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Research Article

Antimutagenic activity of cashew apple (*Anacardium occidentale* Sapindales, Anacardiaceae) fresh juice and processed juice (*cajuína*) against methyl methanesulfonate, 4-nitroquinoline N-oxide and benzo[a]pyrene

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Abstract

Cashew apple juice (CAJ), produced from the native Brazilian cashew tree (*Anacardium occidentale*), and has been reported to have antibacterial, antifungal, antitumor, antioxidant and antimutagenic properties. Both the fresh unprocessed juice and the processed juice (*cajuína* in Portuguese) has been shown to consist of a complex mixture containing high concentrations of anacardic and ascorbic acids plus several carotenoids, phenolic compounds and metals. We assessed both types of juice for their antimutagenic properties against the direct mutagens methyl methanesulfonate (MMS) and 4-nitroquinoline-N-oxide (4-NQO) and the indirect mutagen benzo[a]pyrene (BaP) using pre-treatment, co-treatment and post-treatment assays with *Salmonella typhimurium* strains TA100, TA102, and TA97a. In pre-treatment experiments with strains TA100 and TA102 the fresh juice showed high antimutagenic activity against MMS but, conversely, co-treatment with both juices enhanced MMS mutagenicity and there was an indication of toxicity in the post-treatment regime. In pre-, co-, and post-treatments with TA97a as test strain, antimutagenic effects were also observed against 4-NQO and BaP. These results suggest that both fresh and processed CAJ can protect the cells against mutagenesis induced by direct and indirect mutagens.

Key words: antimutagenicity; BaP, *cajuína*, MMS, 4-NQO, *Salmonella* microsome assay.

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Introduction

It has been reported that some chemical components of fruit and vegetables provide protection against a series of degenerative diseases in humans, such as cancer, cardiovascular diseases, cataracts and brain dysfunction (Ames, 2001; Cambie and Ferguson, 2003). The identification of antimutagenic compounds and the evaluation of their mode of action are extremely important for human health. For instance, polyphenols are a large and diverse class of natural compounds found in fruit and known for their beneficial antioxidant, antimutagenic, anticarcinogenic and anti-inflammatory properties (Duarte-Silva *et al.*, 2000; Gupta *et al.*, 2002; Franke *et al.*, 2004). Polyphenols may also prevent diseases by improving genomic stability (De Flora *et al.*, 2001; Ferguson, 2001). However, some of these compounds have mutagenic and pro-oxidant effects (Ferguson, 2001). There is limited conclusive data regarding the effects of complex mixtures, such as fruit juices and plant extracts, on genomic stability, but it is important to study them since these are the predominant forms in which fruit is consumed by humans.

The cashew tree (*Anacardium occidentale* Sapindales, Anacardiaceae), a tropical plant native to Brazil, produces a pseudo-fruit called the cashew apple that is commonly consumed in the northeastern Brazil as fresh cashew apple juice (CAJ) or as the processed juice (*cajuína* in Portuguese). Studies on the biological activities of these juices have shown that not only do they exhibit antibacterial, antifungal and antitumor activities (Kubo *et al.*, 1993a; Kubo *et al.*, 1993b) but also have antioxidant, antimutagenic and mutagenic properties against hydrogen peroxide (Melo-Cavalcante *et al.*, 2003) and aflatoxins B_1 (Melo-Cavalcante *et al.*, 2005), mainly due to the high concentra-

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tions of anacardic acid, ascorbic acid, carotenoids and phenolic compounds found in the juices.

