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# DNA damage induced by an extract of *Mangifera indica* stem bark using *in vitro* Comet assay

[Daño inducido al ADN por un extracto de la corteza de *Mangifera indica* mediante un ensayo Cometa *in vitro*]

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**Abstract**
The aqueous standard extract of *Mangifera indica* L stem bark (MSBE) is used as a food supplement in Cuba. In this study, the genotoxic effect of MSBE was measured using different variants of the *in vitro* Comet assay in human lymphocytes and rat hepatocytes incubated with MSBE at 37°C for 1 hour. Lymphocytes were incubated with MSBE for the subcellular (at two different pH conditions) and the standard Comet assays, in presence of catalase or $S_9$ microsomal fraction. Hydrogen peroxide, benzo(a)pirene and UV radiation were used as positive controls. Results from standard and subcellular Comet assays clearly showed that MSBE (50 µg/mL) induced primary DNA damage to lymphocytes. This genotoxic effect was slightly reduced when lymphocytes were incubated with MSBE plus catalase, which suggests that hydrogen peroxide is involved in this DNA injury. $S_9$ fraction also decreased MSBE-induced damage to DNA in human lymphocytes. Not genotoxic effect was observed when rat hepatocytes were exposed at MSBE, suggesting that the metabolic activity can be involved in the elimination of the DNA damage generated by the MSBE. In conclusion, MSBE causes primary DNA injury of human lymphocytes *in vitro* Comet assay, but not in rat hepatocytes in similar conditions.

**Keywords:** Comet assay; genotoxicity; *in vitro; Mangifera indica;* Vimang.

**Resumen**
El extracto acuoso de la corteza de *Mangifera indica* L. (MSBE) es usado como suplemento alimenticio en Cuba. En este estudio se determinaron los efectos genotóxicos de MSBE mediante diferentes variantes del ensayo Cometa *in vitro* en linfocitos humanos y hepatocitos de rata incubados con MSBE a 37°C por 1 hora. Los linfocitos fueron incubados con MSBE para la realización de los ensayos Cometa subcelular (a dos condiciones de pH diferentes) y estándar, en presencia de catalasa o fracción microsomal $S_9$. Peróxido de hidrógeno, benzo(a)pireno y radiación UV fueron usados como controles positivos. Los resultados de los ensayos Cometa, tanto subcelular como estándar, mostraron que MSBE (50 µg/mL) indujo daño primario al ADN de los linfocitos. Este efecto genotóxico fue ligeramente reducido cuando las células fueron incubadas con MSBE más catalasa, lo que sugiere que el peróxido de hidrógeno está involucrado en este daño al ADN. La fracción $S_9$ también decreció el daño inducido por MSBE al ADN en linfocitos humanos. No fueron observados efectos genotóxicos cuando los hepatocitos de rata fueron expuestos a MSBE, sugiriendo que la actividad metabólica pudiera estar involucrada en la eliminación del daño al ADN generado por MSBE. En conclusión, MSBE causa daño primario al ADN de linfocitos humanos en el ensayo Cometa *in vitro*, pero no en hepatocitos de rata bajo condiciones similares.

**Palabras Clave**: Ensayo Cometa; genotoxicidad; *in vitro; Mangifera indica;* Vimang.

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## INTRODUCTION

Polyphenolic compounds, including a large group of flavonoids, are abundant in vegetables, fruits, wine and tea. These compounds are generally known to possess potent antioxidant properties, which depend on their free radical scavenging capacity and on their iron chelating activity (Collins, 2005). Many diseases are associated with oxidative stress (Parthasarathy *et al*., 2001) and the use of antioxidant-rich food or food supplements is often recommended to preserve or regain good health. Therefore, large population segments frequently take high dosages of these compounds (Diplock, 1994). However, antioxidant compounds may exhibit important adverse effects after having exerted their presumed protective action, i.e., after having neutralized a reactive species; they may be converted into potentially harmful oxidation products (Boots *et al*., 2002).

