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Original Article

Animal Research Section

Anti-Inflammatory Activity of Oxycarotenoid Extracts Isolated from Coriander Leaves (*Coriandrum Sativum*) and Curry Leaves (*Murraya koenigii*) on Carrageenan Induced Acute Inflammation in Rats

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ABSTRACT

Introduction: Green leafy vegetables are important sources of polyphenols and carotenoids which possess both antioxidant and anti-inflammatory activities.

Aim: To study the anti-inflammatory activity of oxycarotenoid extracts isolated from coriander leaves (*Coriandrumsativum*) and curry leaves (*Murraya koenigii*) in carrageenan induced acute paw oedema in rats.

Materials and Methods: Oxycarotenoid extracts were isolated from the coriander leaves *(Coriandrum sativum)* and curry leaves (*Murraya koenigii)* and they were assessed for anti-inflammatory activities by *in-vivo* methods. The *in-vivo* anti-inflammatory activity was evaluated in carrageenan induced acute paw oedema model. Indomethacin at a dose of 20 mg/Kg body weight was used as standard anti-inflammatory drug.

Results: The results revealed that oxycarotenoids extracted from coriander leaves administered at a dose of 40 mg/kg body weight showed an inhibition of 53.33% whereas the oxycarotenoids extracted from curry leaves showed an inhibition of 60% at the sixth hour after carrageenan injection. The results are comparable with those of indomethacin (20 mg/Kg body weight) administered group which showed an inhibition of 55.53%

Conclusion: These findings suggest that oxycarotenoid extracts isolated from leafy vegetables (coriander leaves and curry leaves) have significant anti-inflammatory activities.

Keywords: Indomethacin, Leafy vegetables, Oxycarotenoids, Paw oedema

INTRODUCTION

Carotenoids are tetraterpenoids derived from 40 C polyenechain. The chain may be ceased upon addition of cyclic groups and may be complemented with oxygen containing functional groups [1]. These are natural pigments synthesised by plants and found in sea food, and some living organisms. The major types of carotenoids are α-carotene, β-carotene, β-crypto-xanthin, lutein, zeaxanthin and lycopene. Carotenoids have been reported to have anti-oxidant [2] and anti-cancer activities [3]. Iman V et al., demonstrated the anti-cancer and anti-inflammatory activities of the alkaloid girinibrine isolated from *Murrayakoenigii* [4]. Yeap SK et al., reported that aqueous extract of M *koenigii* L had chemo-preventive and immuno-modulatory effects on 4T1 breast cancer cell-challenged mice [5]. According to Gupta S et al., the methanol extract of M *koenigii* had anti-inflammatory and analgesic activity in rats [6]. Thabrew MI et al., observed that polyherbal formulation Maharasnadhi Quather had anti-inflammatory and analgesic activities [7].

Crude extract of *Coriandum sativum* L was found to exert antioxidant activity and to protect UVB – induced photoaging of skin [8]. Rajeswari CV et al., observed that coriander leaf powder had antioxidant and antiarthritic potential [9]. Al-Mofleh A et al., demonstrated that crude extract of *Coriandrum sativum* had protective effect on gastric mucosal damage in rats [10]. Laribi B et al., isolated and characterised the bioactive constituents from *Coriandrum sativum* leaves [11]. Yildiz H reported that ethanol extract of *Coriandrum sativum* had antioxidant activities [12]. Sindhu ER et al., investigated the antimutagenic and anticancer activities of commercially available pure oxycarotenoid lectins [13]. There are very few reports available on the isolation of oxycarotenoid rich extracts from curry leaves and coriander leaves and to study their anti-inflammatory activities.

Inflammation is mainly associated with pain, which is the most common symptom that can affect people of any age or gender. To understand the biological principles behind this activity animal model was employed. Pain may occur due to the potential tissue damage caused by free radicals [14,15].

Carrageenan is a hydrophilic colloid containing highly sulphated galactans. It is strongly ionic due to 10-15% of ester-sulphate moiety. Carrageenan induced paw oedema is the commonly used animal model for studying anti-inflammatory property.The carrageenan-induced hind paw oedema is biphasic [16]. Early phase ismediated by histamine, serotonin and bradykinin whereas the late phase is mediated by prostaglandins. Indomethacin was used as reference drug which did not inhibit the initial phase of oedema. The second phase of swelling may be due to the induction of cyclooxygenase (COX-2) [16]. Carrageenan may activate inflammatory markers through BCL10, NF-Κbpathways [17]. These pathways involve phosphorylation and nuclear translocation of phospho-NF-κB and augment the transcription and translation of inflammatory biomarkers such as cyclo-oxygenase, nitric oxide synthase and interleukin-6 [17].

