Absence of mutagenic and citotoxic potentiality of senna (Cassia angustifolia Vahl.) evaluated by microbiological tests.

Silva C.R*; Monteiro M.R; Caldeira-de-Araújo A.; Bezerra R.J.A.C.

Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcântara Gomes, UERJ

Abstract

Senna (*Cassia angustifolia* Vahl.) is widely used as laxative, but data from Ames test and animal and/or human studies with this agent have shown a mutagenic and carcinogenic potentiality. Using thee experimental models (bacterial inactivation test; bacterail mutagenisis assay-Mutoxitest; and growth Inhibition test, we investigated the toxicity of senna. Our data suggest an absence of mutagenic and citotoxic potentiality of senna.

Resumo

Sena (*Cassia angustifolia* Vahl.) é uma espécie amplamente empregada como laxativa, mas dados mutagênicos realizados com teste de Ames e estudos animais e/ou em humanos com esse agente tem mostrado uma potencialidade mutagênica e carcinogênica. Usando três diferentes testes (inativação de bactérias; ensaio de mutagênese em bactérias - Mutoxitest; teste de inibição de crescimento), foi investigada a toxidade dessa planta. Nossos dados sugerem uma ausência da potencialidade mutagênica e citotoxicidade de sena.

The use of medicinal plants has increased in the last decades all over the world. However, little information is available on their potential risk to health. Many plants contain mutagenic and/or carcinogenic substances and their frequent use has been correlated with a high incidence of tumors in the population[1,2].

Senna (*Cassia angustifolia* Vahl) is widely used in clinical practice and as a self-medication for chronic constipation. Chronic use or abuse of this agent can lead to a number of symptoms and signs, such as abdominal pain, nausea and diarrhoea[3,4,5]. Many studies have investigated the mutagenic and carcinogenic effects of senna in bacterial strains, animals and humans[4,5,6]. Ames test detected a weak mutagenic potentiality in *Salmonella typhimurium* TA102 and a stronger mutagenicity in *Salmonella typhimurium* TA97 and TA98[5].

The purpose of this work was to evaluate the cytotoxic and mutagenic effects of senna using *Eschericha coli* strains. Three experimental models were used to perform these studies: (a) bacterial inactivation test; (b) bacterial mutagenesis assays (Mutoxitest)[3] and growth inhibition test.

The data obtained indicate that senna, in the concentrations studied, did not exhibit cytotoxic effect to *E. coli* strains (Table 1). This was observed for *E. coli* AB1157, DNA repair wild-type strain and BW9091, repair mutant strain that is deficient in exonuclease III. This protein is closely associated to the processing step of oxidative damage in the base excision repair system.

Another assay for investigating the toxic effect of senna comprised using Growth inhibition test. The results obtained with *E. coli* IC203, IC204, IC205, IC206 and IC207 strains, specific indicators of lesions induced by ROS attack to DNA, were negative to all the concentrations of senna (Table 2). In addition, the results obtained in Mutoxitest showed that senna did not present mutagenic activity to *E. coli* IC203 and IC205 strains in the concentrations tested (Table 3).

The results obtained in this work, with E. coli strains, reinforce those from literature obtained with *Salmonella typhimurium* TA102 (5), pointing to an absence of toxicity of senna.

Material and Methods

E. coli AB1157 (wild type); BW 9091(*xth*A); IC203 (*uvr*A *oxy*R pKM101), IC204(*uvr*A del[*umu*DC]), IC205 (*uvr*A del[*umu*DC] *mut*M), IC206 (*uvr*A del[*umu*DC] *mut*Y) and IC207(*uvr*A del[*umu*DC] *mut*M *oxy*R) strains were used in this work[7,8,9]. Orient Mix Fitoterápicos do Brasil Ltda. (Rio de Janeiro, Brazil) provided powdered senna leaves in capsules (each capsule contained 2,5% of total sennosides). Senna was dissolved in 0.9% NaCl sterile solution just before use.

The culture media, solutions and procedure for growing cultures were performed as described in Alfaro et al. (2003), Blanco et al. (1998), Martinez et al. (2000) and Silva, et al. (1998)[10,11,12].