Methyl methanesulfonate (MMS) is a monofunctional alkylating agent which can induce gene mutation, unscheduled DNA synthesis and sister chromatid exchange, as well as other genotoxic effects (Eder *et al.*, 2001; Sekihashi *et al.*, 2001). The direct mutagen 4-nitroquinoline-N-oxide (4-NQO), which binds covalently to DNA, has also been widely used in studies of mutagenesis and carcinogenesis. In fact, 4-NQO is an oxidative mutagen that undergoes redox recycling to generate superoxide radical and other reactive oxygen species (Nunoshita and Demple, 1993; Pungartnik *et al.*, 1999; Pungartnik *et al.*, 2002). Both of these adducts can induce misreplication events which result in mutations (Nagao and Sugimura, 1976; Daubersies *et al.*, 1992; Fronza *et al.*, 1992). Benzo[a]pyrene BaP is a polycyclic aromatic hydrocarbon widely distributed in the environment (Hattemer-Frey and Travis, 1991). It is an indirect-acting mutagen that exhibits biological effects after its metabolic activation by P450-dependent monooxygenase enzymes, thus producing electrophilic species capable of reacting with nucleophilic sites of DNA to form adducts (Shoker *et al.*, 2001; Jemnitz *et al.*, 2004). It may induce both *in vitro* and *in vivo* gene mutations and chromosomal aberrations (Waters *et al.*, 1991; Schoket *et al.*, 2001) as well as DNA single-strand breaks (Garry *et al.*, 2003).

During the study described in the present paper we investigate the antimutagenic properties of fresh unprocessed (CAJ) and processed (Cajuína) using the *Salmonella*/microsome assay and MMS and 4-NQO as direct mutagens and BaP as a metabolically activated mutagen.

Materials and Methods

Preparation of fresh and processed CAJ

The juices were produced using cashew apples from Piauí state in northeastern Brazil, the fruit having been bought in an open-air market in the town of Teresina and transported to our laboratory in a refrigerated container. During transport the fruits were kept in thermally insulated containers at 5 °C. Visual inspection showed that all the fruits were in good condition, with no bruising, discoloration or obvious infection by fungi or bacteria. We obtained cashew apples which were pesticide-free and organically cultivated to general ones regarding antimutagenic activities. To produce fresh unprocessed juice the cashew apples were washed, surface sterilized in 70% (w/v) ethanol for about five seconds followed by flaming, macerated and the juice sieved using sterile equipment. The fresh juice was frozen at -20 °C until needed. Before freezing, a sample of the juice was tested for microorganisms by using standard microbiological tests for bacteria and fungi (Thatcher and Clark, 1972). Processed juice (*cajuína*) was produced from the fresh juice according to a commercial process (Lili Doces (Lili Sweets), Teresina, Piauí) which, in addition to the steps described above, included centrifugation, clarification with gelatin, filtration and heating at 100 °C for 1 h (Melo-Cavalcante *et al.*, 2003).

Antimutagenicity assay

We assessed the antimutagenicity capability of the fresh and the processed CAJ using the *Salmonella typhimurium*/microsome assay with the methodological variations described by Melo-Cavalcante *et al.* (2003).The *Salmonella typhimurium* test strains used were TA100 (*his G46, bio chlD uvrB gal, rfa, pKM101*), TA102 (*his G428, rfa, pKM101, pAQ1*) and TA97a (*his 01242, bio chlD uvrB gal, rfa, pKM101*) described by Maron and Ames (1983) and were kindly supplied by Dr. B.N. Ames, University of California, Berkeley, USA. The Moltox microsomal S9 fraction, prepared from the livers of Sprague-Dawley rats treated with the polychlorinated biphenyl mixture Aroclor 1254, was purchased from a commercial company (Molecular Toxicology Inc., USA) and the S9 metabolic activation mixture was prepared according to Mortelmans and Zeiger (2000). The standard mutagens MMS, BaP and 4-NQO were dissolved in dimethyl sulfoxide, all of which were purchased from Sigma. For the antimutagenicity assay, separate overnight cultures of *S. typhimurium* strains TA100, TA102 and TA97a were washed and resuspended in 5 mL of 0.2 M phosphate buffered saline solution PBS (pH 7.4) to produce suspensions containing between 1 x 10^9 and 2 x 10^9 cells mL^{-1}. The strains were chosen according to their sensitivity to mutagens with strains TA100 and TA102 being used, separately, in conjunction with 2 μg $plate^{-1}$ of MMS plus 500 μL per plate of the S9 mix while strain TA97a was used with 0.5 μg $plate^{-1}$ of 4-NQO without the S9 mix and, in a separate assay, with 1 μg $plate^{-1}$ of BaP plus 500 μL per plate of the S9 mix. Doses of fresh juice (10 μL, 25 μL and 50 μL $plate^{-1}$) and processed juice (100 μL, 500 μL and 2000 μL per plate) were arbitrarily selected in preliminary assays designed to ascertain the dose range using a 20 min pre-incubation procedure at 37 °C with the addition of 100 μL $plate^{-1}$ of the appropriate bacterial strain with and without 500 μL $plate^{-1}$ of the S9 mix. The final criterion used to select the doses of fresh juice was non-toxicity. The following controls were used: for mutagen, H_2O + mutagen + bacteria ± S9 mix; for juice, H_2O + juice + bacteria ± S9 mix; for S9 mix, juice + bacteria + mutagen, with omission of S9 fractions; and for bacteria, H_2O + bacteria ± S9 mix.