The aim of the present study was to isolate oxycarotenoid rich ((hexane/acetone 80/20 fraction) extracts of curry leaves and coriander leaves employing column chromatography and to assess their anti-inflammatory activity.

MATERIALS AND METHODS

Test material information

The test material is the oxycarotenoid rich fraction of curry leaves (*Murraya koenigii)* and coriander leaves (*Coriadrum sativum)*

extracts prepared as described earlier [18]. The extracts were in the form of dark brown to yellowish brown viscous liquid. The extracts prepared from coriander leaves are mentioned as Coriander Leaf Extract (COLE) and the extract prepared from curry leaves as Curry Leaf Extract (CULE) using 5% Dimethyl Sulfoxide (DMSO) as vehicle. The test item forms good suspension with 5% DMSO.

Study design

The study was conducted with 48 male albino rats groups (body weight(bwt) =180-220 g) with 6 animals in each of the 8 groups. Animals were maintained under standard laboratory conditions (22±3ºC room temperature and 50-60% humidity) with alternating light and dark cycles of 12 hours and provided with food and water *(ad libitum)*. The rats were fed with pellet diet and were acclimatized to laboratory conditions for 5 days before the start of the experiment. Animals were divided into eight groups with six animals in each group based on the requirements of study protocol as control, low dose, mid dose, high dose and standard drug (indomethacin) [Table/Fig-1].

Groups	No. of animals	Treatment for 28 days
I Carrageenan Control	6	Carrageenan (0.1 mL of 1% solution, sub-plantar inj., left hind paw)+vehicle (1 mL/100g body weight, per oral)
II Low dose	6	Carrageenan (0.1 mL of 1% solution, sub plantar inj., left hind paw)+CULE 40 mg/kg body weight, per oral
	6	Carrageenan (0.1 mL of 1% solution, sub plantar inj., left hind paw)+COLE 40 mg /kg body weight, per oral
III Mid dose	6	Carrageenan (0.1 mL of 1% solution, sub plantar inj., left hind paw)+CULE 80 mg/kg body weight, per oral
	6	Carrageenan (0.1 mL of 1% solution, sub plantar inj., left hind paw)+COLE 80 mg/kg body weight, per oral
IV High Dose	6	Carrageenan (0.1 mL of 1% solution, sub plantar inj., left hind paw)+CULE 160 mg/kg body weight, per oral
	6	Carrageenan (0.1 mL of 1% solution, sub plantar inj., left hind paw)+COLE 160 mg/kg body weight, per oral
V Standard drug	6	Carrageenan (0.1 mL of 1% solution, sub plantar inj., left hind paw+Indomethacin 20 mg/kg body weight, per oral

[Table/Fig-1]: Study design of carrageenan induced acute inflammation model on rats.
CULE: Oxycarotenoid extract of curry leaves (*Murrayakoenigii*)
COLE: Oxycarotenoid extract of coriander leaves (*Coriandrumsativum*)

The test sample, vehicle and standard drug were administered 30 min prior to injection of carrageenan to the sub-plantar region of left hind paw. The paw thickness was measured before injecting carrageenan at 2, 4 and 6 hours after injection using vernier calliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group. The percentage (%) inhibition of oedema was calculated using the formula.

Percentage Inhibition = $[(T_t - T_0)$ control - $(T_t - T_0)$ treated / $(T_t - T_0)$ control$] \times 100$

where T_t=thickness of paw of rats given test extract, and T_0=paw thickness of rats of control group at the same time [19].

Dose formulation and administration

The weighed test material was suspended in 5% DMSO to get desired concentration as per the dose (mg/kg body weight). Test material was formulated shortly before administration. The homogeneity of the test formulation was maintained by continuous stirring with glass rod. Rat gavaging needle fitted to graduated syringe was used for the administration of test material through oral route. The test material was administered after calculating the dose for each respective group and formulation was made with the dose concentration as mentioned in the study design as per the procedure followed by earlier investigators [20].

Study compliance

The recommendations CPCSEA guidelines for laboratory animal facility (Gazette of India, January 7th, 2010) was followed and the protocol was approved by Institutional Animal Ethics Committee (IAEC) (Approval Certificate No.CKL/TOX/IAEC/2017-4/98).

STATISTICAL ANALYSIS

Data analysis was performed using Microsoft excel2010. The data are presented as mean ± standard deviation (SD).

