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Antinociceptive activity of the ethanolic extract of the root bark of *Cassia sieberiana* (Fam. Caesalpinaceae)

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Abstract

The aqueous ethanolic extract of the root bark of *C. sieberiana*, a plant widely used in Ghanaian traditional medicine for pain relief of abdominal origin, dysmenorrhoea, ulcers and general body pains, was investigated for anti-nociceptive effect in a thermal model for pain and anti-colitic actions in rats. Algesia in the form of thermal stimuli was induced using the hotplate model and colitis by intra-colonic instillation of 2, 4, 6-tri-nitrobenzene sulphonic acid (TNBS). Both morphine (1 - 5 mg/kg, i.p.) and *C. sieberiana* extract (10 - 40 mg/kg, p.o.) caused dose-dependent anti-nociceptive effects in rats on the hotplate. The mean maximal analgesic effects occurred 30 minutes after administration of either morphine (1 - 5 mg/kg, i.p.) or extract. There was no statistical difference between the analgesic potency of the extract (EC50: 9.7±3.9) and that of morphine (EC50: 6.5±0.5). However, the analgesic action of *C. sieberiana* (40 mg/kg, p.o.) was less sensitive than that of morphine to the effect of naloxone (0.5 mg/kg, i.p.) which caused 14.7% and 75.3% reductions of the effects of *C. sieberiana* and morphine respectively. In rats with TNBS-induced colitis, whereas treatment with both Prednisolone (10 mg kg^{-1} p.o.) and 5-Aminosalicylic acid (5-ASA) (100 $mgkg^{-1}$ i.r.) significantly reduced the inflammatory features of the damaged colons, the extract (160 mg kg^{-1} p.o.) effect was less obvious. We conclude that the aqueous ethanolic extract of *C. sieberiana* possesses potential analgesic compounds but lacks compounds with promise for anticolitic actions.

Keywords: *Cassia sieberiana;* Analgesia; Anticolitic; Antinociceptive

Introduction

Pain is an important biological reaction of defence and a warning to prevent excessive tissue damage. Persistent and excessive pain however serves no useful purpose and requires interventions for relief. Thus, chronic pain is described as a major public health issue that affects the quality of life and productivity (Burgoyne, 2007). Pain is currently largely managed with pharmacological agents broadly classified as either opioid or non-steroidal anti-inflammatory drugs (NSAIDs). Unfortunately, current analgesics are associated with several undesirable and sometimes life threatening effects (Brooks *et al.*, 1982.). These side effects occur in about 30% of people taking NSAIDs (Hawkey,

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1996). Thus, as part of the continuing search for effective and safer analgesics from natural products, we investigated the aqueous ethanolic extracts of the root bark of *Cassia sieberiana* for antinociceptive actions in a thermal nociceptive assay using the hotplate test in rats (Dambisya and Lee, 1995). *C. sieberiana* is a tree that grows up to 50 ft high. The leaves are pinnate and often purplish and hairy when young. The flowers are prominent and yellow. It is widely distributed throughout the Savannah forest, and Coastal scrubs of West, Central and East Africa. Several uses have been reported for the root bark infusion when administered orally in traditional medicine including diuretic actions, aphrodisiac, anthelmintic actions and effects against gonococcal infections (Irvine, 1961). In Ghanaian traditional medicine, the root bark of *C. sieberiana* is used in to treat abdominal pains, dysmenorrhoea, ulcers and general body pain (Irvine, 1961; Mshana *et al.*, 2000). This information, coupled with reports of amelioration of colonic damage induced by 2,4,6- tri-nitro benzene sulphonic acid (TNBS) in rats through inhibition of PGE_2 production by rofecoxib, a cyclo-oxygenase-2 inhibitor, (Martin *et al.*, 2003), prompted us to also test the extracts of *C. sieberiana* for activity against experimental-colitis using TNBS-induced colitis (Morris *et al.*, 1989) in rats.

We report here profound antinociceptive actions for the aqueous ethanolic extracts of *C. sieberiana* but insignificant activity against colonic damage in rats.