The antimutagenicity of each dose of fresh or processed juice against mutagen was calculated as follows: percentage of inhibition (PI%) = [1 - (B - A/B)] x 100, where A represents the number of revertants $plate^{-1}$ containing the mutagen and B represents the number of revertants $plate^{-1}$ containing the mutagen plus fresh or processed juice. The number of spontaneous revertants was

subtracted from all plates. The antimutagenic effect of the juices at non-toxic doses was given as the fifty-percent inhibitory dose (ID_{50}), *i.e.*, the dose of juice producing a 50% reduction of mutagenicity in the test system. The result "indication of toxicity" was given when the number of *his*$^+$ revertant colonies on plates with juice and mutagen was less than 70% in relation to the number of spontaneous revertants (negative control). Co-mutagenic activities were considered when the number of revertants on the plates with juices and mutagen was higher than that containing mutagen only. Data were expressed as means and standard deviation and statistically analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences between each juice treatment and its positive mutagenic control were considered significant at $*p \leq 0.05$ and $**p \leq 0.01$.

Results and Discussion

The fresh juice showed up to 100% antimutagenic potential against MMS for strains TA100 and TA102, but only for the pre-treatment protocol (Table 1). Dose-dependence relationship did not occur, possibly because the three doses of fresh juice reached their maximum potential as either antimutagens or co-mutagens and, therefore, in most cases a significant difference was not observed between the three doses. In the pre-treatment protocol the cells were grown for 4 h in a nutrient medium with juice, allowing functional components from the fresh juice, such as anacardic acid, present 17.9 ± 0.4 mg 100 g^{-1} juice, and ascorbic acid, present at 120.8 ± 4.1 mg 100 g^{-1} y, to enter into the cells (data from Melo-Cavalcante *et al.*, 2003). These chemicals may have contributed to the antimutagenic effect by increasing the defense against MMS in the pre-treatment due to the induction of protective adaptive systems. Oxidative stress-inducing agents up-regulate DNA polymerase beta and base excision repair (BER), increasing the resistance to alkylating agents such as MMS (Chen *et al.*, 2001; Halliwell, 2001; Cabelof *et al.*, 2002). Another possible mechanism is the protection against MMS by reducing the capacity of this chemical to alkylate DNA. Ascorbic acid may compete with DNA as a target for alkylation, reducing the genotoxicity of alkylating agents (Vijayalaxmi and Venu, 1999). The processed juice, conversely, showed an indication of toxicity in strain TA100 and MMS co-mutagenicity in strain TA102 when the pre-treatment protocol was applied (Table 1). Although the same basic components occur in both the fresh and processed juices (Melo-Cavalcante *et al.*, 2003) we used higher concentrations of the processed juice, which may have elicited pro-oxidative effects from its components.

