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Purgative Activity of *Cassia tora* Leaf Extract and Isolated Aloe-Emodin

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From the 90% methanolic extract of the dried leaves of *Cassia tora* Linn., aloe-emodin, 1,8–dihydroxy–3–(hydroxymethyl)-anthraquinone has been isolated and identified. The purgative activity of the methonalic extract as well as that of isolated aloe-emodin from *C. tora* leaves was evaluated in Wistar rats. The extract as well as isolated material showed significant purgative activity in Wistar rats.

Cassia tora Linn. (Family, Caesalpiniaceae) is a well-known plant widely distributed in India and other tropical countries[1]. It is an annual undershrub and grows wild in wasteland. Various parts of the plant have been known possess medicinal value[1]. Several anthraquinones have been isolated from the seeds of *Cassia tora*[2-4]. Sennosides, which are well known for their medicinal importance, have been detected in the leaves of the plant[5]. The extracts of *Cassia tora* have been used as a remedy for various skin ailments, rheumatic disease and as laxatives[6-8]. The extract of *Cassia tora* leaves has been found to possess significant hepatoprotective activity and antiinflammatory activity[9-11]. Isolation of aloe-emodin from the dried leaves of *C. tora* is reported here. The purgative action of the methanolic extract and the isolated aloe-emodin from *Cassia tora* leaves are also reported.

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The leaves of *Cassia tora* were collected at the flowering stage in the month of mid August and September and shade dried and powdered. Coarsely powered leaves (500 g) were extracted with 90% methanol by cold percolation for 24 h. The extract was concentrated and yield of the dried mass was 18.2 g. Aloe-emodin was isolated from the dried extract and the isolated aloe-emodin were dissolved in 2% aqueous w/v Tween 80 solution to evaluate the purgative potential.

The dried methanolic extract was mixed with 50 ml of water and extracted with 200 ml of petroleum ether (60-80°) followed by extraction with 0.5 N potassium hydroxide. The KOH extract was acidified with dilute HCl to Congo red and further extracted with solvent ether. The solvent ether layer contained major anthraquinone pigments which were detected by TLC on silica gel G (benzene:methanol, 90:10)[12]. The TLC revealed one prominent fluorescent spot along with several minor spots under UV (225 nm) which

turned pink with 5% methanolic KOH[13]. The combined solvent ether extract was washed with water and dried over anhydrous sodium sulphate. The extract was concentrated and the solid residue was dissolved in benzene and chromatographed on silica gel G (E. Merck) column and on elution with benzene:methanol (9:1) an orange yellow coloured band separated out which showed orange fluorescence in the long wave UV. It was eluted with mixture of benzene:methanol (9:1). The fraction was evaporated to dryness. The residue was crystallized from toluene yielding 50 mg of orange coloured needle shaped crystals (m.p. 223–224°) (Found C–66.61; H–3.73, $C_{15}H_{10}O_5$, requires C– 66.57, H–3.71) λ_{max} (EtOH) 225, 254, 276.5, 287, 430 nm. The material showed bathochromic shift in absorption maximum at 430 nm to 510 nm when mixed with 0.01 N ethanolic NaOH which exactly corresponds with aloe-emodin[14]. V_{max} (KBr) 1627.8 and 1676 cm^{-1} (identical with aloe-emodin)[14]. Mass spectrum of the isolated anthraquinone was taken in Hitachi Model RMU–61 mass spectrophotometer. It showed ion peak at m/e 270 (M^+), 239 (loss of CH_2OH), 242 (loss of CO), 214 (loss of CO) and 211 (loss of CH_2OH) which are consistent with aloe–emodin structure.

Male Wistar rats weighing between 150–180 g were used in this study. They were acclimatized to conditions in the animal-housing unit (25±1°, 60–80% relative humidity and 12 h photo period for 1 w prior to the commencement of the experiment. The purgative action of the crude extract (100 and 200 mg/kg, p.o.) and material isolated from *C. tora* leaf (20 mg/kg, p.o.) were studied on two models. The animal experimental protocols were approved by the institutional animals ethics committee.

