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Additional Information

Dried apple enriched with mandarin juice by vacuum impregnation counteracts the liver oxidative effect of tamoxifen in rats.

Abstract

We have investigated the effects of a product made of apple dehydrated enriched by vacuum impregnation of mandarin juice in decreasing oxidative stress biomarkers in rat induced liver injury by tamoxifen. A experimental design was undertaken in six groups of animals. One group fed a standard diet. Two groups were supplemented with 0.745 g/d of apples snacks/day either impregnated or not with mandarin juice during 28 days. Three groups of rats were treated with tamoxifen 1.54 mg/kg/day during 21 days; of these, one group was supplemented with the apple snacks and the other with the apple snacks impregnated with mandarin juice. Plasma aminotransferases were determined as index of hepatocellular damage and interleukin-6 as measure of inflammation. Plasma and liver carbonyl groups, indicative of protein oxidation, and α-tocopherol as evaluation of antioxidant defense were measured. The modified base 8-hydroxydexyguanosine as indicative of DNA damage was determined in liver. Tamoxifen induced an increase in aminotranferases and both plasma and liver markers of oxidative stress and DNA damage that decrease significantly after apple snack consumption; in most of cases they even reach normal values as those achieved previous to induce the injury with tamoxifen. The effect of the mandarin juice incorporated to the structure of apple mainly accounts for increasing levels of α-tocopherol in plasma and liver. The present study shown the potential protective action of the products made of dehydrated apple either impregnated or not with mandarin juice by vacuum impregnation for counteracting the chemically induced liver injury in rats.

Keywords: antioxidants, apple, citrus, 8-dehydroxydeoxyguanosine, protein oxidation, tamoxifen.

1. INTRODUCTION

In recent years, special interest has been directed towards the antioxidant capacity of fruits and vegetables. Prevention from oxidative stress plays a beneficial role in the development of many process related to free radical production such as degenerative diseases and mutagenesis or the action of certain drugs whose metabolism induces the formation of reactive oxygen species. The intake of a diet rich in antioxidant compounds can prevent some oxidative-related disorders and organ toxicity events either directly neutralizing reactive oxygen species (Kujawska et al., 2011) or modulating gene expression that contributes to oxidative stress (Soyalan et al., 2011).

Between the fruits highly consumed, citrus and apples have been proven to have beneficial effects against a number of diseases, such as cardiovascular disease, diabetes and some cancers as well as some age-related disorders (Buscemi et al., 2012; Jedrychowski et al., 2010). These protective effects can be related to the actions of specific compounds such as vitamin C and polyphenols on inflammation and oxidative stress (Bouayed et al., 2011). Although during the last years a lot of studies have demonstrated the beneficial effect of phytochemicals on health (Eilat-Adar and Goldbourt, 2010) there is a growing knowledge about arguing that consumption of foodstuffs can exert more beneficial effects than

active components alone, the various nutrients provided by foodstuffs can have a relevant synergistic action (Garg et al., 2001).

As a consequence, fruit derived products are an emerging area within functional foods. Recently, we have developed an apple snack rich in flavonoids from mandarin juice by using vacuum impregnation (VI) and air drying technologies (Betoret et al., in press). The product can combine the beneficial effect of both sources into one food item, or even enhance the beneficial compounds in the juice by protecting them through inclusion into a food structure. The snack developed can be an alternative within the existing functional food products enhancing the fruit based functional ones. This product can be consumed as a snack or as ingredient in breakfast products mainly by children. In this sense, functional food development requires assessment for its efficacy and safety through in vivo proof-of-concept testing for potential health claims (Jones et al., 2008). Therefore, in the present study, we examined the effects of adding to the experimentation animals' diet a foodstuff made by apple dehydrated enriched by VI of mandarin juice. We assessed the bioavailability by measuring the antioxidant capacity of serum by their ferric reducing antioxidant potential (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods.

Furthermore, we conducted a study to evaluate the effects of this foodstuff consumption in decreasing oxidative stres mainly due to a counteraction of free radicals. With this end we have designed an experimental study in rats using a prooxidative agent to induced liver injury, tamoxifen (TAM), 1-[4-(2-dimethyl-aminoethoxy) phenyl]-1,2-diphenyl-1-butene, a non-steroidal anti-estrogen drug, one of the therapies most commonly used in breast cancer but that can be accompanied with adverse effects, particularly liver toxicity as a result of the reactive oxygen species generated during its metabolic process (Nazarewicz et al.,2007). In this way, we have determined the protection in a model in vivo used for evaluating hepatotoxicity in rodents.

2. MATERIAL AND METHODS

2.1 Reagents and standards.

ABTS and 2,4,6-tris(2-pyridyl)-s-triazine was acquired from Sigma (Sigma Aldrich Co., St. Louis, MO, USA). Methanol, acetonitrile and dichloromethane (HPLC grade) were purchased from Scharlau (Scharlab S.L., Barcelona, Spain). Citrate tamoxifen (T9262) was acquired from Sigma (Sigma Aldrich Co., St Louis, MO, USA). Other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A) and Fluka (Buchs, Switzerland).

