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Assessment of anti-nociceptive and anti-inflammatory potentials of *Dracaena Reflexa* leaves

Pravalika Kuchana
Department of Botany, Osmania University, Telangana, India, 500007.

Sujatha E
Department of Botany, Osmania University, Telangana, India, 500007.
Corresponding author email: sujatha@osmania.ac.in

Abstract*---Dracaena reflexa*** (family: Asparagaceae), commonly known as Song of India is a tropical plant that is traditionally claimed to have high medicinal values. The current exploration was intended to assess the pain-relieving and anti-inflammatory potentials of methanolic extract of *Dracaena reflexa* (MEDR) leaves in rodents. Acute oral toxicity studies were performed for the MEDR as per OECD guidelines 423. Anti-inflammatory action was assessed by the carrageenan incited paw edema method. The anti-nociceptive effect was explored by using acetic acid incited writhing responses, tail immersion, and hot plate technique in rodents. Acute oral toxicity investigation for 14 days reveals the safety of MEDR with no mortality at 2000 mg/kg. Hence 200 & 400 mg/kg was selected for in vivo studies. MEDR at both doses exhibited significant anti-inflammatory action by diminishing paw volume (ml) (0.29±0.016, 0.17±0.014) when compared to control (1.71±0.015) at the 4th hour of the study. Acetic acid incited writhing technique indicates the anti-nociceptive effect as the number of writhing responses with leaf extract significantly decreased (12.33±0.57) in comparison to control rats (27.33±1.52). The latent period of tail flicking responses was remarkably increased in *Dracaena reflexa* extract (400 mg/kg) treated rats (8.62±0.46) when equated with control (4.53±0.12). The reaction time in the hot plate technique was also enhanced with extract (7.835±1.72) compared to control rats (4.15±0.04) indicating analgesic activity of *Dracaena reflexa* leaves. Hence it is evident from the above findings that *Dracaena reflexa* leaves possess significant anti-inflammatory and antinociceptive potentials.

Keywords*---Dracaena reflexa*, anti-inflammatory, antinociceptive, writhing responses, acetic acid, acute toxicity, reaction time.

Introduction

Inflammation is associated with cardiovascular, neurological, lifestyle, and life-threatening diseases including cancer. Several inflammatory mediators and associated markers have been studied to understand the inflammatory pathways and therapeutic targets.[1] Inflammation or tissue damage progresses by the afferent and efferent transmission of the responses of inflammatory mediators to the Central nervous system.[2-3]

Pain is a blend of complex, unpleasant emotions and dreadful experiences that affect living quality.[4] NSAIDs are the primary choice for managing acute pain but cause ulcers and liver toxicity.[5] The search for safer alternative agents for pain and inflammation is an evergreen area of research.[6] Detailed pharmacological screening of natural products against selective targets of pain and inflammation shows better efficacy and selectivity that can assure fewer side effects.[7]

The genus Dracaena is represented by approximately 40 species and 150 varieties. *Dracaena reflexa* belongs to the Asparagaceae family. Basically, it is known as the song of Jamaica or song of India. The plant is indigenous to islands of the Indian Ocean, Mauritius, Madagascar, and Mozambique. The leaves of this famous ornamental plant are thin, deep, linear, bright-green with red edges and irregular stems. The plant can grow in dry soil with minimum temperature, and more tolerant to irregular watering.[8] The plant is abundant in phytochemicals such as quinines, terpenoids, cardiac glycosides, saponins, flavonoids, and tannins. Traditional medicinal uses of the plant include diuretic, anti-diarrheal, and anti- dysenteric, to reduce fever, hemostatic treatment and to reduce dysmenorrhea. Decoction of aerial parts is effective as a febrifuge.[9] The plant is also known for reviving fatigue & muscle pain, malaria fever, and to treat intoxication. Pharmacologically the plant is proved to have antibacterial and antioxidant.[10-11] It can remove formaldehyde, xylene and trichloroethylene residues from the air and approved by NASA as an air purifier.[12] Flavonoids, saponins, tannins, lecithin, phytate, and calcium oxalate are abundant in the plant. Flavonoids exhibit exceptional anti-inflammatory and antinociceptive properties.[13] Hence, our research aims to explore the anti-inflammatory and analgesic potentials of MEDR by the carrageenan-induced paw edema technique, the acetic acid-induced writhing test, the tail immersion test, and the hot plate method in Wistar rats.