*Mangifera indica* L. stem bark aqueous extract (MSBE) is a natural product obtained in Cuba with the formulations under the brand name of Vimang (Center of Pharmaceutical Chemistry, 2002). Chemical studies performed with this extract have led to the isolation and identification of polyphenols, terpenoids, steroids, fatty acid and microelements (Núñez *et al*., 2002; Núñez *et al*., 2007). MSBE has demonstrated an antioxidant activity both *in vitro* and *in vivo* (Martinez *et al*., 2000; Martinez *et al*., 2001; Sanchez *et al*., 2000), and for this reason is used mainly as a nutritional or food supplement. General toxicological screening of the extract showed that it has low toxic potential, since it was only toxic after the acute exposure intraperitoneally (Garrido *et al*., 2009). On the other hand, genotoxicological evaluations previously showed that MSBE is not mutagenic or genotoxic *in vitro* (Ames test and Micronucleus assay) (Rodeiro *et al*., 2006) and *in vivo* (Comet and Micronucleus assays) (Gonzalez *et al*., 2007). Nowadays the determination of the genotoxic potential of complex mixtures obtained from plants has gained increasing interest, due to these mixtures may contain toxic and genotoxic compounds (Sánchez-Lamar *et al*., 2002). The aim of this work was to deepen on the effects of MSBE on primary DNA damage using several variants of the *in vitro* Comet assay.

## MATERIALS AND METHODS

### *Plant material*

*Mangifera indica* Linneo (Anacardiaceae) was collected from a cultivated field located in the region of Pinar del Rio, Cuba. Voucher specimens of the plant (Code: 41722) were deposited at the Herbarium of the Academy of Sciences, guarded by the Institute of Ecology and Systematic from Ministry of Science, Technology and Environment, La Habana, Cuba and authenticated by MSc Ramona Prieto curator and MSc Isora Baró, Director of the Herbarium. Stem bark extract of *M. indica* was prepared by water decoction for 1 h, and then the extract was concentrated by evaporation and sprays dried in a Niro Atomizer Standard Spray Drying (Soeborg, Denmark) to obtain a fine brown powder (MSBE), which melts at 210 - 215 $^{o}$C, with decomposition (Acosta-Esquijarosa *et al*., 2009). MSBE is used as the standardized pharmaceutical active ingredient of Vimang formulations. The chemical composition of this extract has been characterized by chromatographic (planar, liquid and gas) methods, mass spectrometry and UV/VIS spectrophotometry (Núñez *et al*., 2002; Núñez *et al*., 2007). MSBE was dissolved in 0.2% dimethylsulfoxide (DMSO) for the tests. DMSO has been shown to have no genotoxic effect (Aye *et al*., 2010).

### *Chemicals*

Collagenase and β-glucorinadase/arylsulfatase were obtained from Roche (Barcelona, Spain), Ham's F-12, Lebovitz L-15 medium, calf serum bovine, serum albumin, and fraction V were acquired from Gibco (Madrid, Spain). Hydrogen peroxide ($H_2O_2$), benzo-a-pirene, dimethylsulfoxide and catalase were purchased from Sigma (St. Louis, MO, USA) and sodium phenobarbital and 5, 6-β-naphtoflavone from BDH, (Poole, England).

### *Cells*

#### *Isolation of human lymphocytes*

Human peripheral blood was obtained by venous puncture from healthy, adult, young and non-smoking volunteers (Marianao Blood Bank, Havana). Lymphocytes were separated using a modification of the centrifugation method of Boyum (1968). Briefly, 100 µL of heparinised whole blood was mixed with ice-cold RPMI-1640 (900 µL). Then, 100 µL of cold lymphocyte separation medium (LSM) was layered at the bottom of the tube and the sample was centrifuged at 4500 rpm for 3 min at room temperature. The lymphocytes in the upper part of the LSM layer were transferred to a new vial. At this stage, the cells were washed twice with RPMI-1640 (0.5 mL), using

centrifugation for 3 min at 4500 rpm. The final pellet, consisting of approximately 0.4 - 2.0 × $10^5$ lymphocytes, was resuspended in PBS. Cellular viability was determined by exclusion method with Trypan Blue (0.4%). The cellular concentration was adjusted to 1 × $10^5$ cells/mL.