(1) Bacterial inactivation test: aliquots (1ml) of the *E. coli* AB 1157 or BW9091 strain, in the exponential phase, were incubated with shaking for 60 min. at 37°C with different concentrations of senna (0, 12.5, 25, 50, 125, 250 and 500µg/ml of culture). A sample was incubated in 0.9% NaCl sterile solution, under the same conditions, as a control. After the treatments, aliquots (100µl) were taken, diluted with 0.9% NaCl sterile solution and spread, in duplicates, on LB-plates, following incubation for 18 hours at 37°C. The colony-forming units were then scored and surviving fractions were expressed as the averages obtained from at least three experiments, perfoming six determinations[12].

(2) Mutoxitest: various concentrations of senna (0, 12.5,

25, 50, 125, 250 and 500µg/plate) were added to the overnight-cultured *E. coli* IC 203 or IC205 strain (100µl) and, afterwards, the entire mixture was added to molten top agar (2.5 ml) at 45°C. The mixture was then poured onto a minimal medium agar plate. The Trp^+ revertant colonies were counted after incubation at 37°C for 48h. In these experiments, each sample was assayed using triplicate plates and the data presented were the means of two experiments, amounting to 6 determinations[9,11].

(3) Growth inhibition test: 100 l of overnight cultures in nutrient broth were added to 3 ml of molten top agar at 45ºC and poured on LB medium plates supplemented with antibiotic, according to strain studied. Paper discs (5mm in diameter) were impregnated with 10µl of solutions containing one of the following concentrations of senna (0; 5; 12.5; 25; 50; 100; 125; 250 or 500µg/paper disc), placed on the solidified top agar plates and allowed to incubate overnight at 37oC. The diameter of the inhibition zone (in mm) was obtained by measuring the zone diameter and subtracting the disc diameter[10].

Table 1. Effect of different senna concentrations on the bacterial survival after 60 min. treatment.

	senna (µg/ml culture)						
Survival fraction	0	12.5	25	50	125	250	500
E. coli AB 1157	1,06	0,96	0,95	0,81	0,79	1,24	0,80
E. coli BW9091	0,90	0,91	1,1	0,99	0,99	1,1	1,2

Exponentially growing *E. coli* cultures were centrifuged, washed in 0.9% NaCl sterile solution and suspended in the same solution. Aliquots (1ml) of these suspensions were incubated with different senna concentrations for 60 min, at 37°C, with shaking. Afterwards, aliquots (100µL) were taken, diluted and plated onto LB medium for determinig surviving fractions for each strain at different senna concentrations. Values are the mean of three isolated experiments (six determinations) with standard deviations not exceeding 15%.

Table 2. Zone of inhibition diameter (mm) in *E. coli* strains after treatment with different senna concentrations

	Senna (µg/plate)							
E. coli strains	0	12.5	25	50	125	250	500	Control H_2O_2 (300µg/plate)
IC203	0	0	0	0	0	0	0	48
IC204	0	0	0	0	0	0	0	30.5
IC205	0	0	0	0	0	0	0	20
IC206	0	0	0	0	0	0	0	22
IC207	0	0	0	0	0	0	0	37.5

Aliquots (100µl) of exponentially growing cultures of *E. coli* were mixed with 3ml of top agar (45°C) and spread on LB medium plates supplemented with antibiotic (ampicillin or chloranphenicol, in accordance with the strain resistance). After 15 minutes, paper disc (5 mm of diameter) impregnated with different concentrations of the senna (10µl/plate) was placed in the center of the plate. After 24 hours of incubation, at 37°C, the growth inhibition zone formed around the disc was measured. Values are the mean of three isolated experiments (six determinations) with standard deviations not exceeding 15%.

Table 3. Mutoxitest: Mutagenic activity induced by senna in *E. coli* strains

Number of $Trp^- \rightarrow Trp^+$ revertants colonies/plate (mean± SD)		
senna (µg/plate)	*E. coli* IC203	*E. coli* IC205
0	119±16	18±4
5	127±21	20±1
25	134±25	20±3
50	135±25	22±1
100	103±15	23±6
125	120±18	18±2
250	142±10	17±2
500	157±29	17±1

Aliquots (100µl) of exponential growing *E. coli strains* were mixed with 100µl of different concentrations of the senna and top agar (2,5ml) at 45°C. The total volume was poured on minimal glucose agar plates supplemented with 0.5 mg tryptophan/litre.The mutagenic responses were expressed as the absolute number of Trp^+ revertants/plate, after incubation at 37°C, for 48h. Values are the means of three isolated experiments. Standard deviations did not exceed 15%.

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