Materials and Methods

Dracaena reflexa Lam. leaves were collected from Osmania University campus, Telangana and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati and voucher specimen (Pt 0733) was preserved in the herbarium.

Extraction

D. reflexa leaves were dried in the shade and finely ground to a coarse powder. Before the Soxhlet extraction with methanol under controlled temperature (50-60 °C), the powder was defatted with petroleum ether. The extracts were

concentrated at 40 °C and dried under reduced pressure. For subsequent analysis, the dried extracts were stored in a desiccator.

Preliminary phytochemical screening

The preliminary phytochemical investigation of MEDR was carried out by adopting standard protocols.[14]

Experimental animals

The anti-inflammatory and antinociceptive activity were tested on Wistar rats (180-220 g). The rats were accustomed for 10 days after being randomly assigned. Rodents were maintained in polypropylene cages with 26°C temperature, 45-55%relative humidity, and a 12-hour dim/light cycle. The rats were fed with rat pellet food (Golden Mohur Lipton India Ltd.) and water ad libitum. The protocol approval was taken from the institutional animal ethics committee (IAEC), prior to the study.

Determination of acute toxicity

The oral acute toxicity of MEDR was investigated in Wistar rats at doses of 5, 50, 300, and 2000 mg/kg (OECD 423). Rodents were observed for behavioral, autonomical and neurological symptoms, and mortality for four hours. Body weight was examined after six hours of dosage. Cognitive variation, detrimental manifestations, or mortality were measured consistently (for 1 hour) and body weights were measured on the eighth and fourteenth day after drug administration. A therapeutic dosage of $1/5^{th}$ and $1/10^{th}$ of the safe dose was selected.[15]

Influence of MEDR on Carrageenan induced paw edema in Wistar rats

The rodents were randomly divided into five groups, each with five rats.

Group 1: (Normal saline 0.5 ml orally),
Group 2: Carrageenan (0.1 ml solution (1.5%), subplantar injection)
Group 3: Standard (Dexamethasone 5 mg/kg, p.o.)
Group 4: Low dose (200 mg/kg MEDR, p.o.)
Group 5: High dose (400 mg/kg MEDR, p.o.)

Except for the normal group, paw edema was induced in rat's right paws by injecting 0.1 ml of 1.5 percent carrageenan. A plethysmometer was used to measure paw volume after injecting carrageenan and at 0, 1, 2, and 4 hours after injecting carrageenan.

$$\% \text{ Edema inhibition} = [(Vc - Vt)/Vc] \times 100$$

Where Vc =Paw volume of control carrageenan injected (ml) rodents, Vt = Paw volume of test treated rodents. The outcomes have been portrayed in Table 2 and Figure 1.

Influence of MEDR on acetic acid instigated writhing test in rats

The Koster et al. approach was used to direct the writhing test.[16] Twenty rodents (five in each batch) were divided into the following groups:

Group 1: Control bunch (Normal saline),
Group 2: Positive control (Diclofenac sodium, 100 mg/kg body weight)
Group 3: Low dose (200 mg/kg MEDR, p.o)
Group 4: High dose (400 mg/kg MEDR, p.o)

Each rodent was injected intra-peritoneally with 0.7 percent (v/v) acetic acid at a dose of 10 mL/kg body weight. The number of writhing reflexes was measured for each animal for 5 minutes after 15 minutes of acetic acid treatment. In addition, the average abdominal writhings for each group was calculated. The data summarized in table 3 and figure 2 respectively.

