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# *ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF ETHANOL EXTRACT* OF *DRACAENA CINNABARI BALF, AS ENDEMIC PLANT IN YEMEN*

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## ABSTRACT

*Dracaena Cinnabari balf* is species plant in agavaceae family. It is tree endemic to the Island Socotra (Yemen). The resin of this tree, dragon's blood is known in Arabia as « dammalachawin » or Cinnabari. It has been used in traditional medicine for the treatment of gastric sores diarrhea, dysentery, as haemostatic and as anti ulcer remedy, and anti spasmodic, analgesic and anti inflammatory. In the present study, we evaluate the anti-inflammatory and analgesic activities of ethanolic extract obtained from *Dracaena cinnabari balf* resine, using chemical and thermal models which will induce acute pain and inflammation in animal models. The results showed the ethanolic extract has analgesic potential with 56.93% at 50mg/kg and with 67.79% at 150mg/kg as compared to aspirin 44.11% at 200 mg/kg *b*ody weight. As well as significant reduction in inflammation at the doses (50 and 150 mg/kg, p.o) ($P<0.001$) at 3 hours by two method. The results show that the ethanolic extract have not central analgesic effect and have peripheral analgesic as anti –inflammatory activities, supporting the traditional application of this plant in treating various diseases associated with inflammation and pain.

## KEYWORDS

Medicinal plant; *Dracaena cinnabar*i; ethanolic extract; analgesic; Anti inflammatory

## INTRODUCTION

Phytotherapy represents one of the most important fields of traditional in Yemen especially in the Island Socotra. We selected this part of Yemen because this Island is undoubtedly a most precious natural asset not only for the republic of Yemen but also for the whole world and human kinds. 237 plants of about 850 plants are considered to be endemic .The selection of these plants for evaluation was based firstly on wide use for medicinal purposes and secondly on the occurrence as endemic plants on Island Socotra[1, 2]

Many plants are used in folk medicine to treat gastrointestinal disorders ,such as spasms or indigestion and also many people nowadays turn to the use of natural product medicine for treatment of intestinal disorders, Natural products have served as a source of medicines for centuries ,and about half of the pharmaceuticals in use today are rerived from natural products[3]. Dependence on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health care [4]. *Dracaena Cinnabari* balf is a species plant in agavaceae family. It is a tree endemic to the Island Socotra (Yemen) [5].

The resin of this tree, dragon's blood is known in Arabia as « dammalachawin » or Cinnabar. It has been used in traditional medicine for the treatment of gastric sores [6], diarrhea, and dysentery, as haemostatic and as anti ulcer remedy, and anti spasmodic, analgesic [7]. People in Socotra used the resin from dracaena cinnabari for dying wool, glue pottery, breath freshener, to decorate pottery and houses and even as lipstick [8].

External application of it could stop bleeding, promote wound healing, and is mainly used for various skin or mucosal disease [9]. The pharmacological evaluations showed that this plant has anti microbial and cytotoxicity effect [2, 10.] Some constituents of Dracaena cinnabari have been identified: Dracophan, ametacyclophan, Cinnabaron, Abiflavonoids, Numerous phenolic compounds belong to the homoisoflavonoids and chalcons, Sterol, triterpenoids and anew biflavonoids were isolated from this plant. Thus, in the present study attempts were made to investigate its anti-inflammatory and analgesic activities with a view of justifying the use of this plant in treatment of inflammatory diseases and its use as a local analgesic[11, 12, 13, 14, 15].

## MATERIAL AND METHODS

### *(i) Plant Material:*

Dragon's blood from Dracaena Cinnabari was collected from Socotra Island of Yemen on January 2008. A voucher specimen of resin N: (76711) is deposited in the herbarium of Botany Department of Scientific Institute of Rabat.