***Isolation and culture of rat hepatocytes***

Hepatocytes were obtained from Sprague Dawley male rats (200 - 300 g b.w.) by perfusion of the liver with collagenase as described elsewhere (Castell & Gómez-Lechon, 1997). Cell viability of the suspension, assessed by the trypan blue exclusion test, was higher than 85%. Cells were seeded at a density of 8 x $10^4$ viable cells/$cm^2$ in Ham's F-12/Lebovitz L-15 (1:1) medium supplemented with sodium selenite (170 µg/mL), 2% calf serum, 0.2% bovine serum albumin, 50 mU/mL of penicillin, 50 µg/mL of streptomycin and 10 nM insulin and were then incubated at 37 ºC in a 5% $CO_2$ humidified atmosphere. Unattached cells were removed by changing the medium 1 h after plating.

***Genotoxicity of MSBE using Comet Assays (single cell gel electrophoresis: Standard Comet Assay***

Isolated lymphocytes and hepatocytes (centrifuged at 1500 rpm during 5 min and suspended in RPMI 1640 medium) were incubated with MSBE (50, 100, 200, 500 and 1000 µg/mL, respectively) at 37 ºC during 1 hour. Comet assay was essentially performed as described by Singh *et al*. (1988) with a few modifications (Collins *et al*., 1993). Twenty µL of the cell suspensions were embedded in 100 µL of 1% low melting point agarose (LMPA) and, using a cover slip, they were spread on a slide pre-coated with a film of 1% normal melting point agarose. Two slides were prepared for each sample in which agarose cell suspensions were allowed to solidify at 4 ºC. After that, slides were transferred to lysis solution (2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris, 1% Tritón X-100, pH 10) at 4 ºC for 1 hour. Slides were placed in an electrophoresis chamber exposed to alkali (0.3 M NaOH, 0.01 M EDTA, pH 13) for 20 min. Then, electrophoresis was performed for 20 min at 0.8 V/cm and 300 mA. Assay variability was reduced by ensuring that replicate slides were randomly distributed among multiple electrophoresis runs in a fully balanced design. After electrophoresis slides were neutralized (three times) and stained according with the protocol described by García *et al*. (2004). The stained nuclei were classified in five levels of DNA damage and a score was calculated following the formula:

DNA damage score = Nuclei level 1 + 2 (nuclei level 2) + 3 (nuclei level 3) + 4 (nuclei level 4). Then if all nuclei are in level 0 (undamaged) the score is 0 and if all the nuclei are in level 4 (maximum damage) the score is 400 expressed in Arbitrary units (AU) (Collins *et al*., 1993; Collins *et al*., 2004).

***Subcellular Comet Assay***

This assay was performed following the procedure of Kasamatsu *et al*. (1996), except for the silver staining that was conducted according to García *et al*. (2004). Isolated lymphocytes were resuspended in PBS (100 µL), and approximately 10 µL were added to Eppendorf microcentrifuge tubes. Eighty-five µL of pre-warmed (40 ºC) 1% LMPA in PBS were added to each microcentrifuge tube, and gently mixed with the washed cells. Each cell suspension was immediately applied to a microscope slide, which had been precoated with 85 µL of 1% standard agarose in PBS. The slides were placed at 4 ºC until the gel layer solidified. Lysis of cells was then performed by submerging the slides in lysis solution in the absence of light for 1 h at 4 ºC. After lysis, slides were then immersed in 0.4 M phosphate buffer (pH 7.4) for 10 min at room temperature. Each slide was then transferred to a Petri-dish containing 20 mL of MSBE at testing concentrations (50, 100 and 200 µg/mL) and incubated for 1 hour at 37 ºC. After incubation slides were washed in 0.4 M phosphate buffer (pH 7.0) at 4 ºC for 5 min (once) and transferred to two Koplin jars for DNA unwinding and expressing a) single strand breaks (SSB), double strand breaks (DSB) at pH 12.1 and b) alkali-labile sites (ALS) at pH 13, during 20 min at 4 ºC. Electrophoresis was conducted at 4 ºC for 20 min at 0.8 V/cm and 300 mA. The slides were then immersed 3 times in 0.4 M Tris-HCL buffer (pH 7.5) for 5 min. The staining and quantification of damage was already described in Section Standard Comet Assay.

***Comet Assay in presence and absence of catalase enzyme***

Human peripheral blood separated lymphocytes were incubated with 50, 100 and 200 µg/mL of MSBE in presence and absence of catalase enzyme (3500 U/mg), as well as in presence and absence of inactive catalase enzyme (100 ºC during 10 min) at 37 ºC for 1 hour, according with Cemeli et al. (2009). After these

treatments, lymphocytes were embedded in LMPA to carry out the standard Comet assay.