When the both types of juice were incubated with MMS under the co-incubation protocol for 20 min without washing before the addition of bacteria in co-treatment A, potential induction of MMS mutagenicity was observed (Table 1). In this case, possibly, there was insufficient time in this treatment for the induction of protection adaptive systems against MMS by the components of the juices, and/or extracellular reactions may have increased the formation of reactive agents to DNA.

In the post-treatment protocol involving strains TA100 and TA102 both juices produced an indication of toxicity, suggesting that the damage produced by MMS was not repaired by the juices and, in fact, the damage led to cell death (data not shown). It is known that DNA alkylation by monofunctional alkylating agents induces error-prone repair in bacteria, which can induce lethal mutations (Eder *et al.*, 2001).

With 4-NQO, the juices showed antimutagenic activities in the TA97a pre-treatment (Table 2), 4-NQO being a well-known mutagen that undergoes redox recycling to generate the superoxide radical and other reactive species (Nunoshita and Demple, 1993; Brendel *et al.*, 2003) which may be scavenged by antioxidant components of the juices such as phenols and anacardic acid (Melo-Cavalcante *et al.*, 2003; Trevisan *et al.*, 2006). Similar effects were also observed in strain TA97a during co-treatment A, with the antimutagenic potential reaching 99% (Table 2). In this system, antimutagenicity increased with the dose during both pre-treatment and co-treatments, but decreased in the post-treatment with fresh juice. There was no correlation between dose and effect in the treatments with processed juice (Table 2). The differences observed were related to the changes in the experimental procedure characterizing each treatment protocol (see above).

Both juices also showed post-treatment antimutagenic activities against 4-NQO in strain TA97a (Table 2), suggesting the presence of chemical compounds with effects on DNA repair against damage induced by 4-NQO. Several phenolic compounds, including tannic acid, present in both the fresh and processed juices have a role in the regulation of DNA-repair enzymes, reducing the yield of mutations (Cooke *et al.*, 1998). In addition, by scavenging reactive species the antioxidant chemicals in the juices may have protected the TA97a cells against oxidative damage induced by 4-NQO.

Under most treatment protocols both juices showed antimutagenic responses in TA97a against the indirect mutagen BaP (Table 3). The processed juice showed an antimutagenic potential of approximately 100% and no co-mutagenic response, while the fresh juice was a potent antimutagenic agent in co-treatments A and B and in post-treatment and showed a co-mutagenic effect only in during pre-treatment (Table 3). It is known that BaP exhibits its biological effects after metabolic activation by P450-dependent monooxygenase enzymes to produce electrophilic species capable of reacting with nucleophilic sites of DNA to form adducts (Schoker *et al.*, 2001; Hiramatsu *et al.*, 2004; Kim *et al.*, 2005). The components of the juices probably interacted with the active metabolites of BaP gener-

Table 1 - Effects of cashew apple juice (CAJ) against methyl methanesulfonate (MMS) using *Salmonella typhimurium* strains TA100 and TA102 as indicator organisms. Percentage inhibition (PI) calculated as PI = [1 - (B - A/B)] X 100, where A represents plates containing mutagens and B represents the plate containing mutagens and juice. Antimutagenicity = PI ≥ 50%. Indication of toxicity when the number of revertant colonies < 70% compared to number of spontaneous revertants. Co-mutagenicity when there is a significant increase in mutagenicity as compared to positive control. The number of spontaneous revertants was subtracted from all plates. Values are means ± standard deviations (SD). Protocols for CAJ testing: pre-treatment, juice + bacteria in fresh nutrient broth for 4 h, wash bacteria and add mutagen for 20 min, wash bacteria and plate; co-treatment A, juice + mutagen for 20 min, add to the bacteria and plate; and post-treatment A, bacteria + mutagen for 20 min, wash bacteria, add the juice and plate.