### *(ii) Preparation of extracts:*

The powdered resin was successively extracted with ethanol by maceration at room temperature (25C°) over period of 48 hours and were shaken several times .10 g of resin material and 100 ml of ethanol were used in extraction, ethanol containing the extract was then filtered through Whitman paper rand the solvent was vacuum distilled at 65 C° in rotary evaporator. Final extract was red semi-solid in percentage dry weight 90%. This ethanol extract was kept at 4 c until use and the extract was dissolved in (distilled water +drops of Tiween 80) before use

### *Animals:*

Male Swiss mice (20-28g) (Offa-Credo,

France) and Wistar male rats (200-250g) were used in the pharmacological tests (analgesic and anti-inflammatory activities). The animals were acquired from the animal centre of Mohammed V-Souissi University, Medicine and Pharmacy Faculty-Rabat. All animals were kept in a room maintained under environmentally controlled conditions of 23 ±1C° and 12 h light-12 h dark cycle. All animals had free access to water and standard diet. They were acclimatized at least one week before the experiments were started. The animals submitted to oral administration of the extracts or drugs were fasted for 18 h before the experiment (water was available).

***(iv) Analgesic activity:***

The evaluation of the analgesic activity of the ethanol extract of the resin of Dracaena cinnabari balf was carried out by using two different methods that used thermal stimuli (Tail flick test), and chemical stimuli (Koster test).

***(A) : Acetic acid-induced writhing response in mice***

The method used in this test has been described by Koster [16]. The total number of cramps following intraperitoneal administration of acetic acid solution (3% with 300 mg /kg i.p) was recorded over a period of 20 min, starting 5 min after acetic acid injection, the mice were treated with ethanolic extracts of Dracaena cinnabari balf (50 and 150 mg/kg, p.o) or standard drug (aspirin, 200 mg/kg, p.o) , 30 min before administration of acetic acid, the number of cramps and stretching was recorded and permitted to express the percentage of protection using the following ratio (control mean-treated mean) x 100/control mean [17, 18].

***(B): Tail flick test***

The procedure is based on the observation that morphine like drugs are selectively prolonging the reaction time(in second) of the typical tail withdrawal reflex in rats induced by immersing the end of the tail about (4-5 cm) in warm water of 55 C° [1, 19, 20]. Morphine (5 mg/kg s.c), was used as positive control and *Dracaena cinnabari balf ethanolic* extract was administrated (100, 150 mg/kg; p.o.) The tail withdrawal reflex was recorded before and after 15, 30, 60 and 120 min following oral route administration of the ethanolic extract of *Dracaena cinnabari balf* to different groups of six animals.

***(v) Anti inflammatory activity***

The evaluation of the anti-inflammatory activity of *Dracaena cinnabari balf* was carried out by using two different methods that used chemical stimuli (Winter test[21]), and mechanical stimuli (Riesterer and Jaques test) [22] induced paw oedema in rats. In both methods, all animals were fasted 18 hours before testing and received 5 ml of distilled water by gavages to minimize individual variations in response to the swelling of the paws. The right hind paw (RP) is not treated, and it is taken as control.

**% of inhibition=mean [v $_{\text{Left}}$ −v $_{\text{Right}}$] $_{\text{control}}$ - [v $_{\text{Left}}$ −v $_{\text{Right}}$] $_{\text{treated}}$ / [v $_{\text{Left}}$ −v $_{\text{Right}}$] $_{\text{control}}$ × 100.**

V Left means volume of oedema on the left hind paw and v Right means volume of oedema on the right hind paw.

***(A): Carrageenan –induced rat paw oedema***

Carrageenan –induced paw inflammation was produced according to previously described method [21, 23]. One hour after oral administration of the extract (50 and 150 mg. /kg, p.o), reference drug (indomethacin, 10 mg/kg, p.o), an injection of 0.O5ml of carrageenan (1% carrageenan suspended in 0.9% NaCL) was made in to the left hind paw of each rat under the sub-plantar aponeuvrosis .Measurement of paw size was done by mean of volume displacement technic using plethysmometer Digitals 7500 immediately before carrageenan injection and 1h30, 3h and 6 h after carrageenan injection.

***(B): Experimental trauma induced paw oedema in rat***

The anti-inflammatory activity was evaluated according to previously described method[22]. For the experiment the male wistar rats (180-220g) were divided into different groups (n=6). The control group received (5ml/kg of distilled water p.o.), the standard group received the reference drug (indomethacin 20mg/kg, p.o.), and the test group received different concentration of ethanolic extract of *Dracaena cinnabari balf* (50 and 150 mg/kg, p.o). One hour after oral administration of different substances, dropping a weight of 50 gm to all animals on the left hind paw[22, 24]. The right hind paw is not treated; it is taken as a witness. The difference in the foot pad volume between the left and right foot was measured and taken as the edema value by using a plethysmometer Digitals 7500 at 1h30, 3h and 6h after induction of inflammation[24].