***Comet Assay in presence and absence of $S_9$ fraction***

Isolated lymphocytes were mixed with 50, 100 and 200 µg/mL of MSBE in presence and absence of $S_9$ fraction (10% v/v) at 37 ºC for 1 hour. After the incubation time cells were embedded in LMPA for carry out the standard Comet assay. $S_9$ fraction was prepared from young adult male Sprague Dawley rats. According to INVITOX Protocol (1990), animals were sacrificed 5 days after receiving daily an intraperitoneal injection of sodium phenobarbital 30 mg/kg (day 1) and 60 mg/kg (days 2-5). A single administration of 5,6-β-naphtoflavone (80 mg/kg, ip) on third day was also administered. The 9000 ×g liver supernatant ($S_9$) was split into 1 mL aliquots, that were frozen and stored at -80 ºC.

***Statistical analysis***

In all variants of Comet assay the values of descriptive statistics are shown as mean ± SD. The data were evaluated using non-parametric Jonckheere trend and Mann-Whitney tests. The *a priori p* level for statistical significance was $\alpha = 0.05$ in all cases.

## RESULTS

***Standard Comet Assay in human lymphocytes***

In order to get a primary DNA damage profile characterization of the natural extract obtained from *Mangifera indica* L. stem bark the standard Comet assay was conduced. As can be observed in Figure 1, the results of this assay indicate that MSBE was genotoxic at the primary structure level of DNA at the assayed concentrations (50 - 1000 µg/mL). The extract increased DNA damage in a statistically significant and concentration dependent manner (50 - 500 µg/mL) with respect to non-treated control.

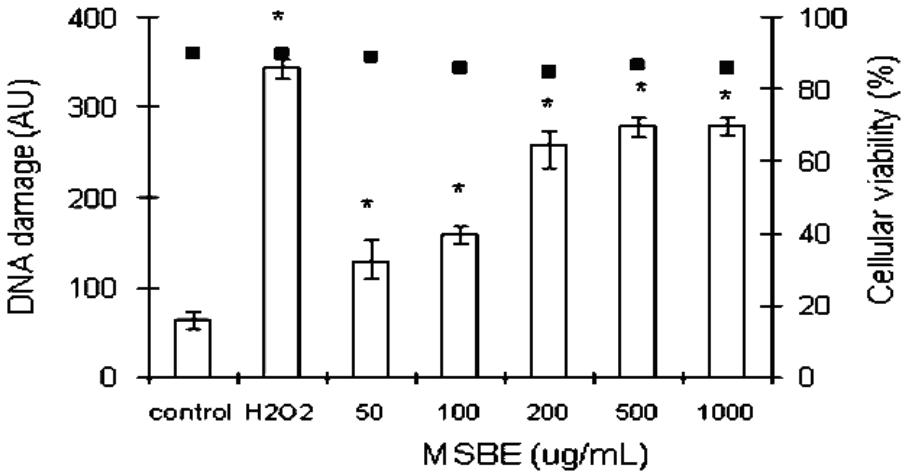


**Figure 1**

**Effect of MSBE on human lymphocytes DNA, after 1 hour incubation. Values are expressed as arbitrary units (mean ± S.D, of at least three independent assays). $H_2O_2$ (100 µM) as positive control and dimethylsulfoxide (0.2%) as negative control. Cellular viability was assessment by the Trypan Blue exclusion method. (%): represents number of life cells x 100/cells total number. *p < 0.01 treatment effect tested by Jonckheere Trend Test.**

***Subcellular Comet Assay in human lymphocytes***

Sub-cellular variant of Comet assay using different pH conditions during electrophoresis (12.1 and 13) can be relevant for showing the nature of DNA damage. This assay demonstrated that MSBE induce damage on naked DNA of isolated lymphocytes in both pH conditions for unwinding and electrophoresis proved, which are statistically significant for 50 and 100 µg/mL of MSBE in pH 12.1 and 13, respectively (Figure 2). These results also showed that the genotoxic effect observed was higher at pH 13 electrophoresis conditions than at pH 12.1 (Figure 2).