Experimental

Animals. Wistar rats of either sex (180 - 250 g) were obtained from the Noguchi Memorial Institute for Medical Research, Accra and housed at a population density of n=5 in wooden cages (34 × 50 × 20 cm) with soft wood shavings as bedding. The animals were allowed water and food (normal rat feed pellets, GAFCO, Tema) *ad libitum* for the duration of the experiment. Room temperature was maintained at 28-30°C, relative humidity 60-70% and overhead fluorescent illumination was provided to maintain a 12-h light–dark cycle. The animals were kept under conditions similar to what one of us (MD) operated under a UK Home Office License for five years (i.e. animals were treated humanely, used once and euthanized at the end of each experiment)

Extract preparation. Pre-authenticated samples of *Cassia sieberiana* by botanists at the Centre for Scientific Research into Plant Medicine, Mampong-Akuapem, Ghana (CSRPM) were air-dried and powdered at the production unit of the CSRPM. The powdered root (550g) was extracted with 70% ethanol in water (2.5 L) at room temperature for 5 days. The extract was filtered and concentrated at 50°C under reduced pressure in a rotary evaporator to a semi-solid mass. The concentrated extract was dried over anhydrous $Ca(OH)_2$ in a desiccator. From a 550g sample of root bark, 73.9g of solid material was obtained, giving a percentage yield of 13.43 %. This was kept in a refrigerator maintained at 4°C. Suitable quantities were suspended in 1% tragacanth mucilage when required.

Selection of animals for analgesic test. The hotplate analgesic model (Dambisya and Lee, 1995) was used because of its effectiveness in investigating the involvement of both spinal and supra-spinal nociceptive processing. Prior to testing, all animals were subjected to a pre-screening test for homogeneity of response by subjecting them to heat stimulus from a hotplate (Harvard, Model #52-8570) maintained at a temperature of 55-56°C. A timer was started when all four paws made contact with the surface of the apparatus. Pain threshold was measured as the latency (seconds) to flutter or lick a hind paw or perform an escape response (i.e., jumping or

scurrying). Rats with pain threshold scores greater or less than two standard deviations from the group mean (critical reaction time) were omitted from the study. A cut-off time of 45 seconds was used to minimize the risk of tissue damage.

Evaluation of Cassia sieberiana extract for analgesia. Groups of rats (n=5) were given doses of either C. *sieberiana* suspension (10, 20, and 40 mg/kg, p.o) or morphine solution (1.0, 2.5, and 5.0 mg/kg i.p). Extract suspension and morphine solution were given in volumes not exceeding 100 ml/kg. The control group received normal saline (100 ml/kg, p.o.). Each rat was subjected to thermal stimuli from the hotplate (55-56°C) and its pain threshold was then taken at 0 and 15-minute-intervals for 60 minutes.

Analgesia, monitored as the percentage increase in pain threshold, was plotted against the time after drug administration. Drug or extract effects were assessed by either comparing the mean peak analgesia or total pain endured over the experimental period (monitored as area under the time-course curves) of extract- and morphine-treated groups with the corresponding values in the vehicle-treated control group.

In a separate experiment, the effects of naloxone (0.5 mg/kg, i.p) on morphine- (5 mg/kg) and extract- (40 mg/kg) induced analgesia were determined 30 minutes after administering the drugs.

Effect of extract on TNBS-induced colitis. Colitis was induced according to the method of Morris *et al.*, (1989). Briefly, the rats were lightly anaesthetised with ether to allow for intra-colonic insertion of a sterile polypropylene catheter (diameter 2.5 mm.) to a distance of approximately 8 cm from the anus. Then 0.25 ml of 30% 2, 4, 6-tri-nitrobenzene sulphonic acid (TBNS) in 50% (v/v) ethanol was instilled into the colon. Following the intra-colonic instillation of TNBS (the hapten), the animals were held in a head-down position for a few minutes to prevent leakage of hapten. Five groups (n = 5) of rats were used:

Group 1: Healthy control rats treated with saline only.

Group 2: Colitic control rats receiving 1% tragacanth mucilage (100 ml kg^{-1}, p.o.).