***(vi): Statistical analysis:***

The results were reported as mean±S.E.M and analyzed by one-way Analysis of Variance (ANOVA) followed by student's t-test. A value of $p<0.05$ or 0.01 was considered significant.

## RESULTS

Effect of ethanolic extract on acetic acid-induced writhing reflex in mice as shown in( Fig.1)The ethanol extract (50mg/kg and 150 mg/kg) of *Dracaena cinnabari balf* restrained the writhing reflex induced by acetic acid with an inhibition percentage of (56.93%,67.79) respectively- The positive drug Aspirin also significantly inhibited the writhing response 44,11**%(**Fig.2.)**.**

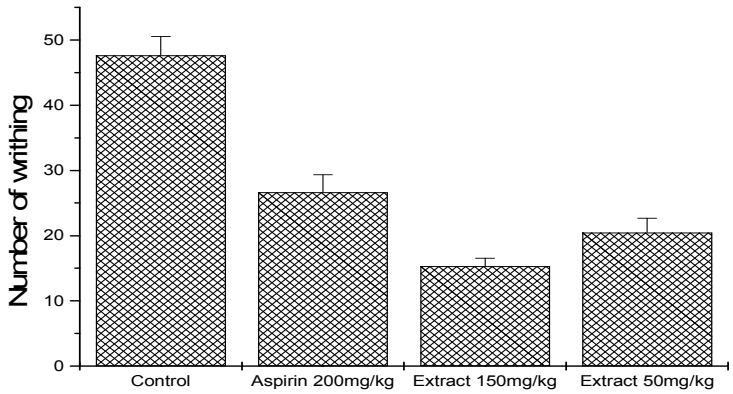


**Figure .1.**

***Effect of ethanol extract of Dracaena cinnabari balf and aspirin on acetic acid-induced writhing reponse in mice. Comparisons are made with control groups. Values are expressed as mean ±S.D. (n=6). Symbols represent statistical significance at P<0.001***

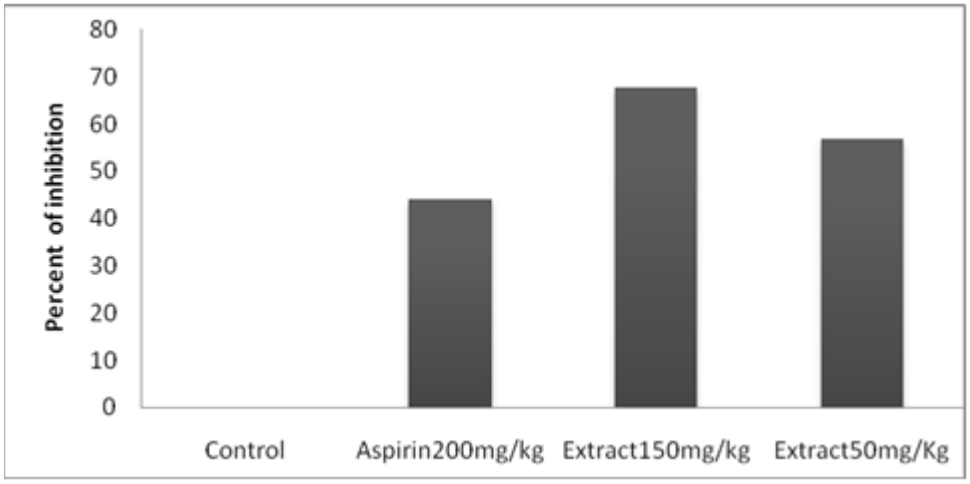


**Figure. 2**

***Effect of ethanol extract of Dracaena cinnabari balf and aspirin on acetic acid-induced writhing response in mice.***

***Effect of ethanolic extract on Tail Flick test:***

In the TAIL Flick test, the analgesic effects were observed (Fig .3), indicating that the ethanol extracts of D.C.C-R could not increase the animal reaction time to the heat stimulus and showed weak analgesic activity with (50 and 150mg/kg, p.o) compared to the control. While the latency time was significantly increased by morphine (5mg/kg) at 45 and 60 min.