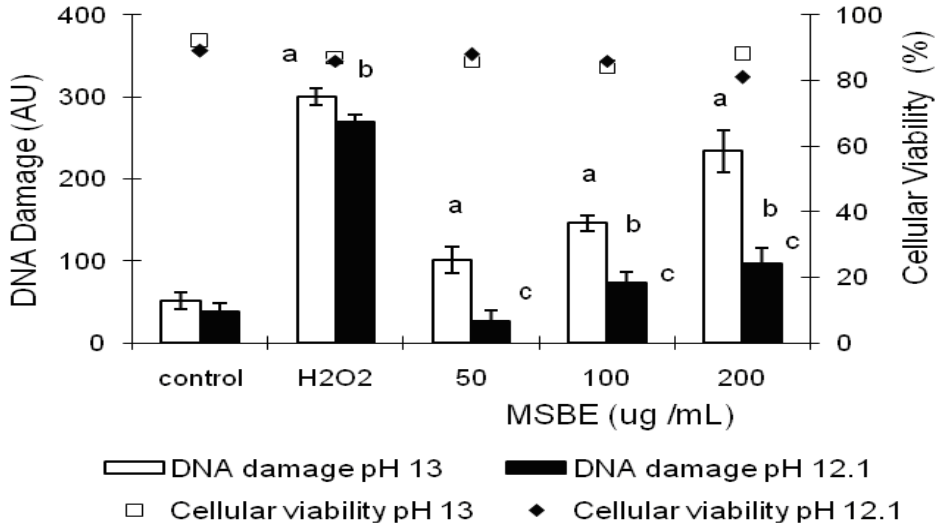


**Figure 2**

**Effect of MSBE on naked DNA of human lymphocytes after 1 hour of incubation. Values are expressed as arbitrary units (mean ± SD, n = 3). $H_2O_2$ (100 µM) as positive control and dimethylsulfoxide (0.2%) as negative control. Cellular viability was assessment by the Trypan Blue exclusion method. (%): represents number of alive cells x 100/ number total cells. [a]p < 0.01, treatment effect (pH 13) tested by Jonckheere Trend Test. [b]p < 0.01, treatment effect (pH 12.1) tested by Jonckheere Trend Test. [c]p = 0.05, comparison between treated cells under different electrophoresis conditions (pH 13 and pH 12.1) by exact Mann Whitney U test.**

***Comet Assay in presence and absence of catalase enzyme in human lymphocytes***

The Comet assay in presence and absence of catalase enzyme was performed to investigate hydrogen peroxide is involved in DNA damage. The results show that MSBE up to 50 µg/mL induced DNA strand breaks at these experimental conditions (Figure 3), but when the Comet assay was conduced in presence of catalase the damage was significantly reduced at 100 and 200 µg/mL of the extract, which could be suggest the DNA damage effects are probably mediated by hydrogen peroxide, which is removed of the medium by the protective activity of this enzyme.

