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Anti-Inflammatory Activity Of Oxycarotenoid Extracts Isolated From Coriander Leaves And Curry Leaves On Formalin Induced Chronic Inflammation Rat Model

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Abstract

Carotenoids are found in abundance in green leafy vegetables. Carotenoids help in photosynthesis by absorbing light energy in plants. They also have an important antioxidant function of deactivating free radicals. Carotenoids are of two types - carotenes and oxycarotenoids. Oxycarotenoids are the carotenoids which contain one or more oxygen atoms such as lutein, zeaxanthin, cryptoxanthin, etc. The present study evaluates the anti-inflammatory activity oxycarotenoids isolated from the coriander leaves and curry leaves. The anti-inflammatory activity was determined using formalin induced chronic paw oedema model. The oxy- carotenoids extracted from coriander leaves administered at a dose of 40mg/kg body weight showed an inhibition of 44.76% and 61% on 3rd day and 7th day after formalin injection whereas the oxycarotenoids extracted from curry leaves showed an inhibition of 59.05% and 63.77% on 3rd day and 7th day after formalin injection. The results are comparable with that of indomethacin standard administered group which showed an inhibition of 60% and 65.04%. These findings suggest that the oxycarotenoids isolated from leafy vegetables (coriander leaves and curry leaves) have significant anti-inflammatory properties.

Keywords: Oxycarotenoids, Chronic inflammation, Anti-inflammatory activity

Introduction

Inflammation, the body's response to an injury is associated with symptoms like redness, swelling and pain. Edema leads to distortion of tissues which in turn is attributable to pain, which is also induced by certain chemical mediators of inflammation. Increased blood flow results in heat generated through the area and is experienced only in the extremities (skin). Rise in temperature at the injury area is attributable to fever, which is induced by chemical mediators of inflammation. Pain/ severe swelling results in loss of function or prevent movement in the area [1]. An inflammatory response which lasts either for few days or for long duration and are called as acute and chronic inflammation respectively. Acute inflammation is a part of healing process in which white blood cells are sent to the injured area whereas chronic inflammation is considered to be the root cause of

many degenerative diseases like diabetics, arthritis and heart diseases. The anti-inflammatory agents are helpful as therapeutic agents for those pathological conditions. Because of the side effects associated with most of the available anti-inflammatory agents, more focus is growing towards the herbal remedies [3]. During the process of inflammation mediators are released which have the ability to generate dangerous and reactive species like reactive oxygen species, reactive nitrogen species and other free radicals. These reactive species affect the nucleic acids, proteins and carbohydrates present in the cellular biomolecules causing damage to the tissues [4]. There are series of events involved in the mechanism of inflammation. The metabolism of arachidonic acid has the key role and which is metabolized to prostaglandins and thromboxane A2 by cyclooxygenase (COX) pathway or to hydroperoxy-eicosatetraenoic acids (HPETE's) and leukotrienes (LTs) by lipoxygenase (LOX) pathway [2]. There are several studies available to establish the role of lipoxygenases in cancer progression. In 2010 Wang and Dubois [10] proved that lipoxygenases and their catalytic products are also associated with apoptosis and tumour cell proliferation.

A diet which includes leafy greens is one of the best inflammation fighting agents because it contains significant amounts of vitamins, carotenoids and other nutrients which help to reduce the chronic inflammation. Dietary carotenoids serve as antioxidant and protect the tissues against damages. Increased focus needs to be given for herbal drugs, due to decreased side effects of herbal drugs as well as increased side effects and intake of synthetic drugs. Anti-inflammatory activity of lutein molecule is known and that of crude extracts containing polyphenols, flavonoids and vitamins are also known but there is no study which reported the anti-inflammatory action of xanthophylls extracted from coriander and curry leaves. The present study is aimed at exploring the anti-inflammatory action of oxycarotenoids extracted from the coriander and curry leaves on formalin induced rat models.

Materials and methods

Test material

The test material is the oxycarotenoid rich fraction of curry leaves and coriander leaves extract prepared as described earlier [9]. The extracts are in the form of dark brown to yellowish brown viscous liquid. The extract prepared from coriander leaves are mentioned as coriander leaf extract (COLE) and the extract from prepared from curry leaves as curry leaf extract (CULE).

Vehicle

5% Dimethyl sulfoxide (DMSO) was used as vehicle for formulation preparation.

Justification for selection of vehicle:

The test material forms good suspension with 5% DMSO. Hence 5% DMSO was used as suspending agent for test material formulation. DMSO is universally accepted and routinely used as vehicle in oral (gavage) efficacy studies.