Influence of MEDR on tail immersion test in rats

The method used by Toma et al. was used to determine the central antinociceptive activity. Twenty rats were divided into four groups each having five animals each:
Group 1: Control (Normal saline),
Group 2: Positive control (Diclofenac sodium, 100 mg/kg body weight, orally)
Group 3: Low dose (200 mg/kg MEDR, orally)
Group 4: High dose (400 mg/kg MEDR, orally)
The painful responses induced in rodents by giving them a warm boost by plunging the tip of their tail in warm water. After each group had been treated, the baseline response time was calculated by immersing the rat's tail ends (last 1-2 cm) in hot water (55±1) °C. The flick reaction of rodents was measured, i.e., the time it took (in a flash) to move the tail away from a warm water, and the results were compared to the control group. To prevent harm to the rodent, a latent time of 15 seconds was selected as the upper limit. The latent time was calculated to be 30 min, 60 min, and 1 hour after the treatment.

Influence of MEDR on hot plate test in rats

Twenty rodents were divided into two groups for this study.
Group 1: Control group (Normal saline, oral),
Group 2: Positive control (Diclofenac sodium, 100 mg/kg body weight, oral)
Group 3: Low dose (200 mg/kg MEDR, oral)
Group 4: High dose (400 mg/kg MEDR, oral)
Rodent's paws are extremely sensitive to temperature at 55 ± 0.5 °C, which are also not harmful to the skin. The animals were placed on a heated plate that was kept at 55 ± 0.5 °C of its maximum temperature. To avoid causing injury to the paw, a cut-off time of 20 seconds was used. At 0, 30, 60, and 90 minutes after oral injection of the extract, the time when rodents started licking their front or back paws was noted. Table 5 and Figure 4 show the results of the study.

Statistical investigations

The results are presented as mean ± S.E.M., and statistical significance between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with ** P<0.01 and *P<0.05 being statistically significant.

Results

Preliminary phytochemical analysis

The presence of flavonoids, tannins, phytosterols, and triterpenoids was detected during preliminary phytochemical screening of leaf extracts (Table1).

Table 1: Preliminary Phytochemical investigation of MEDR

Phytoconstituents	MEDR
Carbohydrates	+
Steroids	-
Glycosides	-
Flavonoids	+
Saponins	+
Alkaloids	+
Tannins	+
Proteins	-

- Absent + Present

Acute toxicity study of MEDR

MEDR was subjected to an acute toxicity study in compliance with OECD-423 guidelines. Female rodents were treated at doses of 5, 50, 300, and 2000 mg/kg via oral route and showed no signs of toxicity. The rodents were examined further, twice a day, and showed no toxic symptoms. As a result, the oral LD50 of MEDR was determined to be greater than 2000 mg/kg. Accordingly, 2000 mg/kg was considered as the safest higher dose for methanolic extract, and $1/10^{th}$ and $1/5^{th}$ of 2000 mg/kg, i.e., 200 mg/kg (lower dosage), 400 mg/kg (higher dosage), were selected for further study.

Anti-inflammatory potentials of MEDR by Carrageenan instigated paw edema method in rodents

When compared to control (1.71±0.015) at the 4th hour after carrageenan administration, MEDR (both doses) significantly reduced the carrageenan incited paw volume in rats (0.29±0.016 and 0.17±0.014). The paw volume decreased with 400 mg/kg dose and is comparable to the paw volume reduction in rats treated with dexamethasone (5 mg/kg). The edema inhibition with MEDR 400 mg/kg was determined to be 90.5%, which is fairly similar with Dexamethasone.