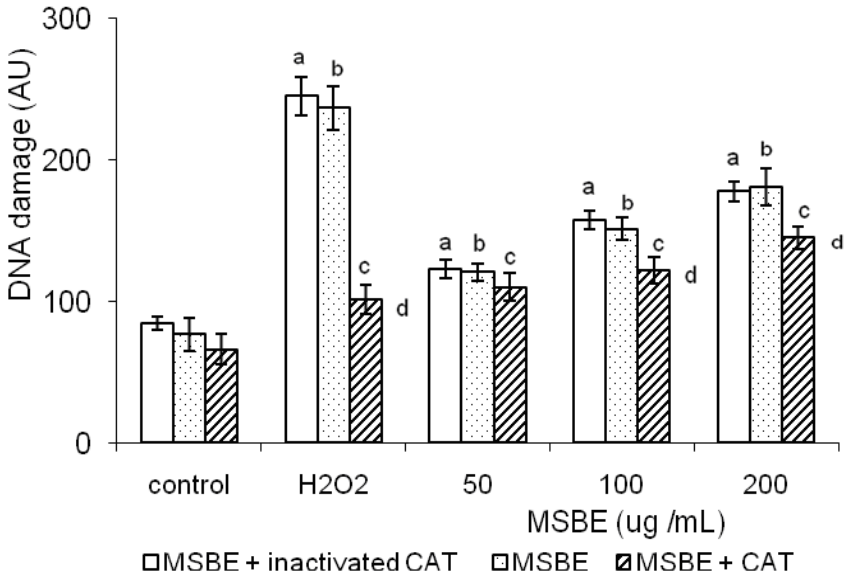


**Figure 3**

**Effect of MSBE on DNA of human lymphocytes in presence or absence of catalase after 1 hour of incubation. Values are expressed as arbitrary unit (mean ± DS, n = 3). $H_2O_2$ (100 µM) as positive control and dimethylsulfoxide (0.2%) as negative control. Cellular viability was assessment by the Trypan Blue exclusion method. (%): represents number of life cells x 100/ number total cells (viability was superior to 85%). $^{a}p < 0.05$, comparison between treated cells and control (MSBE + inactivated CAT) by Test Trend Jonckheere. $^{b}p < 0.01$, treatment effect (MSBE) by Jonckheere Trend Test. $^{c}p < 0.01$, treatment effect (MSBE + CAT) by Jonckheere Trend Test. $^{d}p = 0.05$, comparison between MSBE and MSBE + CAT treatments by exact Mann Whitney U test.**

***Comet Assay in presence and absence of $S_9$ fraction in human lymphocytes***

Figure 4 shows the results obtained when the variant of Comet assay supplemented or not with the $S_9$ fraction was performed. As this data show the DNA damage induced by MSBE was significantly reduced in the presence of $S_9$ fraction at all the concentrations tested.

***Standard Comet Assay in rat hepatocytes in culture***

With the aim to corroborate it, if hepatic biotransformation reactions are responsible of the reductions of the genotoxic effect induced by MSBE, rat hepatocytes were used as model of DNA damage for conducing Comet assay. According to results observed in Figure 5, no genotoxic effect was detected after 1h of exposure between 10-500 µg/mL of MSBE under these experimental conditions.

**DISCUSSION**

The Comet assay is a straightforward and highly sensitive method for measuring DNA damage and repair at the level of individual cells. The DNA strand breaks and alkali-labile sites have been generally associated with the increase of reactive oxygen species (Labieniec *et al*., 2003; Wasson *et al*., 2008). MSBE is a complex mixture where polyphenols are the major fraction. The main polyphenol of the MSBE is mangiferin (Núñez *et al*., 2002), which is a well-established antioxidant (Martinez *et al*., 2000; Martinez *et al*., 2001; Sanchez *et al*., 2000). Most polyphenols, specifically flavonoids and tannins, that possess antioxidant activities, can also act as pro-oxidants, in presence of transition metals and depending on the redox environment (Labieniec *et al*., 2003). For instance, flavonoids can increase or decrease the formation of hydroxyl radical in Fenton type reactions, depending on experimental redox environment (Khan *et al*., 2000). In fact, flavonoids are considered genotoxic agents in a variety of prokaryote and eukaryote systems (Boos & Stopper, 2001; Heo & Sohn, 2001). Flavonoids have also been found in MSBE and they have been reported as scavengers of highly reactive species as peroxynitrite

and the hydroxyl radical (Heijnen *et al*., 2001). This activity gives rise to the oxidized products semiquinone and quinone radicals, which exhibit potential toxicity due to their oxidant capacity (Awad *et al*., 2001).