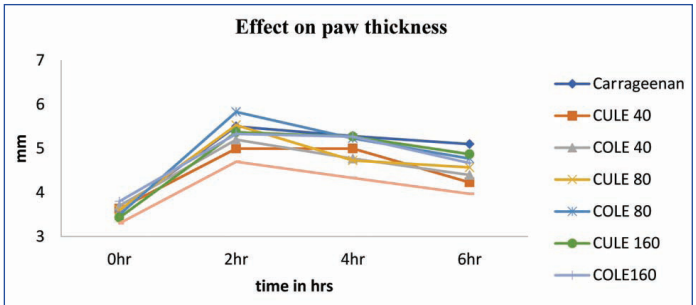
RESULTS

Effect of oxycarotenoid extracts on carrageenan induced acute inflammation in rats were observed as effect on paw thickness:

Effect on paw thickness: Carrageenan injections lead to marked increase in paw thickness in rats throughout the study period. However, at sixth hour, rats treated with indomethacin (20 mg/kg bwt.), showed significant reduction in the paw thickness (3.967±0.153 mm, $p<0.05$) compared to the carrageenan control group (5.10±0.70 mm). Treatment with CULE at 40 and 80 mg/kg bwt and COLE at 40, 80 and 160 mg/kg bwt, resulted in marked decrease in paw thickness which were comparable to that of indomethacingroup. Paw thickness of rats treated with CULE at 40,80 and 160 mg/kg bwt were 4.233±0.115,4.567±0.603 and 4.867±0.115 mm, respectively and those of rats treated with COLE at 40, 80 and 160 mg/kg bwt were 4.40±0.20, 4.767±0.404 and 4.767±0.153 mm, respectively [Table/Fig-2,3].

Groups		Paw thickness (mm)			
		0hr	2hr	4hr	6hr
Group- I (Carrageenan control)		3.600±0.100	5.500±0.755	5.267±0.751	5.100±0.700
Group- II (Low dose)	CULE	3.633±0.208	5.000±0.436	5.000±0.557	4.233±0.115
	COLE	3.700±0.173	5.200±0.200	4.767±0.321	4.400±0.200
Group- III (Mid dose)	CULE	3.600±0.200	5.533±1.097	4.733±0.702	4.567±0.603
	COLE	3.500±0.100	5.833±0.379	5.233±0.473	4.767±0.404
Group- IV (High dose)	CULE	3.433±0.058	5.367±0.451	5.267±0.321	4.867±0.115
	COLE	3.800±0.265	5.333±0.115	5.267±0.153	4.667±0.153
Group- V (Indometacin 20 mg/kg bwt)		3.300±0.100	4.700±0.100	4.300±0.265	3.967±0.153*

[Table/Fig-2]: Values of paw thickness (mm) at different time intervals before and after carrageenan injection in treated rats (Values are expressed as mean±SD).
CULE: Oxycarotenoid extract of curry leaves *(Murrayakoenigii)*
COLE: Oxycarotenoid extract of coriander leaves *(Coriandrum sativum)*
Standard drug: Indomethacin 20 mg/kg bwt., No. of animals in each group – 6
Extracts: Low dose – 40mg/kg bwt; Mid dose – 80mg/kg bwt; High dose -160 mg/kg bwt
* P<0.05, when compared with control (Group I).



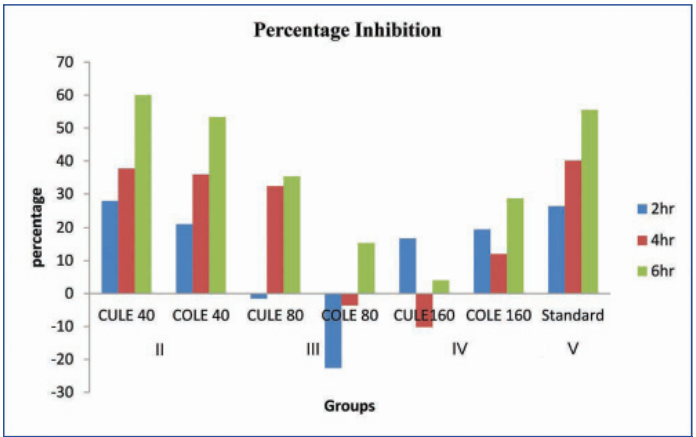
[Table/Fig-3]: : Effect of oxycarotenoid extracts of curry leaves (*Murrayakoenigii*) (CULE) and coriander leaves (*Coriandrum sativum*) (COLE) on paw thickness (mm).

Percentage inhibition of paw oedema: There was no marked reduction of paw oedema, in the early hours of study in any of the CULE and COLE treatment groups. However, administration of CULE and COLE at a dose of 40 mg/kg bwt produced 60.0% and 53.33% inhibition respectively; administration of CULE and COLE at

a dose of 80 mg/kg bwt produced 35.33% and 15.33% inhibition respectively; whereas administration of CULE and COLE at a dose of 160 mg/kg bwt produced 4.0% and 28.67% inhibition respectively at the sixth hour. At the sixth hour, there was 55.53% inhibition of paw oedema formation in animals treated with indomethacin (20 mg/kg bwt) [Table/Fig-4,5].