Table 2: MEDR and Dexamethasone inhibited paw volume and edema (percent) in rats with carrageenan-induced paw edema

Groups	Paw Volume (ml) at various time intervals (hrs)				
	0	1	2	4	% Inhibition of edema
Normal (Normal saline 0.5 ml orally)	0.15±0.012	0.16±0.052	0.17±0.035	0.17±0.075	-
Carrageenan (0.1 ml of solution 1.5%, subplantar inj.)	0.64±0.018	0.74±0.014	0.92±0.002	1.71±0.015	-
Standard (Dexamethasone 5 mg/kg, orally)	0.29±0.014	0.27±0.015	0.19±0.017*	0.15±0.024**	91.22
Low dose (200 mg/kg MEDR, orally)	0.47±0.043	0.42±0.036	0.36±0.019	0.29±0.016*	83.04
High dose (400 mg/kg MEDR, orally)	0.35±0.017	0.31±0.028	0.24±0.014*	0.17±0.014**	90.05

The significance of the differences between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with ** P<0.01 and *P<0.05 being considered statistically significant.

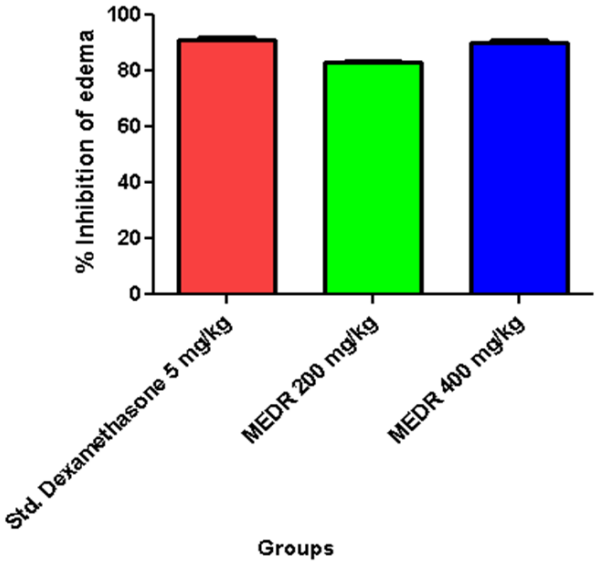

Figure 1: Percentage of edema inhibition in rats after treatment with dexamethasone and MEDR in carrageenan induced paw edema method

The analgesic activity of MEDR using acetic acid induced writhing test in rats

When compared with control (27.33±1.52), MEDR at 400 mg/kg bw had substantially lowering the abdominal writhings (12.33±0.57). The MEDR 400 mg/kg results are comparable to Diclofenac sodium (100 mg/kg) writhing responses (8.66 ±0.57). Table 3 and Figure 2 depict the results.

Table 3: In an acetic acid induced writhing test, abdominal writhings in rats were administered with saline, diclofenac sodium, or MEDR

Treatment	Abdominal writhings
Normal (Normal saline 0.5 ml orally)	27.33±1.52
Standard (Diclofenac sodium 100 mg/kg, p.o)	8.66±0.57***
Low dose (200 mg/kg MEDR, orally)	16.66±2.08*
High dose (400 mg/kg MEDR, orally)	12.33±0.57 **

Findings are reported as mean ± S.E.M., and statistical significance between the treated and control groups was determined using One Way ANOVA, followed by Dunnett's test, with *P<0.001, ** P<0.01, and *P<0.05 found to be statistically significant.

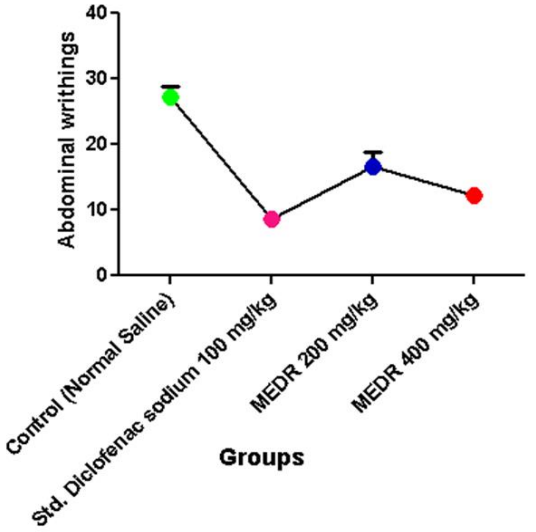

Figure 2: Abdominal writhings in Control, standard diclofenac, and MEDR treated rats in acetic acid stimulated writhing test