Test system and Management

- Wistar albino rats
- Rat is one of the recommended species by regulatory agencies for conducting toxicological studies among rodents Source
- Small animal breeding station, Mannuthy, Thrissur, Kerala
- 48 male rats
- 180-220 g
- 8 to 12 weeks
- Cage cards

Husbandry

- Animals were housed under standard laboratory conditions
- Air-conditioned environment with adequate fresh air supply with IVC system (Air changes 15 per hour), room temperature 21 to 24.0°C, relative humidity 57-65%, with 12 hours light and 12 hours dark cycle. The temperature and relative humidity were recorded daily.
- Single animal was housed in a standard polysulphonate cage (Size: L 300 x B 170 x H 140 mm) with stainless steel top grill mesh having facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube. Sterilized paddy husk was provided as bedding material.
- The animals were acclimatized for a minimum period of five days laboratory conditions and were observed for clinical signs daily. Veterinary examination of all the animals was recorded on the day of receipt and on 5th day of acclimatization.
- The animals were fed *ad libitum* throughout the acclimatization and study period. Laboratory animal feed (Manufactured by Feed plant of School of Animal Nutrition and Feed Technology, Kerala Veterinary & Animal Sciences University) was provided.
- Water was provided ad libitum throughout the acclimatization and study period. Deep bore-well water passed through activated charcoal filter and exposed to ultraviolet rays in Aquaguard water filter cum purifier (Manufactured by Eureka Forbes Ltd., Mumbai) was provided in plastic water bottles with stainless steel sipper tubes.

Methods

Determination of anti-inflammatory activity of formalin induced chronic inflammation models:

48 male albino rats (180-220 g) were utilized in this study. Animals were maintained under standard laboratory conditions (22 ± 3ºC room temperature and 50-60% humidity) with alternating light and dark cycles of 12 hrs and provided with food and water (*ad libitum*). The rats were fed with pellet diet and were acclimatized to laboratory conditions for 5 days prior to the experiment. Animals were divided into eight groups (six animals in each group) and the test sample, vehicle and standard drug were administered after the injection of formalin to the sub-plantar region. The paw thickness was measured before injecting formalin and on day 3 and 7 post-injection using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the formalin control group.

Study design

A total of 48 Male albino rats were utilized in this study. Animals were maintained under standard laboratory conditions, fed with pellet diet and were acclimatized to laboratory conditions for 5 days prior to the experiment. After acclimatization period rats were divided into eight groups (six each in groups) and were treated as in the following manner listed in table 1.

Table 1: Study design of formalin induced model on rats.

Group	No. of animals	Treatment for 28 days
I Formalin Control	6	Formalin (20µl of 1% solution, sub-plantar inj., right hind paw) + distilled water (1ml/100g body weight, per oral, for 7 days)
II Low dose	6	Formalin (20µl of 1% solution, sub-plantar inj., right hind paw) +**CULE*** 40mg/kg body weight, per oral
	6	Formalin (20µl of 1% solution, sub-plantar inj., right hind paw) +**COLE**** 40mg /kg body weight,

		per oral
III Mid dose	6	Formalin (20µl of 1% solution, sub-plantar inj., right hind paw) +**CULE*** 80mg/kg body weight, per oral
	6	Formalin (20µl of 1% solution, sub-plantar inj., right hind paw) +**COLE**** 80mg/kg body weight, per oral
IV High Dose	6	Formalin (20µl of 1% solution, sub-plantar inj., right hind paw) +**CULE*** 160mg/kg body weight, per oral
	6	Formalin (20µl of 1% solution, sub-plantar inj., right hind paw) +**COLE**** 160mg/kg body weight, per oral
V Standard	6	Formalin (20µl of 1% solution, sub-plantar inj., right hind paw) + Indomethacin 20mg/kg body weight, per oral

* CULE: Oxycarotenoids extract of curry leaves

** COLE: Oxycarotenoids extract of coriander leaves

The test sample, vehicle and standard drug were administered after the injection of formalin to the sub-plantar region. The paw thickness was measured before injecting formalin and on day 3 and 7 post-injection using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the formalin control group.

The percentage (%) inhibition of oedema was calculated using the formula

% inhibition = $[(T_t - T_0)$ control - $(T_t - T_0)$ treated / $(T_t - T_0)$ control$] \times 100$

where T_t is the thickness of paw at corresponding time and T_0 is the initial paw thickness.

