

## Anti-allergic, anti-inflammatory and anti-lipidperoxidant effects of *Cassia occidentalis* Linn.

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Received 19 May 2009; revised 11 December 2009

*Cassia occidentalis* Linn. mast cell degranulation at a dose of 250 mg/kg, showed dose dependent stabilizing activity towards human RBC, with is widely used in traditional medicine of India to treat a number of clinical conditions including allergy and inflammatory manifestations. In the present study anti-allergic, anti-inflammatory and anti-oxidant properties of *C. occidentalis* whole plant ethanolic extract (CO) was investigated. Effects of CO on rat mast cell degranulation inhibition and human red blood cell (HRBC) membrane stabilization were studied *in vitro* following standard methods. The anti lipidperoxidant effects of CO were also studied *in vitro*. Effect of CO on carrageenan-induced mouse paw oedema inhibition was also assessed. CO significantly decreased maximum protection of 80.8% at 15 µg/ml. The extract also caused significant reduction in malondialdehyde (MDA) levels of murine hepatic microsomes at 100 µg/ml (56%) and significantly reduced carrageenan induced inflammation in mice at a dose of 250 mg/kg. Results of the present study indicated that CO inhibited mast cell degranulation, stabilized HRBC membrane thereby alleviating immediate hypersensitivity besides showing anti oxidant activity.

**Keywords:** Anti-allergy, Anti-inflammatory, Anti-lipid peroxidation, *Cassia occidentalis*, Mast cell degranulation, Malondialdehyde

India has an ancient history of the use of plants in the indigenous systems of medicine (Ayurveda, Unani and Siddha) dating back to over 5000 years. It has been estimated that over 8000 plants are used in traditional, folk and herbal medicines<sup>1</sup>. *Cassia occidentalis* Linn. belongs to the family Leguminosae and sub family Caesalpinaceae. It is known as 'Negro Coffee' or 'Stinking Weed' in English, 'Kasondi' in Hindi and 'Oolan Takara' or 'Ponnaiam' in Malayalam. The plant is found growing wild throughout India and is an erect, annual undershrub, about 60 – 150 cm in height. *C. occidentalis* is used for treating various allergic and inflammatory diseases in Indian traditional medicine and it has been reported to have anti-biotic<sup>2</sup> and hepatoprotective activity<sup>3</sup>. Phytochemical studies of *C. occidentalis* have shown the presence of anthroquinones, polyphenolics, flavonoids, tannins etc<sup>4</sup>. Despite its therapeutic use, its antiallergic and related

bioactivities have not been reported so far. In present study, anti-allergic, anti-inflammatory and anti-lipidperoxidant effects of ethanolic extract of the whole plant of *C. occidentalis* have been reported.

### Materials and Methods

*Plant material and preparation of extract*—The whole plants of *C. occidentalis* was collected from Aryankavu, Kollam district, Kerala, India during January 2007 when they were in flowering. Plant material was authenticated by Dr Mathew Dan, plant taxonomist of the institute. A voucher specimen has been deposited at the institute herbarium (TBGT 57018 dated 19/01/07). The plant material was washed thoroughly in tap water, shade dried and powdered. The powder (100 gm) was cold extracted with 1000 ml of ethanol overnight, at room temperature with constant stirring, as per the procedure adopted by Suja *et al*<sup>5</sup>. The extract was filtered and the filtrate concentrated under reduced pressure in a rotary evaporator (Superfit, India) to yield 8000 mg of the crude extract (8.0% with respect to the dried plant material). This crude extract (CO) was reconstituted in 0.5% Tween-80, to desired concentrations and used in the experiments.

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*Animals*—Wistar albino rats, males (200–300 g) and Swiss albino mice, males (20 – 30), were obtained from the institute's animal house. They were maintained under standard laboratory conditions (temperature 24° - 28°C, RH 60 - 70% and 12 h LD cycles) and fed commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, *ad libitum*. All experiments were carried out according to NIH guidelines, after getting the approval of the institute's Animal Ethics Committee. (No B-7/1/2008/123).