Groups		Inhibition of paw edema (%)		
		2hr	4hr	6hr
Group- I (Carrageenan control)		-	-	-
Group- II (Low dose)	CULE	27.89	37.7	60.01
	COLE	21.05	35.92	53.33
Group- III (Mid dose)	CULE	-1.58	32.34	35.33
	COLE	-22.63	-3.60	15.33
Group- IV (High dose)	CULE	16.74	-10.18	4
	COLE	19.47	11.98	28.67
Group- V (Indomethacin 20mg/kg body wt)		26.32	40.12	55.53

[Table/Fig-4]: : Percentage inhibition (% calculated from measurement of oedema in mm) of paw oedema at different time intervals after administration of oxycarotenoid extracts of curry leaves (CULE) and coriander leaves (COLE)
(Values are expressed as %)
CULE: Oxycarotenoid extract of curry leaves(*Murrayakoenigii*)
COLE: Oxycarotenoid extract of coriander leaves(*Coriandrumsativum)*
Low dose - 40mg/kg bwt; Mid dose - 80mg/kg bwt; High dose -160 mg/kg bwt
No.of animals in each group –Six



[Table/Fig-5]: Percentage Inhibition (% calculated from measurement of oedema in mm) of paw oedema caused by oxycarotenoid extracts of curry leaves (*Murrayakoenigii*) (CULE) and coriander leaves (*Coriandrumsativum)*(COLE).

It could be observed from the present study that administration of the oxycarotenoid extracts showed significant reduction of paw thickness and inhibitory effect also. There was no marked inhibition of paw oedema, in the early hours of study in any of the CULE and COLE treatment groups. But, marked percentage inhibition of paw oedema, 55.53%, 60% and 53.33%, was noted at 6th hour by standard drug (Indomethacin-20mg/kg bwt.), CULE (40mg/kg bwt.) and COLE (40mg/kg bwt.), respectively. Therefore, it can be assumed that the effect of CULE and COLE on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclooxygenase (COX) with resultant reduction in prostaglandin synthesis.

On the basis of findings made in the present study, it may be inferred that oxycarotenoid rich fraction of curry leaf extract (CULE) and coriander leaf extract (COLE) have significant anti-inflammatory activity and it could justify the use of these plants in the management of inflammatory conditions.

DISCUSSION

Carrageenan-induced rat paw oedemahas been widely used as an in vivo experimentalmodel to study the anti-inflammatory effect of new therapeutic agents. The developmentof carrageenan-induced paw oedema in rats is generally represented by a biphasic curve [21]. The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and is mediated by histamine, serotonin, and an increasing synthesis of prostaglandins in the damaged tissue surroundings [22]. The second phase is sustained by prostaglandin release [23,24] and subsequent inflammatory reaction [25].

No marked inhibition of paw oedema was observed [Table/Fig-2] in the study groupsin early hours in any of the CULE and COLE treatment groups. Hence, it can be inferred that release of histamine and serotonin are not inhibited by these extracts. But, there was marked percentage inhibition of paw oedema, 55.53%, 60% and 53.3%, at 6th hour by indomethacin, CULE (40 mg/kg bwt.) and COLE (40 mg/kg bwt.) respectively.

Lutein is found to down-regulate prostaglandin E2 (PGE2) synthesis [26]. Chang, et al, (2006) reported that synthesis of nitric oxide, an inflammatory mediator is related to induction of COX-2 activity and subsequent generation of PGE2 [27-29].Thus, the results obtained in this study suggest that oxycarotenoid rich fraction of curry leaf extract (CULE) and coriander leaf extract (COLE) possess anti-inflammatory activity against carrageenan induced paw oedema in rats which is comparable to that of indomethacin at a dose of 20 mg/Kg bwtand this could be due to the inhibition of the enzyme cyclooxygenase, leading to the inhibition of prostaglandin synthesis.

Limitation(s)

The study was based on oxycarotenoid rich extracts isolated form curry leaves and coriander leaves. The extracts may contain minimal amounts of alkaloids, flavonoids and other xanthophylls and hence the anti-inflammatory activity may not be fully attributed to oxycarotenoids.

CONCLUSION(S)

The results from the present study indicate that oxycarotenoids isolated from coriander leaves and curry leaves have significant anti-inflammatory action and hence the inclusion of these plants in the diet may have beneficial effects on human health.

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