Dose formulation

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 just before administration. The homogeneity of the test formulation was maintained by continuous stirring with glass rod.

Administration of test material

The test material was administered through oral route by gavage to the animal after formulation preparation using rat gavaging needle fitted to graduated syringe. The administration of the test material was done after calculating the dose for each respective group and formulation was made with the dose concentration as mentioned in the study design.

Study compliance

The study was performed in accordance with the following:

The recommendations of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, January 7th 2010 and the protocol approved by Institutional Animal Ethics Committee (IAEC).

Safety Precautions

Gloves, cap and face mask were used in addition to protective body garments and rubber slipper to ensure adequate personal health and safety and to avoid inhalation and skin contact with the test material.

Results

Effect of oxycarotenoid extracts of coriander and curry leaves on formalin induced chronic inflammation models:

In the present study, administration of CULE reduced formalin-induced paw oedema showing significant anti-inflammatory effect on 3rd day and 7th day, percentage inhibition was found to be 59.05% and 67.79% respectively, at dose of 80 mg/kg body weight, COLE also reduced formalin-induced paw oedema showing significant anti-inflammatory effect on 3rd day and 7th day,

percentage inhibition was found to be 44.76% and 61.03% respectively, at dose of 40 mg/kg body weight. Therefore, it appears to act by inhibiting proliferative phase of inflammation. Treatment with CULE at 80 and 160 mg/kg bwt. and COLE at 40 and 160 mg/kg bwt. showed reduction in paw thickness which was comparable to that of standard group (indomethacin group). On the other hand, treatment with CULE at 40mg/kg bwt. and COLE at 80 mg/kg bwt. showed reduction in paw thickness which was comparable to that of control group.

The results obtained from the present study showed anti-inflammatory potential of the oxycarotenoid rich fraction of curry leaf extract (CULE) and coriander leaf extract (COLE) against formalin induced inflammation. On the basis of the findings made in the present study, it may be inferred that oxycarotenoid rich fraction of curry leaf extract (CULE) and coriander leaf extract (COLE) might have significant anti-inflammatory activity and it could justify the traditional use of these plants in the management of inflammatory conditions.

Effect on paw thickness

Sub-plantar injection of formalin into the hind paw elicited notable edema and inflammation in the experimental animals. However, on 3rd day, rats treated with standard drug, indomethacin (20 mg/kg bwt.), CULE extract at doses (80 and 160 mg/kg body weight) and COLE at doses (40 and 160 mg/kg body weight) showed significant reduction in the paw thickness compared to the formalin control group (5.57±0.153mm). Paw thickness of rats treated with CULE at 80 and 160mg/kg bwt were 4.733±0.115 ($P<0.01$) and 4.90±0. 361 mm ($P<0.05$) respectively, paw thickness of rats treated with COLE at 40 and 160 mg/kg bwt. were 4.933±0.153 and 4.933±0.306 mm ($P<0.05$) respectively and paw thickness of rats treated with indomethacin was 4.467±0.306 mm ($P<0.001$). Paw thickness of rats treated with CULE at 40mg/kg bwt and COLE at 80 mg/kg bwt were 5.267±0.153 mm and 5.4±0.10 mm respectively and paw thickness of rats treated with formalin control was 5.567±0.153 mm (Table 2; Fig 1).

On 7th day post formalin injection, treatment with CULE at 80 mg/kg bwt and indomethacin at 20 mg/kg bwt resulted in significant decrease in paw thickness, (3.900±0.20 mm and 4.10±0.173 mm respectively, $P<0.01$) compared to formalin control group (4.833±0.208 mm). However, treatment with CULE at 40 and 160 mg/kg bwt and COLE at 40, 80 and 160 mg/kg bwt showed reduction in paw thicknesses which were comparable to that of control group.

Percentage inhibition of paw oedemal

On 3rd day after formalin injection, CULE extract at doses (80 and 160 mg/kg body weight) and COLE at doses (40 and 160 mg/kg body weight) extract showed marked inhibition of paw oedema, with more pronounced effect produced by CULE 80 mg/kg (59.05%) and COLE 160 mg/kg(50.95%). Meanwhile, 20 mg/kg indomethacin exhibited the highest inhibition of 60%. Treatment with CULE extract at doses (40 mg/kg body weight) and COLE at doses (80 mg/kg body weight) extract showed an inhibition of paw oedema as 17.14% and 20.48% respectively.