Group 3: Positive control I, colitic rats receiving prednisolone (10 $mgkg^{-1}$ p.o.) daily

Group 4: Positive control II, colitic rats receiving ASA (100 mg kg^{-1} i.r.) daily

Group 5: Test group, colitic rats receiving extract (160 mg)

Drugs were administered 18 h and 1 h prior to the induction of colitis and repeated daily throughout the experimental period. Rats in groups 1 and 2 received drug vehicle (1% tragacanth mucilage, p.o.) in a comparable volume (100 ml/kg body weight). Each rat was checked daily for changes in body weight and stool consistency. On day 5, the animals were sacrificed by cervical dislocation. Colonic tissue (8-10 cm) proximal to the anus was excised, opened longitudinally, and washed in saline to remove faecal matter. The tissue was then weighed and examined macroscopically for damage. After gross examination, the tissues were stored in 10% formalin in phosphate buffered saline and processed for histo-pathological examination.

Macroscopic assessment of colonic damage. Colonic damage for each rat was assessed using criteria described by Bobin-Dubigeon *et al.*, (2001). Damage scores (0–10) were assigned based on clinical features of the colon, the presence of tissue adherences and/or stool consistency (see Table 1).

Statistical analysis

Group means were compared using one-way ANOVA with Bonferroni's multiple comparison post test (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA).

Results

Analgesic activity of C. sieberiana on the hotplate. Both morphine (1 - 5 mg/kg, i.p.) and *C. sieberiana* (10 - 40 mg/kg, p.o.) produced dose-dependent increases in pain thresholds. The mean peak pain threshold for animals given either morphine or *C. sieberiana* occurred 30 minutes after extract administration (Figure 1). The maximal increases in pain threshold attained in the presence of morphine at dose levels of 1.0, 2.5, and 5.0 mg/kg i.p were 21.1±2.3%, 41.1±3.5%, 74.4±5.7% ($P < 0.01$) respectively (Figure 1A).. *C. sieberiana, at* 10, 20, and 40 mg/kg, p.o. caused maximal increases of 24.0±2.9%, 54.7±4.1%, and 57.3±4.3% ($P < 0.05$) respectively (Figure 1B).

Figure 2 represents the effects of the drugs on the total pain endured (monitored as area under the time-course curve) over the experimental period. The EC50 values for the extract and morphine were 9.7±3.9 and 6.5±0.5 respectively confirming the null hypothesis that the analgesic potency of the extract is the same as that of morphine. Figure 3 shows the effect of naloxone (0.5 mg/kg, i.p.).on the antinociceptive effects of morphine (5 mg/kg, i.p.) and *C. sieberiana* (40 mg/kg, p.o.). In the morphine-treated group, the mean pain threshold was significantly ($P<0.05$) reduced in the presence of naloxone by 75.3 %. The effect of *C. sieberiana* (40 mg/kg, p.o.) was reduced by only 14.6 %.

Effect of extract on TNBS-induced colitis

Control groups. Intra-colonic injection of tri-nitrobenzene sulphonic acid in rats produced persistent bloody and mucoid diarrhoea starting from day 1 to day 5. The gross histological features indicated an intense inflammatory response with oedema, extensive hyperaemia and ulceration (Figure 5: Panel A5). There was also extensive tissue adhesion in several areas of the colon to surrounding tissues. The mean colitic damage score was 9 (range: 8-10, Figure 4). Microscopic examination of the group revealed colons with evidence of severe colitis characterised by extensive disruption and deep haemorrhagic ulcers (Figure 5: Panel A5 & F). The animals were mostly prostrate with reduced movements and piloerection. Oedema, assessed as the wet colon weight/colon length was also greatest in this group (Table 2). Colons of rats in the sham group receiving normal saline did not show any abnormal histopathological features (Figure 5: Panel A1 & B) compared to the colitic control group (Panel A5 & F).

Effect of prednisolone and 5-aminosalicylic acid (5-ASA) on colitic damage. The inflammatory response was markedly reduced in these two groups. The histo-pathological features indicated a healing process with near normal mucosa and less obvious ulcerations (Figure 5: Panel A2 & 3, Panel C & D). The mean colonic damage score in the prednisolone-treated (10 $mgkg^{-1}$ p.o.) group was 3 (range: 3–5, Figure 4). Corresponding values in the ASA-treated group were 5 (range: 4-8). Motility in this group of animals was not affected. Oedema, (measured as wet colon weight/colon length) was significantly less (5-ASA: 140.7±18.7, Prednisolone: 123.8±11.1) than that of the colitic control group (Colitic control: 210.2±15.3; Table 2).