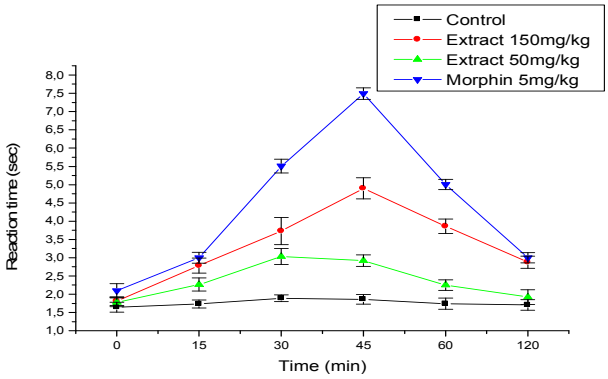


**Figure. 3 .**

***Central Analgesic activity of ethanolic extract of Dracaena cinnabari balf by (Test TAILflick)***
***Each value represents the mean ± SEM (n = 6) p<0.001, statistically significant relative to control at 45 min.***

***Effects of ethanolic extract on carrageenan – induced rat paw oedema***

The results are presented in( Fig .4)The ethanol extract (50mg/kg and 150 mg /kg , p.o)showed reduction in the carrageenan-induced paw oedema in rat at 1h30, 3h and 6 h after carrageenan injection with(41.84% ,48.20% ,47.70%)of the inhibition rat ,respectively at dose 50mg/kg and with( 71.57% ,72.47% ,71.12%) of the inhibition rat, respectively at the dose 150mg/kg .Indomethacin (10mg/kg, p.o)also inhibited paw oedema in rat after carragenan injection with69.74% ,75.21%, 63% of the inhibition rat ,respectively(Fig .5).

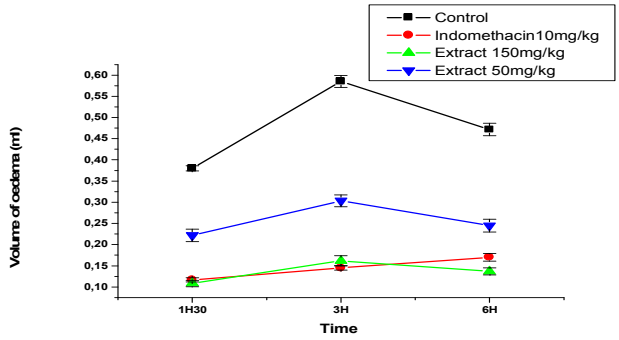


**Figure. 4** .

***Effects of ethanolic extract of Dracaena cinnabari balf on carrageenan –induced rat paw oedema. Each value represents the mean ± SEM (n = 6) p<0.001, statistically significant relative to control at 3 hours.***