The analgesic activity of MEDR using tail immersion test in rats

The flick response in control rats was 4.53±0.12 seconds. After 90 minutes of treatment, rats administered with standard diclofenac sodium had a response of 9.33±0.42 seconds, demonstrating an analgesic effect. MEDR at 400 mg/kg bw elicited a flick reflex at 8.62±0.46 seconds 90 minutes after treatment,

demonstrating that the leaf extract has a statistically significant analgesic effect. Table 4 and Figure 3 show the final results.

Table 4: Latent period of tail-flick response

Treatment	The latent period of flick response in seconds			
	0 minutes	30 minutes	60 minutes	90 minutes
Group 1- (Normal saline)	4.55±0.09	4.56±0.09	4.56±0.19	4.53±0.12
Group 2- Diclofenac sodium100 mg/kg, orally	4.59±0.14	9.81±0.38**	9.85±0.42**	9.33±0.42**
Group 3 Low dose (200 mg/kg MEDR, orally)	4.36±0.22	4.94±0.37	5.48±0.36	5.56±0.25
Group 4 High dose (400 mg/kg MEDR, orally)	4.48±0.13	6.69±0.4	8.74±0.54*	8.62±0.46*

Findings are addressed as mean ± S.E.M and statistical importance among treated and control sets was dissected utilizing One way ANOVA, trailed by Dunnett's test were *P<0.001, ** P<0.01, and *P<0.05 were viewed as statistically remarkable.

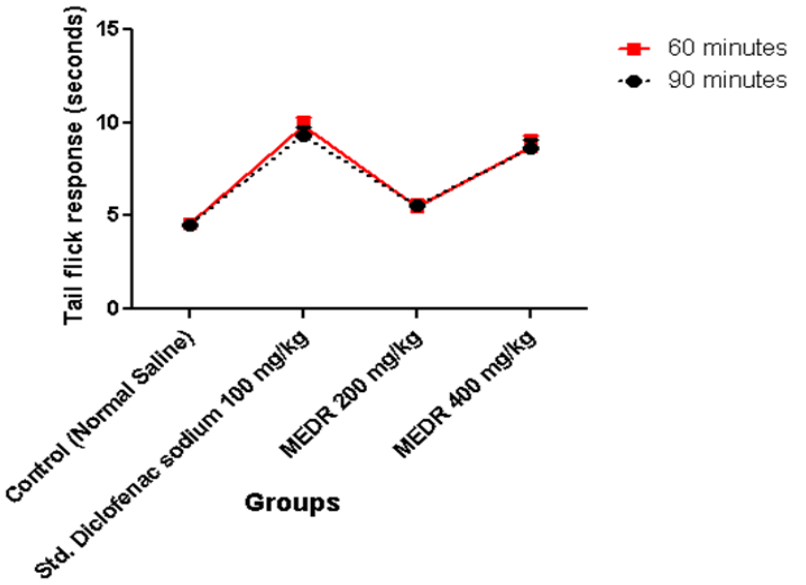

Figure 3: The flick responses (seconds)

Analgesic activity of MEDR using hot plate analysis in rats

After 90 minutes of treatment, MEDR at 400 mg/kg delayed the reaction time to 7.835±1.72 seconds, compared to 4.15±0.04 seconds in control rats. Whereas,

Diclofenac sodium produced the response after 7.95±0.1 seconds (Table 5 and Figure 4).