Effect of extract on TNBS-induced lesions. Daily administration of the extract produced a marginal and statistically insignificant ($P<0.05$) reduction in the inflammatory response (Figure 5: Panel A4 & E). The mean damage score of the resulting tissue with focal ulcerations and oedematous mucosa was 8 (range: 5 – 9, Figure 4). Though the oedema induced by TNBS was also reduced (180.9±17.5, it was not significantly different from that of the vehicle-treated colitic control group (210.2±15.3, -Table 2).

Table 1: Criteria for scoring macroscopic damage to intra-colonic instillation of tri-nitrobenzene sulphonic acid

Criteria	Score
Ulceration	
No damage	0
Focal hyperaemia	1
Ulceration without hyperemia or bowel wall thickening	2
Ulceration with inflammation at 1 site	3
≥ 2 sites of ulceration and inflammation	4
Major sites of inflammation < 2 cm along the organ	5
Major sites of inflammation > 2 cm along the organ	6
Adhesions	
No adhesion	0
Minor adhesion	1
Major adhesion	2
Diarrhoea	
Absence	0
Presence but no blood	1
Presence with blood	2
Maximum score	10

Table 2: Body weight change and colon wet weight/colon length on day 5 of colitis induction.

Group (n = 5)	% change in body weight	Colon weight/colon length (mg/cm)
Normal	+6 ± 2.3	106.3 ± 5.7
Colitic control	-5.7 ± 1.2	210.2 ± 15.3
5-ASA	+3.1 ± 1.7	140.7 ± 18.7
Prednisolone	+4.1 ± 0.9	123.8 ± 11.1
Extract	+2.8 ± 0.9	180.9 ± 17.5

Results are presented as mean ± s.e.m. (n=5).

Discussion

In this study we confirmed one of the traditional uses of *C. sieberiana* as an analgesic. *C. sieberiana* exhibited a dose-dependent anti-nociceptive effect as assessed on the hot plate test, a sensitive acute pain model for demonstrating opiate analgesia as well as several types of hyperalgesic reactions from spinal origin (Dambisya and Lee, 1995). Though this model is commonly used to test for analgesic efficacy of experimental drugs in rodents, it is limited by the fact that drugs that affect behaviour (e.g. sedatives, muscle relaxants and psychotomimetics) as well as mixed opiate agonist-antagonists can produce false antinociception in the model (Tjolsen *et al.*, 1991). The folkloric use of *C. sieberiana,* as a pain reliever coupled with preliminary indications in our hands of antinociceptive actions in the abdominal constriction model in mice (results not included here) indicate that our present finding in rats is a true analgesic effect.

The failure of naloxone to significantly antagonise the antinociceptive action of *C. sieberiana,* implies the extract exerts its analgesic actions via mechanisms other than that of the opiate system. This finding implies that the extract from *C. sieberiana* is also unlikely to interact strongly with either GABAergic, serotonergic or noradrenergic systems in the spinal cord as these systems are involved in antinociception induced by opioids (Fürst,, 1999). Opiates exert their analgesic effects *via* supra spinal and spinal receptors. It is not surprising that the effect of *C. sieberiana* extract as an analgesic cannot be clearly defined presently. Being a crude extract, the observed activity is probably a composite of effects attributable to the several constituents in the extract.