Fresh juice					Processed juice				
	Number of *his*$^+$ revertant colonies plate^{-1} (Mean ± SD for three replicates)					Number of *his*$^+$ revertant colonies plate^{-1} (Mean ± SD for three replicates)			
Protocols and CAJ dose (µL plate^{-1})	Strain TA100	PI	Strain TA102	PI	Procedure and CAJ dose (µL plate^{-1})	Strain TA100	PI	Strain TA102	PI
Pre-treatment					Pre-treatment				
10	987 ± 7*	67	202 ± 3*	100	100	220 ± 1*	-	2500 ± 300**	-
25	645 ± 9*	77	234 ± 18*	86	500	63 ± 0.1*	-	2381 ± 300**	-
50	922 ± 9*	64	213 ± 4*	96	2000	0 ± 0	-	2200 ± 102**	-
Response	Antimut		Antimu		Response	Indic		Co-mut	
Co-treatment					Co-treatment				
10	2113 ± 63*	-	2437 ± 283*	-	100	-		2200 ± 69**	-
25	1798 ± 119*	-	2274 ± 138*	-	500	392 ± 4*		2500 ± 170**	-
50	2499 ± 137*	-	2456 ± 86*	-	2000	2500 ± 400*		2500 ± 200**	-
Response	Co-mut		Co-mut		Response	Co-mut		Co-mut	
Post-treatment A					Post-treatment A				
10	0 ± 0	-	0 ± 0	-	100	52 ± 1*	-	0 ± 0	-
25	0 ± 0	-	0 ± 0	-	500	53 ± 9*	-	0 ± 0	-
50	0 ± 0	-	0 ± 0	-	2000	39 ± 1	-	0 ± 0	-
Response	Indic		Indic		Indic	Indic		Indic	
MMS 2 µg plate^{-1}	2257 ± 511	-	430 ± 1	-	MMS 2 µg plate^{-1}	2.316 ± 516		720 ± 1	-
Spontaneous revertants	170 ± 16	-	203 ± 6	-	Spontaneous revertants	232 ± 18	-	240 ± 31	-
Juice control					Juice control				
10	218 ± 25	-	286 ± 5	-	100	235 ± 3	-	245 ± 11	-
25	209 ± 39	-	276 ± 10	-	500	262 ± 7	-	302 ± 12	-
50	290 ± 34	-	263 ± 6	-	2000	390 ± 15	-	307 ± 28	-

Antimut = Antimutagenicity. Co-mut = Co-mutagenicity. Indic = Indication of toxicity.
*Statistically significant compared to the MMS at *p ≤ 0.05 and **p < 0.01 by one-way analysis of variance ANOVA followed Dunnett's Test. A dash (-) indicates data not available.

ated by the presence of the S9 mix, resulting in the formation of non-mutagenic compounds. It is known that phenolic compounds can form complexes with BaP and reduce its bioavailability (Chen and Yen, 1997; Mejía *et al.*, 1999; Gupta *et al.*, 2002). Furthermore, ascorbic acid may inhibit the metabolic activation of BaP (Cooke *et al.*, 1998; Rauscher *et al.*, 1998).

A similar pattern of responses was found in previous studies in which fresh and processed CAJ protected *S. typhimurium* strain TA102 against DNA damage induced by aflatoxin B1. The mechanism responsible for this protection included the possible interaction with S9 enzymes, and the transformation of aflatoxin B1 and its mutagenic metabolites to non-mutagenic compounds (Melo-Cavalcante *et al.*, 2005). The stimulation of repair and/or reversion of DNA damage may have been another antimutagenic mechanism of fresh and processed CAJ during post-treatment. This protection may be attributed to the presence in both juices of chemically-active components shown to be involved in the protection of DNA (Melo-Cavalcante *et al.*, 2003; Melo-Cavalcante *et al.*, 2005). However, fresh juice increased BaP mutagenicity in the