*Mast cell study in vitro*—Rats (4 groups, 6 per group) were used for the study. Group 1, the normal control group was given po, 1 ml of 0.5% Tween-80 whereas groups 2 and 3 were given CO (250 and 500 mg/kg, po, respectively) and group 4 animals were given disodium cromoglycate (DSCG - 10 mg/kg, ip) for 4 days prior to the collection of mast cells. After 4 days of treatment, 10 ml of normal saline was injected into the peritoneal cavity of all the animals and after gentle massage, the peritoneal fluid was collected and transferred into siliconized test tubes containing 7-10 ml of RPMI-1640 (pH 7.2 to 7.4). The collected mast cells were washed 3 times by centrifugation at low speed (400-500 rpm). The mast cells from normal or sensitized groups (control and treated rats) were incubated with egg albumin (1.0 mg/ml) respectively, at 37°C in a water bath for 10 min, stained with toluidine blue (1%), and percentage of protection against granulation was counted under compound microscope<sup>6</sup>.

*Stabilization study of human red blood cell (HRBC) membrane*—Blood was collected from 6 healthy volunteers after obtaining their prior informed consent. It was mixed with equal volume of sterilized Alsever's solution containing dextrose (2%), sodium citrate (0.8%), citric acid (0.05%), and sodium chloride (0.42%) in water, and centrifuged at 3000 rpm. The packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% v/v suspension was made with isosaline.

The reaction mixture contained varying concentrations of CO, 1ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Distilled water (2 ml) was used as control. They were incubated at 37°C for 30 min and centrifuged, and absorbance measured at 560 nm. The hemolysis (%) was calculated by assuming the hemolysis produced in the presence of distilled water as 100%. The HRBC

membrane stabilization or protection (%) was calculated by using the formula<sup>7</sup> given below:

$$\text{Protection (\%)} = \frac{100 - \text{OD of control}}{\text{OD of drug treated sample}} \times 100$$

*Anti-lipid peroxidation studies*—The anti-lipid peroxidant effect of CO was studied *in vitro*, following modified methods<sup>8,9</sup>. Briefly, 0.5 gm of the rat liver tissue was homogenized with 10 ml of 150 mM KCl-Tris-HCl buffer (pH 7.2). The reaction mixture contained 0.25 ml of liver homogenate, Tris - HCl buffer (pH 7.2), 0.05 ml of 0.1 mM ascorbic acid (AA), 0.05 ml of 4 mM FeCl<sub>2</sub> and 0.05 ml of varying concentrations of CO extract. The mixture (in triplicate) was incubated at 37°C for 1 hr in capped tubes. Then 0.5 ml of 0.1 N HCl, 0.02 ml of 9.8% sodium dodecyl sulphate (SDS), 0.9 ml of distilled water and 2 ml of 0.6% thiobarbituric acid (TBA) was added to each tube and vigorously shaken. All the tubes were then placed in a boiling water bath at 100°C for 30 min. After cooling, the flocculent precipitate was removed by adding 5 ml of n-butanol and they were centrifuged at 3000 rpm for 20 min. The TBA-chromogen was measured at absorbance 532 nm, with the spectrophotometer (Remi, India). Lipid peroxides in the liver tissue was expressed in terms of nmoles of thiobarbituric acid reactive substances (TBARS) produced/mg protein<sup>10</sup>.

*In vivo anti-inflammatory activity*—Mice were divided into 4 groups of 6 animals each. Oedema was induced by injection of 0.05 ml of solution of 3% w/v carrageenan type IV (Sigma Chemical Company, USA) in 0.9% saline solution into the subplantar region of the left hind paw. The volume of each paw up to the tibiotarsal articulation, was measured plethysmographically before the injection of carrageenan, and 3, 5, and 7 hrs later. Group 1, the carrageenan control was given only carrageenan, groups 2 and 3 were given 250 and 500 mg/kg of CO, before carrageenan injection and group 4 was given the standard drug, ibuprofen (80 mg/kg)<sup>11,12</sup>.