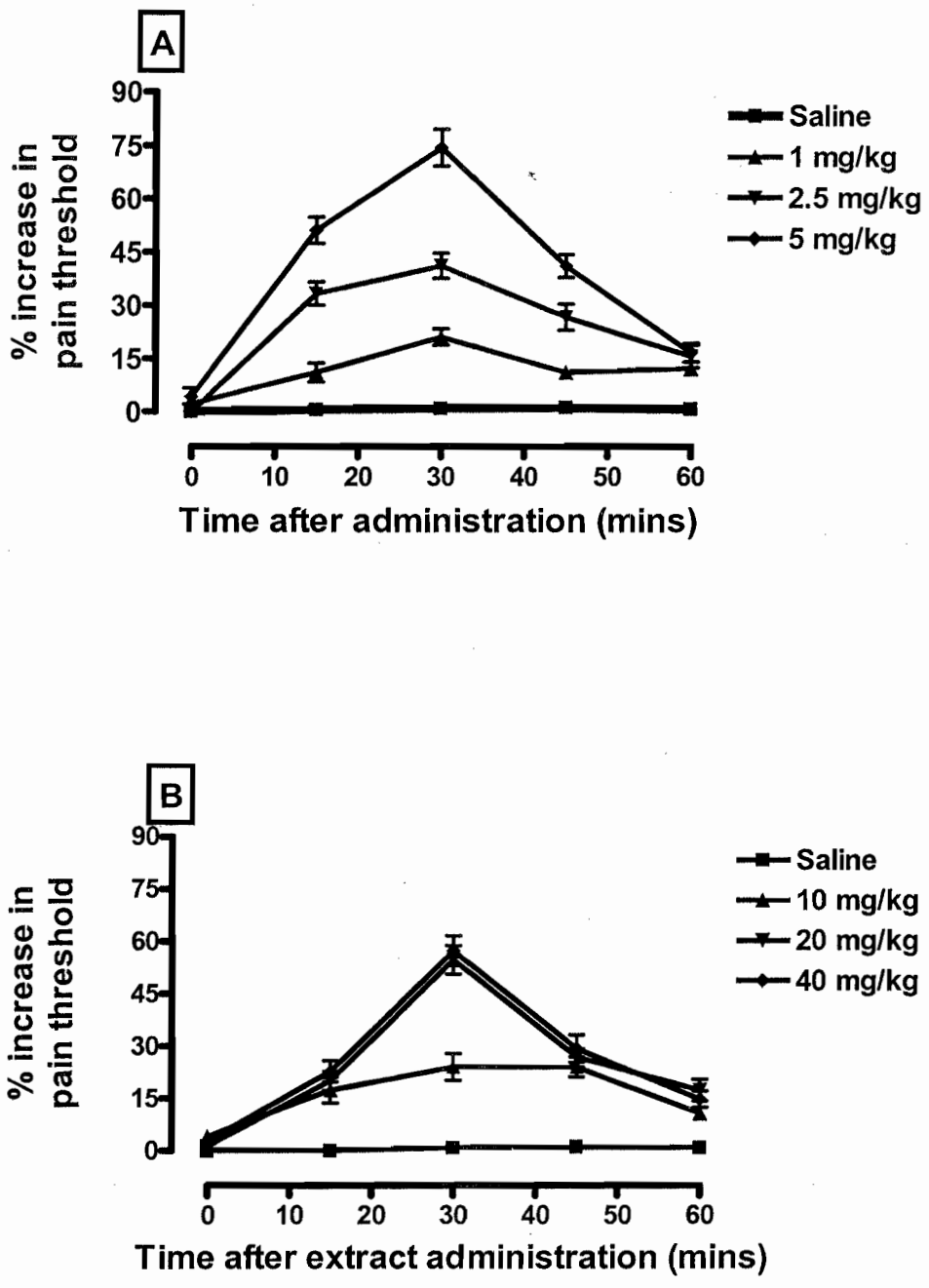


Figure 1: Time-course effect of morphine (1-5 mg/kg, i.p.) (**A**) and *C. sieberiana* (10-40 mg/kg, p.o.), (**B**) on mean pain thresholds of rats on the hot plate. Each rat was subjected to thermal stimuli from the hotplate (55-56°C) and its pain threshold was then taken at 0 and 15-minute-intervals for 60 minutes. The results are presented as mean ± s.e.m. (n = 5).