Table 2 - Effects of cashew apple juice (CAJ) against 4-nitroquinoline N-oxide (4-NQO) using *Salmonella typhimurium* strain TA97a as indicator organism. Percentage inhibition (PI) calculated as PI = [1 - (B - A/B)] X 100, where A represents plates containing mutagens and B represents the plate containing mutagens and juice (Melo-Cavalcante *et al.*, 2003) Antimutagenicity = PI ≥ 50%. Indication of toxicity when the number of revertant colonies < 70% compared to number of spontaneous revertants. Co-mutagenicity when there is a significant increase in mutagenicity as compared to positive control. The number of spontaneous revertants was subtracted from all plates. Values are means ± standard deviations (SD). Protocols for CAJ testing: pre-treatment, juice + bacteria in fresh nutrient broth for 4 h, wash bacteria and add mutagen for 20 min, wash bacteria and plate; co-treatment A, juice + mutagen for 20 min, add to the bacteria and plate; and post-treatment, bacteria + mutagen for 20 min, wash bacteria, add the juice and plate.

Fresh juice			Processed juice		
	Number of *his*$^+$ revertant colonies plate^{-1} (Mean ± SD for three replicates)			Number of *his*$^+$ revertant colonies plate^{-1} (Mean ± SD f or three replicates)	
Protocols and CAJ dose (µL plate^{-1})	Strain TA97a	PI	Procedure and CAJ dose (µL plate^{-1})	Strain TA97a	PI
Pre-treatment			Pre-treatment		
10	389 ± 43*	60	100	250 ± 86*	70
25	346 ± 69*	66	500	234 ± 3*	82
50	321 ± 53*	70	2000	198 ± 24	76
Response	Antimutagenicity		Response	Antimutagenicity	
Co-treatment			Co-treatment		
10	514 ± 57*	43	100	322 ± 67*	70
25	397 ± 26*	60	500	347 ± 34*	66
50	111 ± 2*	99	2000	194 ± 23*	87
Response	Antimutagenicity		Response	Antimutagenicity	
Post-treatment A			Post-treatment A		
10	173 ± 37*	90	100	177 ± 3*	-
25	265 ± 96*	78	500	133 ± 10*	-
50	269 ± 51*	77	2000	-	-
Response	Antimutagenicity		Indication of toxicity	Antimutagenicity	
4-NQO 0.5 µg plate^{-1}	823 ± 184	-	4-NQO 0.5 µg plate^{-1}	823 ± 184	-
Spontaneous revertants	104 ± 18	-	Spontaneous revertants	104 ± 18	-
Juice control			Juice control		
10	74 ± 7	-	10	108 ± 12	-
25	118 ± 17	-	25	104 ± 8	-
50	124 ± 23	-	50	108 ± 12	-

*Statistically significant compared to the 4-NQO at *p ≤ 0.05 by one-way analysis of variance ANOVA followed Dunnett's Test. A dash (-) indicates data not available. ANOVA followed Dunnett's Test. A dash (-) indicates data not available.

pre-treatment, suggesting a co-mutagenic effect (Table 3). Again, a similar response was reported in experiments involving aflatoxin B_1 when fresh juice was tested using pre-treatment of strain TA102 (Melo-Cavalcante *et al.*, 2005). Many known antimutagenic chemicals of juices may also act as co-mutagens after metabolic activation and, moreover, polyphenols can be mutagenic, depending on whether they are present before, during, or after exposure to the relevant mutagen (Ferguson, 2001; Kozubek *et al.*, 2001; Geetha *et al.*, 2005).

In conclusion, the results indicate that both fresh and processed CAJ may be useful for protection against direct and indirect mutagens. The antimutagenic activities of these juices may have been mediated by some or all of the following: the generation of scavenger reactive species in treatments with 4-NQO; the induction of adaptive systems in treatments with MMS; the enhancement of DNA repair in treatments with 4-NQO and BaP; the inhibition of metabolic activation in treatments with BaP; and the interaction with mutagen or pro-mutagen in treatments with MMS and BaP. Therefore, both fresh and processed cashew apple juice may not be merely a source of nutrients but also a complex mixture of chemical compounds with beneficial properties for improving genomic stability.