The characteristic diarrheal droppings were counted by administering the crude extract, isolated material and standard drug. The method followed was essentially that of Lou[15]. Food and water were withdrawn from the male rats weighing between 150-180 g early in the morning and the animals were put singly into each compartment of the cage. After 2 to 3 h, the feces were examined and any rat having soft or wet feces was discarded. They were divided into five groups of 10 rats each. Sennoside tablets (7.5 mg/kg, p.o.) were used as standard. 2% aqueous Tween 80 solution was used as control. The crude extract as well as isolated material was dissolved in 2% aqueous Tween 80 solution. For administering the drug, the rats were held firmly and a stomach tube was then passed gently down the esophagus. As it passed into the stomach, the animal exhibited a definite and characteristic gagging sound. The dose then given by means of a syringe attached to the stomach tube. After dosing the animals were kept under observation for at least 12 h. During the testing period, moistened food and fresh water was supplied in the food container. Purgative action was indicated by the excretion of the wet feces, which were recognized by their somewhat rounded shape and the presence of a brown stain surrounding each drop on the blotting paper. Counting of wet feces usually started 2 h after administration repeated for every half an hour till the end of

TABLE 1: EFFECT OF *CASSIA TORA* LEAF EXTRACT AND ALOE-EMODIN ON DIARRHOEAL DROPPINGS.

Oral treatment	Mean defecation per animal	Frequency of wet feces
Vehicle Control	1.2±0.43	1.2 ± 0.43
Sennoside (7.5 mg/kg,p.o.)	4.8±0.41	4.6±0.47*
C. tora extract (100 mg/kg, p.o.)	3.2±0.52	3.1±0.48
C. tora extract (200 mg/kg, p.o.)	4.4±0.46	4.0±0.42*
Isolated aloe–emodin (20 mg/kg, p.o.)	4.7±0.42	4.3±0.45*

P value was calculated by comparing with control by student's 't' test. *P<0.001. Values are mean±SEM, N=10.

TABLE 2: EFFECT OF *C.TORA* LEAF EXTRACT AND ALOE–EMODIN ON GI MOTILITY.

Treatment after charcoal meal	Movement of charcoal meal as percentage
Vehicle control	51.1±2.71
Sennoside (7.5 mg/kg p.o.)	85.5±2.24*
C. tora extract (100 mg/kg p.o.)	68.4±2.42
C. tora extract (200 mg/kg p.o.)	79.3±2.41*
Isolated aloe - emodin (20 mg/kg p.o.)	83.5±3.1*

P-value was calculated by comparing with control by student's 't' test. *P<0.001. Values are mean±SEM, N=10.

5th or 6th h. The final counting was done early in the following morning. The purgative action was compared to that produced by sennoside tablets, which were administered as a solution prepared by dissolving the powdered tablets in 2% w/v aqueous Tween 80 solution. The standard drug, sennoside, was given at a dose of 7.5 mg/kg. p.o.

Rats were fasted for 18 h and placed in five cages containing 10 in each group, charcoal meal (3% deactivated charcoal in 10% aqueous tragacanth) was administered orally to each animal. Immediately after that, the first three group of animals were administered orally with the extract of *C. tora* suspension (100 and 200 mg/kg, p.o.) and material isolated from it (20 mg/kg p.o.). The fourth group received orally sennoside solution (7.5 mg/kg p.o.) as the standard drug for comparison. The fifth group was treated with 2% aqueous Tween 80 solution alone and served as a control. Thirty minutes after the charcoal meal treatment, each animal was killed and the distance moved by the charcoal meal in the intestine from the pylorus was cut and measured and expressed as a percentage of the distance from the pylorus to the caecum. The results of the experiment are shown in Tables 1 and 2.

The results indicated that the crude extract and isolated material separated from *C. tora* leaf has exhibited purgative action as compared to standard drug as shown in Table 1 and 2. Both the treatments produced significant purgation and severity of diarrhoea in experimental animal model. Both the extract and isolated aloe–emodin increased significantly the frequency of defecation and wetness of fecal droppings when compared to the untreated rats. They also increase significantly the propulsion of the charcoal meal through the gastrointestinal tract when compared with the control group. All the effects obtained were comparable to that of the standard purgative, sennoside.

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