*Behavioural and toxic effects*—Acute oral toxicity study was performed as per Organization of Economic Cooperation and Development (OECD) guidelines<sup>13</sup>. Briefly, four groups of 12 mice were administered p.o. 250, 500, 1000 and 1500 mg/kg of the CO extract. They were observed continuously for 1hr for any gross changes, symptoms of toxicity and mortality if any and intermittently for the next 6hrs and then again, 24 hrs after dosing with CO extract.

**Statistical analysis**—Data were expressed as mean  $\pm$  standard deviation of the mean (SD), and statistical comparisons were performed using Student's 't' test<sup>14</sup>.

## Results and Discussion

**Mast cell degranulation study**—The results of mast cell degranulation showed that CO significantly prevented the degranulation of mast cells at 250 mg/kg dose. The higher dose (500 mg/kg) showed lesser protection from degranulation than the lower dose. This could be due to the fact that at the higher dose CO may even be cytotoxic to the mast cells. Both the doses of CO showed better protection than the standard drug, DSCG at 10 mg/kg dose (Table 1, Fig. 1).

**HRBC stabilization study**—CO showed dose dependent stabilizing activity towards HRBC. 79.21% membrane stability was observed at 25  $\mu$ g/ml concentration, and 80.8% membrane stability at 50  $\mu$ g/ml concentration. Beyond this concentration, no activity was observed (Table 2).

Table 1—Effect of *Cassia occidentalis* whole plant ethanol extract (CO) on mast cell degranulation induced by egg albumin

[Values are mean  $\pm$  SD of 3 experiments]

Groups	Mast cell degranulation (%)	Protection of mast cell degranulation (%)
Egg albumin Control (1.0mg/ml)	61	39
Egg albumin + DSCG (10mg/kg)	27	73**
Egg albumin + CO (250mg/kg)	5	95**
Egg albumin + CO (500mg/kg)	14	86**

\*\* $P \leq 0.01$ , compared to egg albumin control

From the above results it is clear that CO has significant anti allergic activity. CO significantly protected the peritoneal mast cells from antigen-induced degranulation. Disodium cromoglycate (DSCG), a mast cell stabilizer was the standard drug used in the study. DSCG inhibited mast cell degranulation by preventing release of inflammatory mediators from mast cells and enhancing their membrane stability<sup>15</sup>. In fact, the present study showed that CO is far more effective than the standard drug disodium cromoglycate (DSCG) in restoring membrane stability.

The mast cell is a well-known effector cell in allergic diseases. Histamines are mediators released from ruptured mast cells that play a major role in allergic response. The amount of histamine released depends on the number of mast cells that are degranulated or ruptured. Therefore if the number of degranulated mast cells can be controlled, then the allergic reaction can be controlled. The function of mast cells can be manipulated for therapeutic ends by regulating their function with appropriate drugs. In this direction, it appears that *Cassia occidentalis* can be a drug of choice. The mode of action of the active fraction (CO) in eliciting the mast cell inhibition response remains to be studied. Possibly, it influences

Table 2—Effect of ethanol extract of whole plant of *Cassia occidentalis* (CO) on membrane stability of human RBC

[Values are mean  $\pm$  SD of 3 experiments]

Groups	OD at 560 nm	Protection of membrane stability (%)
Control (Distilled water)	1.20	-
CO (25 $\mu$ g/ml)	0.25**	79.21
CO (50 $\mu$ g/ml)	0.23**	80.8
CO (100 $\mu$ g/ml)	0.38**	68.41
CO (200 $\mu$ g/ml)	0.75**	37.65