Table 3 - Effects of cashew apple juice (CAJ) against benzo[a]pyrene (BaP) using *Salmonella typhimurium* strain TA97a as indicator organism. Percentage inhibition (PI) calculated as PI = [1 - (B - A/B)] X 100, where A represents plates containing mutagens and B represents the plate containing mutagens and juice (Melo-Cavalcante *et al.*, 2003) Antimutagenicity = PI ≥ 50%. Indication of toxicity when the number of revertant colonies < 70% compared to number of spontaneous revertants. Co-mutagenicity when there is a significant increase in mutagenicity as compared to positive control. The number of spontaneous revertants was subtracted from all plates. Values are means ± standard deviations (SD). Protocols for CAJ testing: pre-treatment, juice + bacteria in fresh nutrient broth for 4 h, wash bacteria and add mutagen ± S9 mix for 20 min, wash bacteria and plate; co-treatment, juice + mutagen for 20 min + S9 mix for 20 min, add to the bacteria and plate, (method B) mutagen + S9 mix for 20 min, add juice and incubate for 20 min, add the bacteria and plate; post-treatment A, bacteria + mutagen + S9 mix for 20 min, wash bacteria, add the juice and plate; and post-treatment B, bacteria + mutagen + S9 mix for 20 min, wash and incubate with juice in fresh broth for 30 min wash bacteria and plate.

Fresh juice			Processed juice		
	Number of *his*$^+$ revertant colonies plate^{-1} (Mean ± SD for three replicates)			Number of *his*$^+$ revertant colonies plate^{-1} (Mean ± SD f or three replicates)	
Protocols and CAJ dose (µL plate^{-1})	Strain TA97	PI	Procedure and CAJ dose (µL plate^{-1})	Strain TA97	PI
Pre-treatment			Pre-treatment		
10	770 ± 94*		100	222 ± 5*	73
25	1345 ± 123*		500	109 ± 20*	99
50	1350 ± 147*		2000	108 ± 16*	99
Response	Co-mutagenicity		Response	Antimutagenicity	
Co-treatment			Co-treatment		
10	411 ± 82	38	100	385 ± 48*	62
25	364 ± 34*	50	500	514 ± 16*	44
50	365 ± 19*	49	2000	286 ± 59*	75
Response	Antimutagenicity		Response	Antimutagenicity	
Post-treatment A			Post-treatment A		
10	399 ± 22*	42	100	453 ± 73*	53
25	330 ± 97*	57	500	295 ± 40*	74
50	278 ± 61*	69	2000	395 ± 55*	60
Response	Antimutagenicity		Indication of toxicity	Antimutagenicity	
Post-treatment B			Post-treatment B		
10	251 ± 73*	75	100	166 ± 3*	80
25	224 ± 68	81	500	141 ± 50*	95
50	303 ± 75*	65	2000	208 ± 22*	86
Response	Antimutagenicity		Response	Antimutagenicity	
BaP 1 µg plate^{-1}	583 ± 129	-	BaP 1 µg plate^{-1}	837 ± 63	-
Spontaneous revertants	141 ± 26	-	Spontaneous revertants	108 ± 3	-
S9 mix control					
10	138 ± 12			103 ± 5	-
25	130 ± 11			173 ± 5	-
50	298 ± 36			282 ± 16	-
Juice control			Juice control		
10	145 ± 9	-	10	98 ± 13	-
25	137 ± 13	-	25	192 ± 13	-
50	128 ± 17	-	50	215 ± 25	-

*Statistically significant compared to the BAP at *p ≤ 0.05 by one-way analysis of variance ANOVA followed Dunnett's Test. A dash (-) indicates data not available. Followed Dunnett's Test. A dash (-) indicates data not available.

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