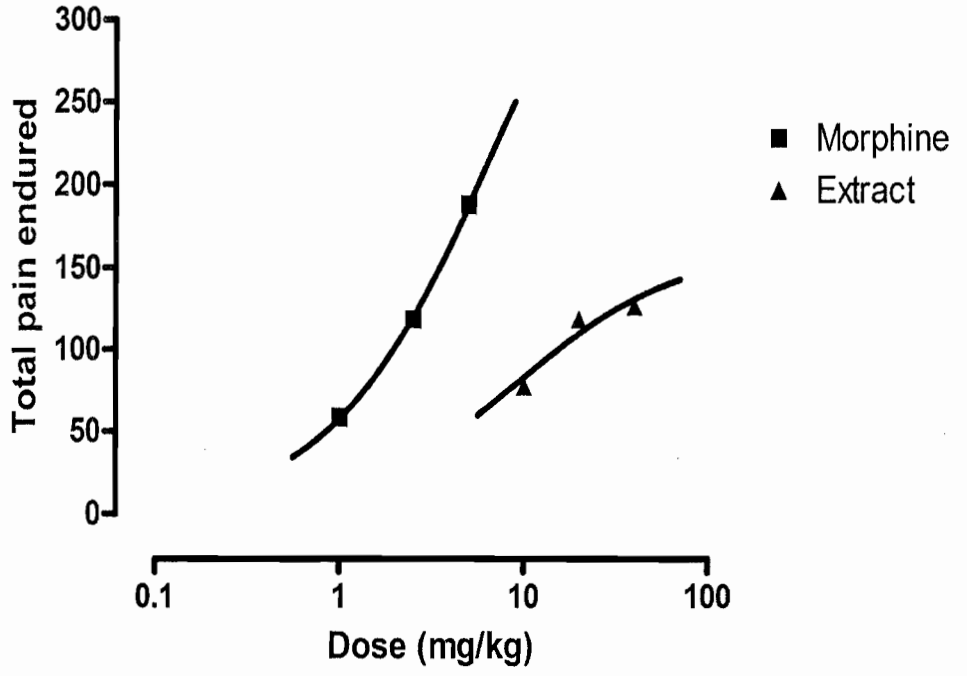


Figure. 2 Dose-effect relationship of *C. sieberiana* (10-40 mg/kg) and morphine (1.0-5.0 mg/kg) on total pain endurance of rats placed on the hotplate (monitored as area under the time-course curve) over the experimental period.

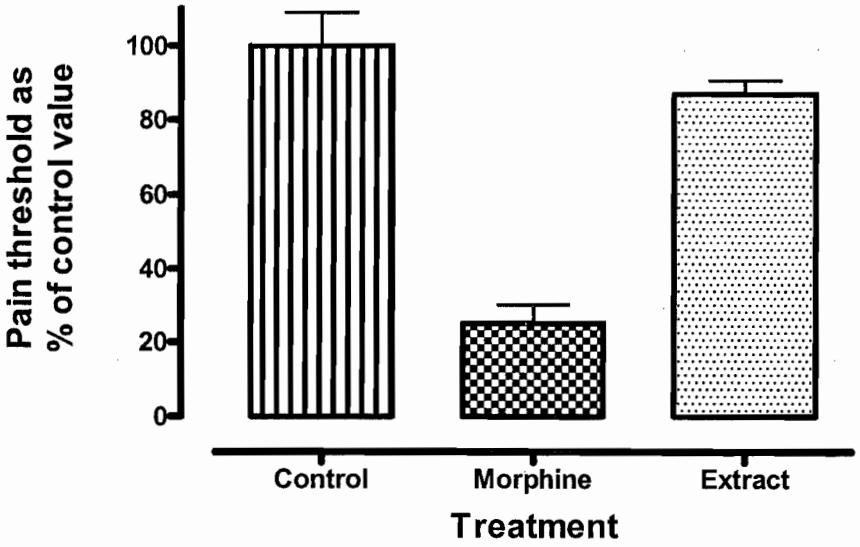


Figure 3: Effect of naloxone on the antinociceptive effects of morphine and *C. sieberiana.* Naloxone (0.5 mg/kg, i.p.) was administered 30 minutes before the administration of morphine (5.0 mg/kg, i.p.) and *C. sieberiana* (40 mg/kg, p.o.). The pain reaction times were determined 30 minutes after drug administrations. The results are presented as mean ± s.e.m. (n = 5).

