ARABIC SUMMARY

التأثيرات المسكنة للألم والخافضة للحرارة والمضادة للإتهاب لسم القوقع المخروطي كونس فيكسيلم

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يحتوي سم القواقع المخروطية على خليط متنوع من الببتيدات النشطة حيويا والتي يستخدمها هذا الجنس من القواقع البحرية في الإفتراس أو الدفاع عن نفسه, عن طريق الاستهداف المحدد والدقيق للمستقبلات والقنوات الأيونية على أسطح خلايا الحيوان المحقون بالسم. وبالرغم ان هناك العديد من الدراسات التي أثبتت التأثيرات الدوائية العصبية لكثير من مكونات سموم حيوانات أخرى مثل العقارب والثعابين على العديد من نماذج حيوانات التجارب, إلا أنه لا توجد دراسات مماثلة على سموم القواقع المخروطية, ومن هنا كان هدف البحث لدراسة التأثير المسكن للألم والخافض للحرارة والمضاد للإلتهابات لسم القوقع المخروطي كونس فيكسيلم. وقد تم استخدام جرعتين مختلفتين في هذه الدراسة (خمس وعشر الجرعة النصف المميتة, ٤,٨٤ و ٢,٢٤ مجم/ كجم وزن جسم على التوالي) عن طريق حقنهما في التجويف البريتوني لحيوانات التجارب. تم دراسة تأثير السم المسكن للجهاز العصبي الطرفي والمركزي للفئران عن طريق تحفيز التقلصات البطنية بواسطة حمض الخليك وتجربة غمر الذيل في الماء الساخن, على التوالي. بينما تم دراسة التأثير الخافض للحرارة على الجرذان من خلال تجربة برودار المحفزة لرفع درجة الحرارة بواسطة الخميرة, والتأثير المضاد للإلتهابات باستخدام الكاريجنان المحفز للإستسقاء في بطن قدم الجرذان. وقد أفضت النتائج بأن لسم القوقع تأثير مسكن للألم ذو دلالة إحصائية معنوية على التقلصات البطنية والحس الألمي على ذيل الفئران المغمور بالماء الساخن عند مقارنته بالمجموعات الضابطة. كما أثبتت النتائج بأن السم يملك تأثير ذو مغزى إحصائي معنوي كخافض للحرارة ومضاد للإلتهاب إذا ما قورن بالمجموعات الضابطة. وكانت تلك التأثيرات الدوائية أكثر قوة ووضوحا عند استخدام خمس جرعة السم النصف المميتة وأقرب لتأثيرات الأدوية المرجعية. ومن هذه الدراسة يمكن الاستنتاج بأن سم القوقع المخروطي كونس فيكسليم له تأثيرات مسكنة للآلام وخافضة للحرارة ومضادة للإلتهابات على حيوانات التجارب.