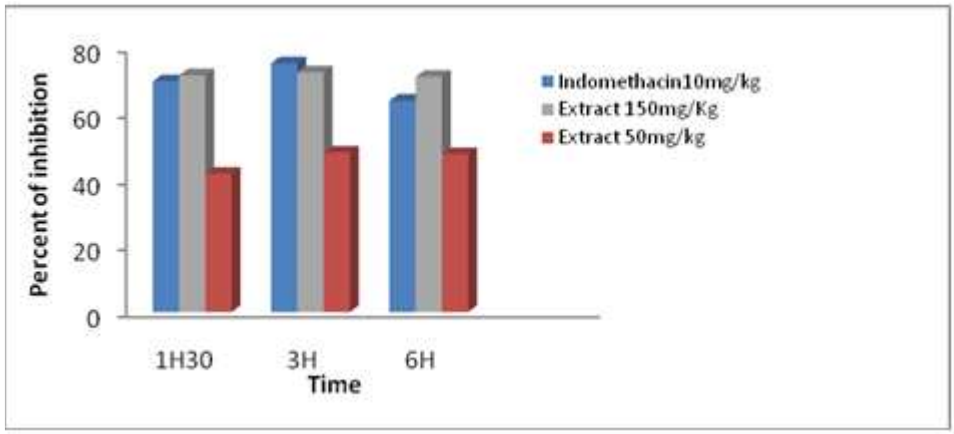


**Figure .5.**

***Effects of ethanolic extract of Dracaena cinnabari balf on carrageenan –induced rat paw oedema***

***Effects of ethanolic extract on experimental – induced rat paw oedema***

The results are presented in (Fig. 6). The ethanol extract (50 and 150 mg/kg, p.o) showed reduction in the experimental -induced paw edema in rat at 1h30, 3h and 6h after experimental –induced rat paw oedema with 38.79% , 45.84%, 40.38% of inhibition rat respectively at dose 50mg/kg and with 66.15%, 67.33%, 64.70% of inhibition rat ,respectively at dose 150mg/kg. Indomethacin (10mg/kg, p.o) also inhibited paw oedema in rat after experimental –induced rat paw oedema with 81.52%, 84.81%, and 74.50% of the inhibition rat .respectively (Fig.7).

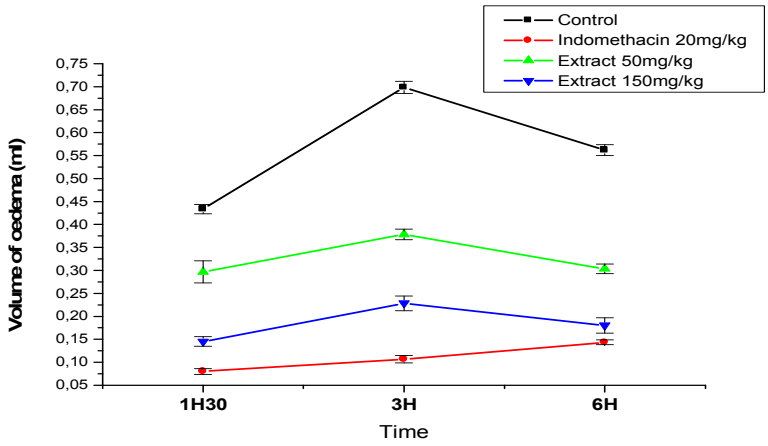


**Figure.6.**

***Effect of ethanolic extract of Dracaena cinnabari balf on experimental –induced rat paw oedema .Each value represents the mean ± SEM (n = 6) p<0.001, statistically significant relative to control at 3 hours.***

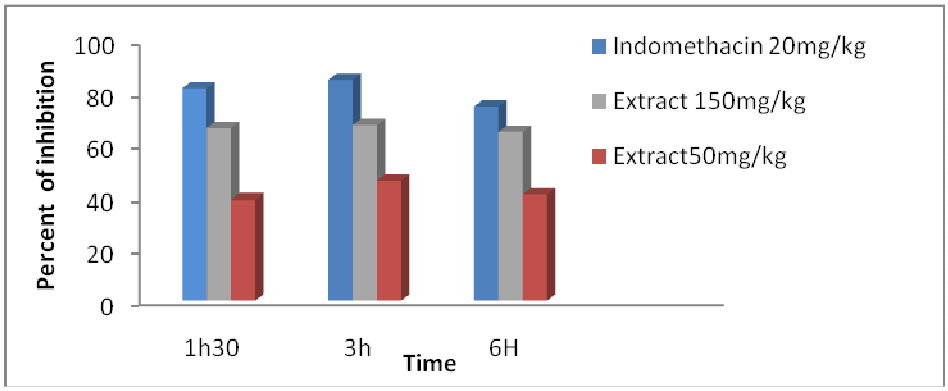


**Figure.7.**

***Effect of ethanolic extract of Dracaena cinnabari balf on experimental –induced rat paw oedema.***

## DISCUSSION

The anti-inflammatory and analgesic effects of the ethanol extract of D.C.B-R were investigated in this study. The analgesic activities were evaluated using two animal models .The Tail flick test was selected to investigate central analgesic activity. Acetic acid-induced writhing response was selected to observe peripheral analgesic effects. In the acetic acid –induced writhing test, the ethanol extract demonstrated a significant analgesic effect, inhibiting pain by 56.39%, 67.79% as compared to the control, respectively (Fig.2).