\*\* $P \leq 0.01$ , compared to distilled water control

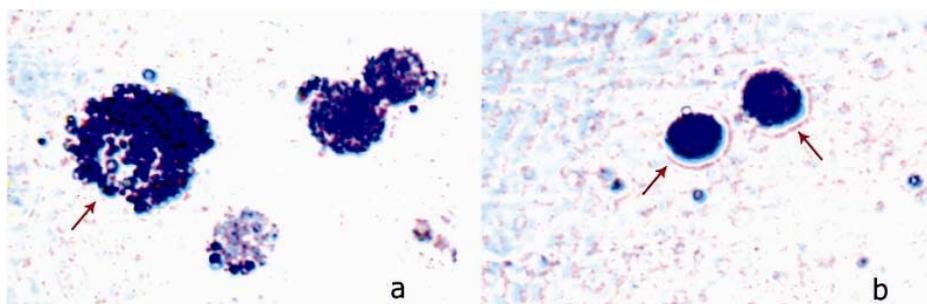


Fig. 1—Microphotographs showing effect of *Cassia occidentalis* whole plant ethanol extract (CO) on rat mast cell degranulation—(a): Rat mast cell degranulation caused by egg albumin (arrow)  $\times 125$ ; (b): Inhibition of mast cell degranulation by CO (arrows)  $\times 125$ .

differentiation into mast cells and/or mast cell chemical composition and/or architecture of mast cell surface membrane. It is also possible that CO may reduce the life span of mast cells.

*Anti-lipid peroxidation studies—In vitro* anti-lipid peroxidation studies using rat hepatic microsomes showed a significant reduction in the amount of malondialdehyde (MDA) released in a dose dependent manner at concentrations of 50 and 100 µg/ml. There was a significant increase of MDA in FeCl<sub>2</sub> – AA treated rat liver homogenate, compared to normal control without FeCl<sub>2</sub> – AA (Table 3).

Thus the present study showed CO significantly inhibited rat liver microsomal lipid peroxidation *in vitro* indicating its anti oxidant potential. Reactive oxygen species (ROS) are intimately related to the onset of inflammation. Free radicals derived from metabolites of unsaturated fatty acids can also induce allergic response by causing histamine release from mast cells<sup>16</sup>.

Protection against free radical mediated lipid peroxidation by plant extracts is of great significance for their traditional use against inflammatory and allergic disorders, many of which are associated with membrane damage and tissue recovery<sup>17</sup>. Disintegration of lysosomes has been correlated to the peroxidation of lysosomal lipids<sup>18</sup>. Therefore the beneficial effects of CO observed in the present study may also be from its role in the stabilization of lysosomes.

*Anti-inflammatory studies in vivo—In the anti-inflammatory study using carrageenan, it was seen that CO significantly reduced the inflammation at 250 mg/kg dose. The higher dose (500 mg/kg) showed a lower level of protection than the lower one (250 mg/kg). The inhibition of inflammatory response produced by the lower dose of CO was almost equal to that produced by 80 mg/kg of the standard drug, ibuprofen (Table 4).*

Treatment with CO inhibited mouse paw oedema induced by carrageenan. There are two phases of carrageenan induced inflammatory reaction, early or first phase and later or second phase. It has been proposed that the early phase results from histamine, serotonin and bradykinin liberation, while the late phase is associated with release of prostaglandin. The carrageenan induced paw oedema model in rat is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit

cyclooxygenase involved in prostaglandin synthesis<sup>19</sup>. Based on these reports, it can be inferred that the inhibitory effect of CO on carrageenan-induced inflammation in mice could be due to the inhibition of enzyme cyclooxygenase, thereby leading to inhibition of prostaglandin synthesis.