However, on 7th day post formalin injection, CULE (80 and 160 mg/kg body weight), COLE(40 mg/kg body weight) and indomethacin (20 mg/kg body weight), resulted in 67.79%, 68.38%, 61.03% and 65.44% respectively, inhibition in paw edema, which was markedly higher than all the effects produced by other treatments such as CULE 40 mg/kg body weight (41.18%) and COLE 80 ,160 mg/kg body weight (43.38% and 38.97%) (Table 3 and Figure 2)

Table 2: Values of paw thickness (mm) at different days - before and after formalin injection (Values are expressed as mean ± SD)

GROUPS		Paw thickness (mm)		
		0th day	3rd day	7th day
Group- I (Formalin control)		3.467±0.058	5.567±0.153	4.833±0.208
Group- II (Low dose 40mg/kg bwt)	CULE	3.533±0.153	5.267±0.404	4.333±0.289
	COLE	3.767±0.208	4.933±0.153*	4.300±0.265
Group- III (Mid dose 80mg/kg bwt)	CULE	3.867±0.208	4.733±0.115**	3.900±0.200**
	COLE	3.733±0.058	5.400±0.100	4.500±0.173
Group- IV (High dose 160mg/kg bwt)	CULE	3.900±0.100	4.900±0.361*	4.333±0.231
	COLE	3.900±0.100	4.933±0.306**	4.733±0.321
Group- V (Standard 20mg/kg bwt)		3.633±0.115	4.467±0.306***	4.100±0.173**

CULE: Oxycarotenoid extract of curry leaves

COLE: Oxycarotenoid extract of coriander leaves

No.of animals in each group – 6. * P<0.05, ** P<0.01, *** P<0.001 when compared with Group I

Figure 1: Effect of oxycarotenoid extracts from curry leaves and coriander leaves on paw thickness

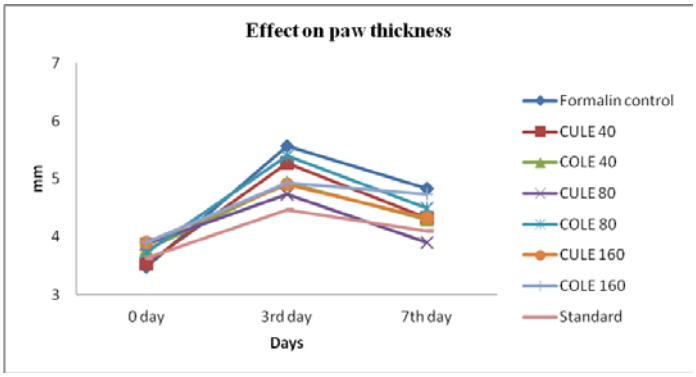

Figure 1: Effect on paw thickness

Table 3: Percentage inhibition of paw edema at different days after formalin injection (Values are expressed as %)

GROUPS		Inhibition of paw edema (%)	
		3rd day	7th day
Group- I (Formalin control)		-	-
Group- II (Low dose 40mg/kg bwt)	**CULE**	17.14	41.18
	COLE	44.76	61.03
Group- III (Mid dose 80mg/kg bwt)	**CULE**	59.05	67.79
	COLE	20.48	43.38
Group- IV (High dose 160mg/kg bwt)	**CULE**	40.12	68.38
	COLE	50.95	38.97
Group- V (Standard 20mg/kg)		60.00	65.44

ULE: Oxycarotenoid extract of curry leaves

COLE: Oxycarotenoid extract of coriander leaves

No.of animals in each group – 6

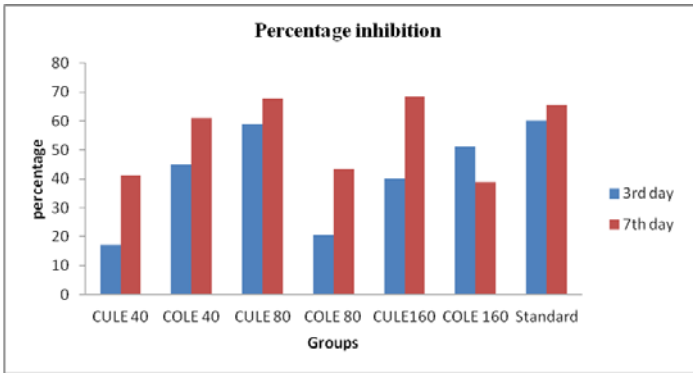

Figure 2: Percentage inhibition of paw oedema caused by oxycarotenoid extracts of curry leaves (CULE) and coriander leaves (COLE) at different days after formalin injection.