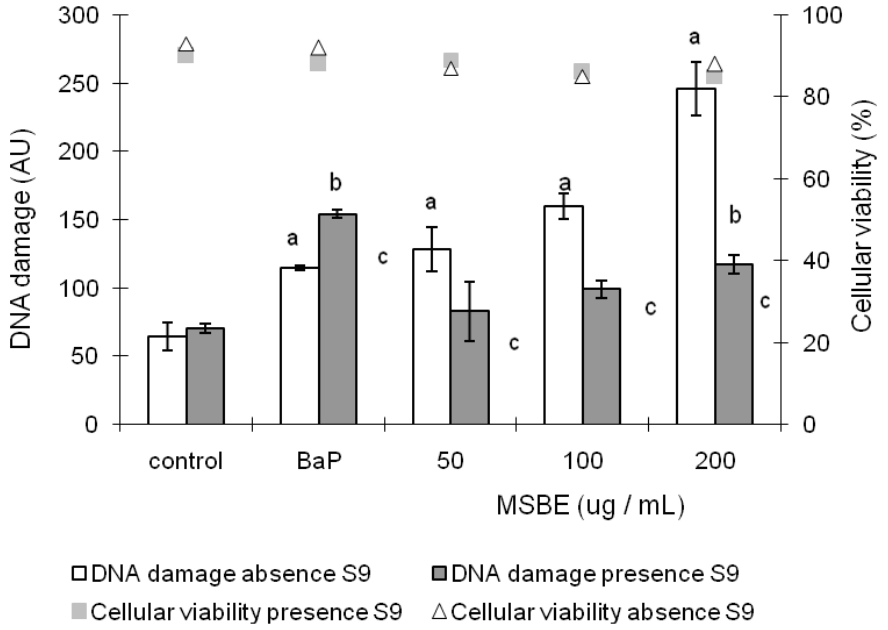


**Figure 4**

**Effect of MSBE on DNA of human lymphocytes, after 1 hour incubation in presence and absence of $S_9$. Values are expressed as arbitrary unit (mean ± SD, n = 3). Benzo(a)pirene (5 µg/mL) as positive control and dimethylsulfoxide (0.2%) as negative control. Cellular viability was assessment by the Trypan Blue exclusion method. (%): represents number of life cells x 100/ number total cells. $^a p < 0.01$, treatment (absence of $S_9$) effect tested by Jonckheere Trend Test. $^b p < 0.01$, treatment (presence of $S_9$) effect tested by. Jonckheere Trend Test. $^c p = 0.05$, comparison between different experimental group (absence and presence of $S_9$) by exact Mann Whitney U test.**

The observed DNA damage with the sub-cellular Comet assay was obtained without activation/deactivation through the microsomal system or enzymatic supplementation (Figure 2). It can be related to the unwinding and electrophoresis at pH 13, which permit to detect different types of DNA damage such as single strand breaks, double strand breaks and alkali-labile sites (ALS). Meanwhile, the same step at pH 12.1 does not detect ALS (Silva *et al*., 2000). Consequently, this result suggests that MSBE has active principle(s) that possess genotoxic effect *per se*. This situation could be explained by the auto-oxidation in the absence of transition metals (Holz *et al*., 1995; Awad *et al*., 2001). In these experimental conditions MSBE induces the formation of ALS, among other different types of DNA damage. ALS is produced by the action of the determinate radical oxygen species and by the action of alkilant agents (Silva *et al*., 2000). Recently, it was demonstrated that mangiferin did not induce cytotoxic or genotoxic effects (Rodeiro *et al*. 2012). So this glycosylated xanthone would not be responsible for these effects. It is possible that there are other polyphenols in the extract (e.g., flavonoids) that could be exerting this *in vitro* genotoxic effect.