During inflammation, lysosomal enzymes are released into the cytosol, causing damage to the surrounding tissues, thereby producing a variety of disorders<sup>20</sup>. Studies have confirmed that non-steroidal anti-inflammatory agents act by stabilizing lysosomal membranes or inactivating already released enzymes. It was observed in the present study the CO showed human RBC membrane stabilizing effects. Our results are consistent with those of other workers on human RBC protection against heat and hypotonic induced lysis by saline by ethanolic extracts of medicinal plants<sup>21,22</sup>. As a result of structural similarity between lysosomal membranes and red blood cell components, the protection of HRBC membrane against heat and isotonic induced stress are taken as a measure of anti-inflammatory activity of the extracts.

Table 3—Inhibitory effect of ethanol extract of whole plant of *Cassia occidentalis* (CO) on FeCl<sub>2</sub> – AA induced lipid peroxidation in rat liver homogenate

[Values are mean ± SD of 3 experiments]

Group	CO (µg/ml)	MDA (n mole/mg protein)	Inhibition of MDA (%)
Normal control	-	1.35	-
FeCl <sub>2</sub> -AA control	-	2.77	-
FeCl <sub>2</sub> -AA + CO	25	1.91	31.01
FeCl <sub>2</sub> -AA + CO	50	1.58	43.15**
FeCl <sub>2</sub> -AA + CO	100	1.22	56.02**

\*\**P* ≤ 0.01, compared to FeCl<sub>2</sub> - AA control

Table 4—Anti-inflammatory activity of ethanol extract of whole plant of *Cassia occidentalis* (CO).

[Values are mean ± SD of 6 animals]

Treatment	paw volume (ml)		
	3 hr	5 hr	7 hr
Carrageenan control	0.30 ± 0.01	0.30 ± 0.02	0.30 ± 0.03
CO (250 mg/kg)	0.16 ± 0.02 (46.66)	0.15 ± 0.02** (50.00)	0.14 ± 0.01** (53.33)
CO (500 mg/kg)	0.21 ± 0.03 (30.00)	0.20 ± 0.01 (33.33)	0.19 ± 0.01 (36.66)
Ibuprofen (80 mg/kg)	0.16 ± 0.01 (46.66)	0.14 ± 0.01** (53.33)	0.13 ± 0.02** (56.66)

Values in parentheses indicate the % reduction of inflammation. \*\**P* ≤ 0.01, compared to carrageenan control

**Toxicity studies**—In the toxicity study, no mortality occurred within 24 hr with the 4 doses of CO tested. The LD<sub>50</sub> was therefore greater than 1500 mg/kg p.o. in mice (data not shown).

The results of the acute toxicity study therefore indicated that CO was fairly nontoxic, upto 1500 mg/kg p.o. This is not surprising as it is extensively used in traditional medicine as an anti-inflammatory and anti allergic agent.

Phytochemical studies of *Cassia occidentalis* have shown the presence of anthraquinones, polyphenolic compounds like flavonoids, tannins etc<sup>4</sup>. Tannins and their related compounds are well known to have antioxidant activity and also inhibit histamine release induced by superoxides in rat mast cells<sup>23</sup>. These findings indicate that antiallergic activities are closely associated with radical scavenging activities. There is a positive correlation between the presence of polyphenols and anti-inflammatory activity of plant extracts<sup>24</sup>. The anti-inflammatory, antioxidant and antiallergic effects of CO observed in the present study may therefore be due to the presence of flavonoids in it, since flavonoids are known to exert inhibitory effect on enzymes related to the production of inflammatory mediators<sup>4</sup>.

Findings of this study indicate antiallergic, antioxidant and anti-inflammatory effects of CO extract as evidenced by its capacity for inhibition of oedema formation, for mast cell degranulation inhibition and stabilization of HRBC membrane. The results suggest the potential of CO for the treatment of allergic and inflammatory diseases. Further studies are required to understand the exact mechanism of action of CO for the observed bioactivities.

### Acknowledgement

Thanks are due to Sri. K P Pradeepkumar for photographic assistance and S Radhakrishna Pillai for technical assistance.

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