Discussion

Formalin-induced paw edema is one of the most suitable test procedures to evaluate chronic anti-inflammation, as it closely resembles human arthritis [5]. Two distinct biphasic nociception in the paw is initiated by formalin-induced experimental models, first being acute neurogenic pain, which lasts for first 5minutes and second being inflammatory pain response, lasting from 15 to 30 minutes [6]. Increased vascular permeability and blood flow due to various inflammatory mediators is the root cause for edema formation in the paw [7]. Early neurogenic effect,

followed by later tissue mediated response is observed in nociceptive effect of formalin [10] Formalin induced paw edema in rats represents the proliferative phase of inflammation [1]. Thus formalin-induced paw oedema is a model used for the evaluation of an agent with probable anti-proliferative activity.

In the present study, sub-plantar injection of formalin into the hind paw elicited notable oedema and inflammation in the experimental animals. However, pre-treatment with CULE extract showed a significant inhibition in the late phase of formalin induced pain which was well comparable with the standard drug indomethacin and also corroborates with the findings of Young, et al, [12]. As shown in Table 5.5 and 5.6, administration of CULE prevented formalin-induced paw edema showing significant anti-inflammatory effect on 3rd day and 7th day, percentage inhibition was found to be 59.05% and 67.79% respectively, at dose of 80 mg/kg bwt. Therefore, it appears to act by inhibiting proliferative phase of inflammation. Treatment with CULE at 80 and 160 mg/kg bwt. and COLE at 40 and 160 mg/kg. bwt also showed reduction in paw thickness which was comparable to that of standard group.

Conclusion

In conclusion, the study revealed that the oxycarotenoid present in the coriander leaves and curry leaves exert significant anti-inflammatory action.

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References

1. Ahamed Z, Ramabhimaiah S. (2012). Anti-inflammatory activity of Aqueous Extract of Carica papaya seeds in Albino Rats. *Biomed Pharmacol J.* 5(1), 173-177.
2. Alitonou GA, Avlessi F, Sohounhloue DK, Agnaniet H, Bessiere JM, Menut C. (2006). Investigations on the essential oil of Cymbopogon giganteus from Benin for its potential use as an anti-inflammatory agent. *Int.J.Aromatherapy*. 16(1), 37-41.
3. Brown C, Fezoui M, Selig WM, Schwartz CE, Ellis JL.(2004).Antitussive activity of sigma-1 receptor agonists in the guinea-pig. *Br J Pharmacol*.. 141, 233–240
4. Firdous AP, Kuttan G and Kuttan, R. (2015). Anti-inflammatory potential of carotenoid meso-zeaxanthin and its mode of action. *Pharm. Biol*. 53(7), 961-967.
5. Greenwald RA. (1991). Animal model for the evaluation of arthritic drugs. *Methods Find Exp Clin Pharmacol.* 13(2), 75–83.
6. Huang MH, Huang SS, Wang BS, Wu CH, Sheu MJ, Hou WC *et al.* 2011. Antioxidant and anti-inflammatory properties of *Cardio spermum halicacabum* and its reference compounds *ex vivo* and *in vivo*. *J Ethnopharmacol*. 133(2), 743-750.
7. Lalenti A, Lanaro A, Moncada S, Di Rosa M. (1995). Modulation of acute inflammation by endogenous nitric oxide. *Eur. J Pharmacol*. 211(2), 177-84.
8. Shah BN, Patel NP and Pandya P. (2008a). Role of leukotriene in inflammation and antileukotriene therapy.*J.Pharmacy Res*. 1(2), 113-123.
9. Sherena PA, Annamala PT, Mukkadan JK, Dinesha Ramadas. (2018). Biochemical assays of crude and purified oxycarotenoid extracts isolated from coriander leaves and their effect on oxidative stability of oils by Rancimat assay.*Research and reviews: A journal of biotechnology*. 8(1) , 1-10.
10. Wang D and Dubois RN (2010). The role of COX-2 in intestinal inflammation and colorectal cancer.

Oncogenes.29(6), 781-788.

11. Wheeler-Aceto H and Cowan A. (1991). Neurogenic and tissue mediate components of formalin-induced oedema. *Agents Actions.* 34, 264.
12. Young H, Luo Y, Chang H, Hsieh W, Liao J, and Peng W (2005). Analgesic and anti-inflammatory activities of Gingerol. J Ethnopharmacol. 96(1), 207 – 210.