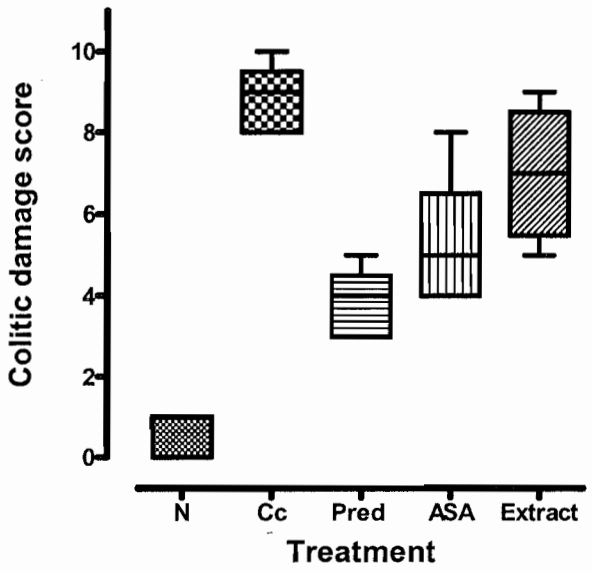
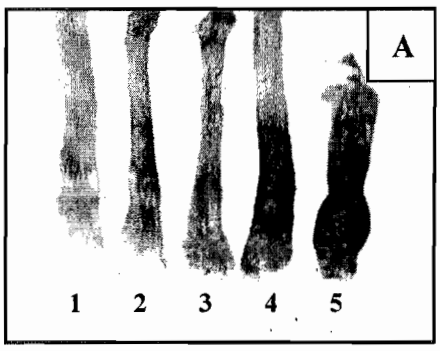


Figure 4: Effect of daily administration of *C. sieberiana* extract on colonic damage caused by trinitro-benzene sulphonic acid. N represents macroscopic damage in saline treated normal rats, Cc for colitic control rats, (Pred) for prednisolone-treated, ASA for aminosalicylic acid and extract for extract-treated group

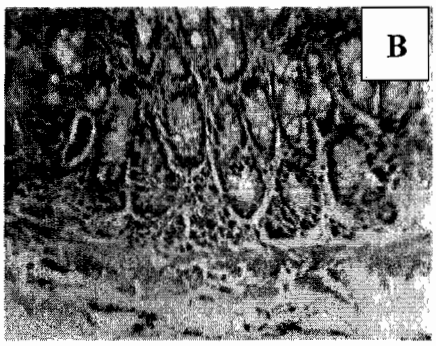
The resultant effect observed in this study appears to be more like an NSAID effect since naloxone had little effect on it.

On the assumption that the extract of *C. sieberiana* exerts its analgesic effects partly as a cyclooxygenase inhibitor, we tested it for activity against TNBS-induced colitis. The pathogenesis of inflammatory bowel disease is associated with prostanoid generation which negatively modulates the course of the disease (Carty *et al.*, 2000). Martin *et al.*, (2003), demonstrated up-regulation of cyclo-oxygenase-2 and a consequent elevation of prostaglandin E_2 production in TNBS-induced colitis. Prostaglandin E_2, a pro-inflammatory mediator, induces epithelial cell chloride secretion, causing the diarrhoea observed in inflammatory bowel disease. PGE_2 also modulates the intestinal immune response, including differentiation of T cells and the production and release of pro-inflammatory cytokines such as TNFα and interleukin (IL)-1h (Newberry *et al.*, 1999). Rofecoxib, a cyclo-oxygenase-2 inhibitor, was shown to attenuate the colonic damage induced by TNBS in rats through inhibition of PGE_2 production (Martin *et al.*, 2003). Other workers have reported beneficial actions of other COX-2 inhibitors, such as celecoxib or nimesulide, as through marked reduction of cellular infiltration and inflammation of the colon (Cuzzocrea *et al.*, 2001; Kankuri *et al.*, 2001). Following these reports and our finding that *C. sieberiana* could have components acting like NSAIDs we hypothesised that the extract would be effective against experimental colitis in rats.