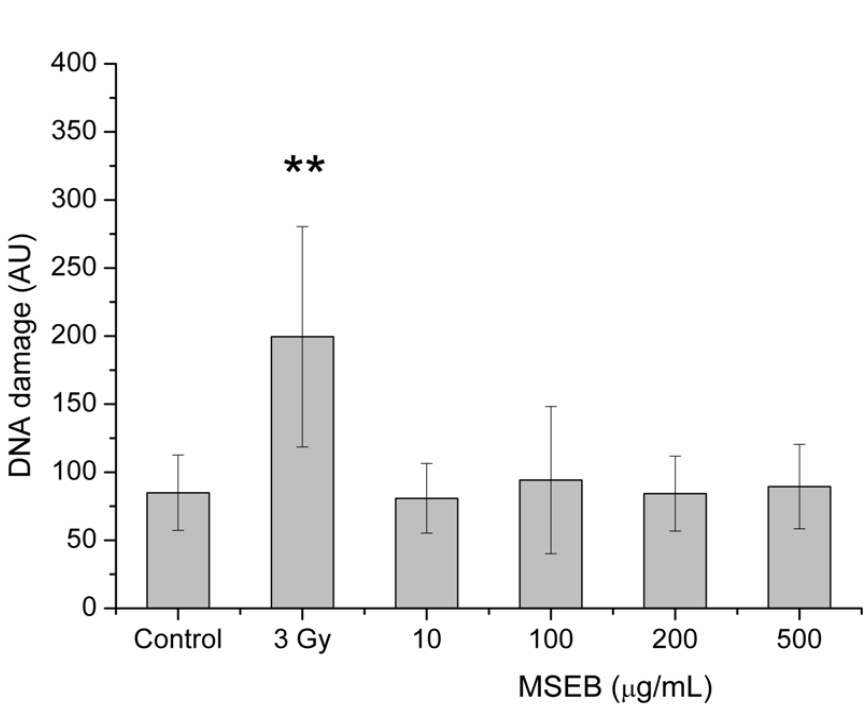


**Figure 5**
**Effect of MSBE on rat hepatocytes DNA, after 1 hour incubation. Values are expressed as arbitrary units (mean ± SD, of at least three independent assays). Gamma-irradiation (3 Gy) as positive control and dimethylsulfoxide (0.2%) as negative control. Cellular viability was assessment by the Trypan Blue exclusion method, hepatocytes viability was in all the cases above of 95%, **p = 0.01, comparison between different experimental group (absence and presence of $S_9$) by exact Mann Whitney U test.**

It is well established that the hydrogen peroxide generates DNA single strand breaks at concentrations as low as 25 µM, and produces a clear effect related to the dose at higher concentrations in different cell types (De Flora, 1998). The use of catalase to protect against the genotoxic effects of hydrogen peroxide has also been applied in other *in vitro* systems (Bradley & Erickson, 1981; Díaz-Llera *et al*., 2002). Although, hydrogen peroxide is a rather stable molecule in aqueous solution, it can cause strand breaks in purified DNA (Rice-Evans, 2001) hence, it can be involved in the primary DNA damage induced by MSBE in sub-cellular Comet assay (Figure 3).

Polyphenols are extensively metabolised *in vivo*, resulting in a significant alteration in their redox potentials (Awad *et al*., 2002; Williams *et al*., 2004). Metabolic modifications of polyphenols will alter their classical antioxidant nature, which will be very different to the original molecular effects (Spencer *et al*., 2004). Catalase is the main intracellular catalytic function for removing of hydrogen peroxide. Our results in this research suggest that genotoxic effect of MSBE in catalase presence was slightly reduced and the $S_9$ microsomal fraction decreased the genotoxic effect induced by MSBE at tested studied concentrations (Figure 3).

The results obtained using the Comet assay supplemented or not with the $S_9$ fraction show that MSBE in the presence of $S_9$ fraction induce a reduction of the DNA damage in the experimental culture (Figure 4). Regarding the latter finding, it could be suggested that the components of MSBE were metabolized by the liver microsomal enzymes contained in the $S_9$ fraction, which could indicate that these enzymes are related to the detoxification of the genotoxic compounds in the MSBE. In addition, it has been demonstrated that fraction $S_9$ contain glutathione, peroxidase, and catalase which are very important in the elimination of hydrogen peroxide and

should be involved into the mechanism of damage of MSBE.

Cultured hepatocytes are considered to be a good model for studying the metabolic capacity of liver (Castell & Gómez-Lechón, 1997); consequently the hepatic metabolism has probably a decisive role detoxifying genotoxic compounds of this natural extract. With the aim to corroborate, if hepatic biotransformation reactions are responsible of the reductions of the genotoxic effect induced by MSBE, rat hepatocytes were used as model of DNA damage. According to results, no genotoxic effect was detected when rat hepatocytes in culture were incubated with MSBE during 1h (Figure 5). The results of this study are consistent with the findings previously obtained by our research group, which not primary DNA damage neither damage clastogenic were induced by MSBE *in vivo* conditions (Rodeiro *et al*., 2006; Gonzalez *et al*., 2007).

In conclusion, MSBE induces DNA strand breaks as well as ALS in human lymphocytes treated *in vitro* and hydrogen peroxide could be involved in the mechanism of DNA damage. In contrast, genotoxic effect was not induced by the extract in rat hepatocytes using standard Comet assay. These results suggest that the efficiency with which the MSBE is converted to its metabolites by mean of the rat liver $S_9$ fraction is comparatively high, but the exposure time and concentration of the mixture should be set taking into consideration the rate of metabolism of the test compound and the limit of detection for DNA damage depending on the type of cell being used. This also indicates that MSBE probably contain genotoxic compounds such as flavonoids susceptible to be metabolized to non genotoxic compounds.

This study confirms the use of a battery of Comet assays is a powerful tool in the determination and partial characterization of the primary DNA damage provoked by natural products that could be used as functional food or supplements.

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