Table 5: Reaction time (seconds) with MEDR treated rats in hot plate test

Treatment	Reaction time in seconds			
	0 minutes	30 minutes	60 minutes	90 minutes
Group 1- (Normal saline)	3.725±0.25	3.815±0.27	4.025±0.07	4.15±0.04
Group 2- Diclofenac sodium100 mg/kg, orally	3.715±0.22	7.15±0.15**	7.635±0.24**	7.95±0.1**
Group 3 Low dose (200 mg/kg MEDR, orally)	3.795±0.12	5.515±0.19	5.245±0.66	5.545±0.15
Group 4 High dose (400 mg/kg MEDR, orally)	3.755±0.19	6.025±0.15	6.585±0.38*	7.835±1.72**

Findings are addressed as mean ± S.E.M and statistical importance among treated and control sets was dissected utilizing One way ANOVA, followed by Dunnett's test were *P<0.001, ** P<0.01, and *P<0.05 was viewed as statistically remarkable.

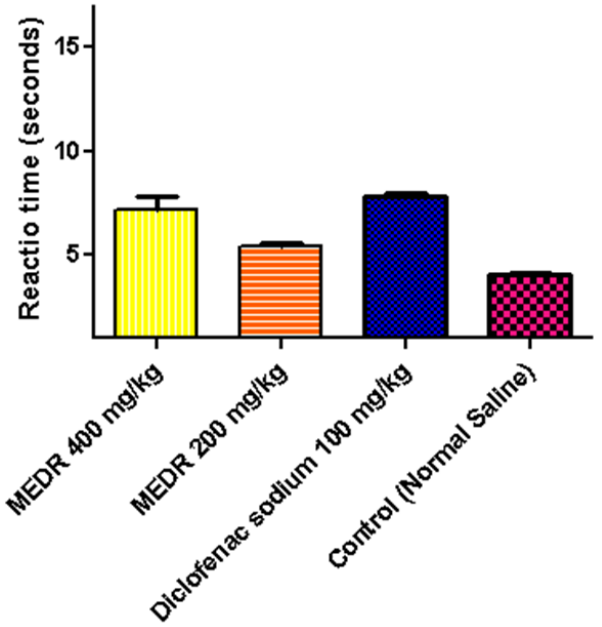

Figure 4: Reaction time (seconds) with Control, standard Diclofenac and MEDR treated rats in hot plate test

Discussion

Carrageenan is a strong chemical, used to stimulate the release of proinflammatory and inflammatory mediators (TNF-α, bradykinin, histamine, leukotrienes, prostaglandins, etc.) It is used to induce inflammation in experimental animals to test the anti-inflammatory activities of extracts. In the current study anti-inflammatory activity was measured after four hours of treatment and the results were displayed as percentage inhibition of edema in the rats and compared with dexamethasone. MEDR exhibited 90.5% inhibition at 400 mg/kg body weight whereas, Dexamethasone showed 91.22 % inhibition at 5 mg/kg body weight.

The peripheral and central analgesic effects of the drug can be estimated by the acetic acid induced writhing test. Intraperitoneal administration of acetic acid may stimulate the release of PGs or mediators of pain or some of endogenous nociceptive stimulants. The analgesic effect was tested by the writhing responses like extension of hind limbs, trunk twisting, abdominal constrictions. NSAIDS peripherally blocks COX enzyme and produce analgesic effect.

When compared to control and standard, the MEDR displayed significant ($P<0.001$) analgesic response. The MEDR at 400 mg/kg dose had indicated higher percentage inhibition than the Diclofenac sodium. The observed flick response of MEDR at 400 mg/kg bw, were comparatively higher (8.62±0.46 seconds) than the response at 200mg/kg bw (5.56±0.25). Similarly, in the hot plate method MEDR at 400 mg/kg bw, exhibited 7.835±1.72 seconds of delayed tail withdrawal time.

Conclusion

The current study found that a 400 mg/kg dose of MEDR significantly reduced inflammation and pain in experimental rats in the given models. It is evidenced by a decrease in paw volume, writhing responses, an increase in tail flicking time, and reaction time. As a result, we conclude that MEDR have notable anti-inflammatory and antinociceptive properties. However, further research is needed to isolate the active ingredients responsible for anti-inflammatory and anti-nociceptive activities, as well as to determine the mechanism underlying in them.

Conflict Of Interest

The authors disclose that they have no conflict of interest regarding the current research work.

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