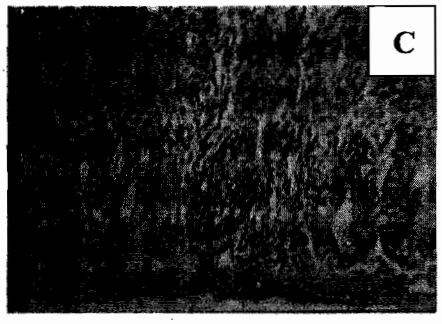
We demonstrated severe mucosal damage caused by TNBS instillation with histo-pathological features indicative of transmural necrosis, oedema and diffuse inflammatory cell infiltration in the mucosa. The gross morphological examination showed focal areas of ulceration of the colonic mucosa extending through the muscularis mucosae and loss of the epithelium.



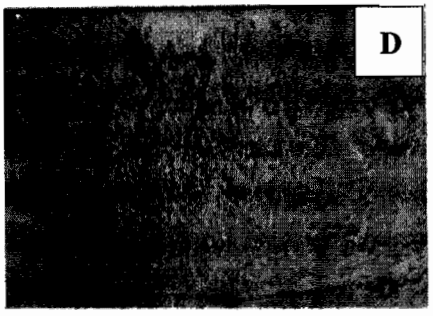
Gross histology of TNBS-induced colitis in the rat..



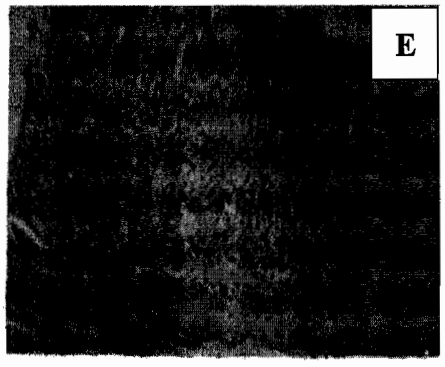
Normal colon



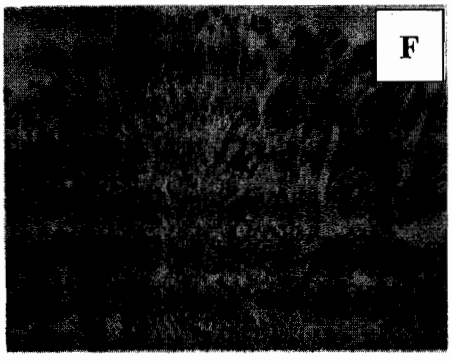
Prednisolone-treated



5-ASA - treated



Extract-treated



Colitic control

Figure 5: Effect of pre-treatment with cassia extract, prednisolone, and 5-ASA on the gross (panel A) and microscopic (panel B-F) features of TNBS-induced colitis in the rat colon. Agents were administered 18 h and 1 h before and every 24 h after induction of colitis. Each colon was excised and opened along the mesenteric border 5 days later. In the gross histology (Panel A), 1, 2, 3, 4, 5 represent saline-treated control, prednisolone-treated, 5-ASA-treated, *Cassia sieberiana*-treated, and vehicle-treated colonic control respectively. Panel B-F shows the corresponding microscopic histology.

Ulceration results in loss of the epithelium and mucin depletion thus depriving the mucosa of the protective actions of mucin and perpetuating the damage. Treatment of rats with prednisolone and ASA predictably markedly attenuated the extent and severity of the colonic injury, reducing the macroscopic indicators of damage. Treatment with *C. sieberiana* extract marginally reduced the colonic injury. The effect was extremely low in comparison with prednisolone and ASA. The use of a single dose of extract in the study is a major weakness. However, the dose (160 mg/kg)

used was four times the highest dose used for analgesic studies. Looking for activity at much higher doses than .this would at best indicate the presence of weakly acting constituents. Our inability to demonstrate profound protection against experimental colitis with an extract that possesses analgesic actions is consistent with the report of Lesch *et al.*, (1999) that despite potent extra-intestinal anti-inflammatory actions, COX-2 inhibitors do not appear to have any beneficial effect on TNBS colitis.

Conclusion: Our findings indicate that the ethanolic extract of *C. sieberiana* posses potent analgesic constituents but lacks components with anti-colitic actions at the dose levels tested.

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