In the Tail Flick test ,the analgesic effects were observed (Fig.3),indicating that the ethanol extracts of D.C.B-R could not increase the animal reaction time to the heat stimulus and showed weak analgesic activity with( 50 and 150mg/kg, p.o )compared to the control. It is known that, abdominal constriction response is very sensitive and able to detect anti-nociceptive effect of extracts or compound that may appear inactive in other methods like tail-flick test local peritoneal receptors are postulated to be partly involved in abdominal writhing response[25, 26].

The mechanisms of the reaction to this nociceptive stimulus seem to be related with prostanoid system. Experimental results obtained by several researchers indicated increased levels of lipoxygenase product[27]. Related studies have demonstrated that acetic acid indirectly induces the release of endogenous mediators of pain (such as prostaglandin, kinin, histamine,…. etc) that stimulate the nociceptive neurons, which are sensitive to non-steroidal anti-inflammatory drugs and opioïds [28]. These observations suggest that, the ethanolic extract of *Dracaena cinnabari balf* have a significant inhibitory activity in inflammatory pain, and this activity may be related with the suppression of synthesis and/or release of endogenous pro-inflammatory substances[29]. Inflammation responses occur in three distinct phases and apparently mediated by different mechanisms: acute phase by local vasodilatation and increase capillary permeability, subacute phase by infiltration of leukocytes and phagocytic cells and chronic proliferative phases, in which tissue degeneration and fibrosis occur[30, 31]. Accordingly, anti-inflammatory tests are divided in to those measuring acute inflammation, subacute inflammation and chronic repair processes[32, 33]. Carrageenan-induced oedema falls in the category of acute inflammation, which involved the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever[34, 35]. The paw oedema induced by carrageenan and experimental test has been extensively studied in the assessment of anti-inflammatory action involving several chemical mediators such as histamine, serotonin, bradykinin, and prostaglandins[24, 36, 37].The results show that the ethanol extract of D.C.C-R inhibited edema of the paw induced by carrageenan in rats (Fig.4, Fig.5).The ethanol extract at an oral dose of 50mg/kg and 150 mg/kg can inhibit paw oedema. These results indicate that the ethanol extract of D.C.C-R exert significant anti-inflammatory activity, especially in the acute phase of inflammation .The activity may be related to the inhibition of inflammation's chemical mediators.

Vascular permeability was induced by acetic acid, which was another acute inflammatory model. Acetic acid can cause the increase of chemical mediators such as prostaglandin =E2 (PGE2), histamine and serotonin in peritoneal fluids, leading to the increase in vascular permeability[38]. The ethanol extract (50 and 150mg/kg, p.o) significantly inhibited the acetic acid-induced increase in vascular permeability in mice. These results suggest that D.C.C-R extract exerts an anti-acute inflammatory action .The mechanism may be due to inhibiting the inflammatory mediators. Several compounds have been separated from D.C.B-R, which were identified as: Dracophan, ametacyclophan [11], Cinnabaron, Abiflavonoids [12], Sterol, triterpenoids and anew bioflavonoid [14] and anew bioflavonoid [15].

The presence of flavonoides with tri trepenoids in the extracts may enhance the analgesic effect by inhibition of COX and lipo-oxygenase enzymes[39] and this property has been observed in quercetin, luteolin, kaempferol and bioflavonoid[40]. The analgesic and anti inflammatory activity is a common property of many terpenoids and sterols[41, 42]. As such, the observed analgesic and anti-inflammatory properties of ethanol extract of D.C.B-R in the present study may be due to the presence of photochemical compound. Therefore, it is concluded that D.C.B-R extract are capable of inhibiting inflammatory reaction as well as pain. The results provided experimental evidence for it is traditional use in treating various diseases associated with inflammation and pain.

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***Abbreviations***

D.C.B-R:*Dracaena cinnabari balf* - Resin, i.p:intraperitoneal, RP : right paw, LP : left Paw, A SA : Acetyl Salicylic Acid*, COX ; cyclooxygenase, PG:prostaglandnin*

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