2.2 Food materials.

Mandarin juice was obtained as previously described by Betoret et al. (2009). In brief, Ortanique fruit was harvested in an orchard located in Turís (Valencia), Spain, and squeezed immediately for juice preparation. The fruits were washed by immersion in tap water, after which they were drained, and squeezed in an industrial extractor with finger cups (Exzel, Luzzysa; El Puig, Valencia, Spain). Raw juice was divided into two fraction and the low pulp juice fraction was homogenized with a Manton-Gaulin pilot homogenizer (model 15M8TBA) at 15 MPa, centrifuged with a Westfalia centrifuge (model SAOH 205), and pasteurized at 63 ºC for 15 s.

Apples (cv. Granny Smith) were obtained from a local market. Peeled apples were cut into-disc-shaped samples (5 mm thick, with a 65 mm external diameter and 20 mm internal diameter) following their vertical axis. Three samples were obtained from each apple and utilized for VI.

2.3 Snack preparation.

We prepared two types of snack: a snack made of apple dehydrated exclusively and a snack made of apple dehydrated enriched with mandarin juice by VI. The snacks were obtained following the methodology previously described in (Betoret et al., in press). The VI experiments were performed on a pilot scale using equipment designed in the Institute of Food Engineering for Development of the Polytechnic University in Valencia, Spain (Fito et al., 2001). A vacuum pressure of 50 mbar was applied for 10 min to the apple samples immersed in the mandarin juice after which atmospheric pressure was restored. The samples were left submerged in the mandarin juice for a further 10 min. Apple samples were dried for 24 h using an air dryer (LIZ®, Construcciones mecánicas José Lizondo, Barcelona, España) at 40 ºC under a flow rate of 4 m/s. Final concentration achieved was 20 mL of juice/100 g of apple. 40 grams of dehydrated snack impregnated with mandarin juice contains the same amount of flavonoids as 250 ml of fresh mandarin juice (Betoret et al., in press). Composition of the snacks are shown in Table 1.

2.4 Animals.

Female Wistar rats (12 weeks old and around 250-300 g weight each) were used in this study. Animals were kept on standard laboratory diet (IPM R-20 marketed by Panlab, Barcelona, Spain) and tab water ad libitum through the experiments. Temporary cannulation of the lateral tail vein (23G cannula, Chiron Biosciencies, Kent , UK) was performed in the biovailability study. To obtain a larger amount of plasma, animals were anesthetized with halothane and quickly the intracardiac blood sample was collected into a heparinized tube and centrifuged. To obtain liver we proceeded to subsequent cervical dislocation of the animal. Then, it was sectioned longitudinally the animal and the liver was removed

immediately by dissection and washed in ice-cold isotonic saline. The liver was wrapped in aluminum foil and freeze up to -80ºC. The method outlined by Santos et al. (2002) was used to obtain liver homogenates. Livers were homogenized with 5 mL of buffer (250 mM sucrose, 1 mM EGTA, 5 mM Hepes-KOH pH: 7.4)/1 g of tissue, using Junke and Kunkel homogenizer (Staufen, Germany). Subsequently, it was centrifuged 900 g for 10 minutes at 4 °C and was filtered through of three layers of gauze to obtain the liver homogenate. All animal experiments were approved by the Institutional Animal Ethics Committee.

2.5 Bioavailability of the snack antioxidants.

The total antioxidant capacity was determined in plasma at times 0, 30, 60 and 90 minutes with ABTS and FRAPS methodologies after supplementation of the snacks enriched with mandarin juice.

The ABTS assay was followed the method proposed by Miller and Rice Evans (1997) and modified by Re et al. (1999), based on the oxidation of ABTS with potassium persulfate to form the radical monocation ABTS•+, which is reduced in the presence of hydrogen-donating antioxidants. The reagent ABTS•+, was generated by the reaction 7 mM solution of ABTS with 2.45 mM potassium persulfate solution in a proportion 1:1. The assay was made up with 2.970 mL of ABTS•+ and 30 µL of plasma. The method determine the decolorization of the ABTS•+, through measuring the reduction of the radical cation in the absorbance at 734 nm.

The FRAP assay was determined using the method proposed by Benzie and Strain (1996).This method is based on ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex. The reaction mixture was prepared by mixing 10 volumes of 300 mM sodium acetate to pH: 3.6, 1 volume of 40 mM 2,4,6-tris(2-pyridyl)-s-triazine and 1 volume of 20 mM FeCl3. 50 µl of plasma was added to 2.950 mL of the reagent mixture. The mixture reactive was incubated at 37 °C for 30 minutes in darkness. FRAP values were obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration.

2.6 Experimental design.

The snack supplementation period was 28 days and the period of tamoxifen treatment (21 days) started one week after supplementation. Each animal was eating 0.745 g of snack per day, which is equivalent to 40 g of snack consumed per day by a child of 30-40 kg.

The rats were randomly assigned into 6 groups of 10 animals each as follows:

- CONTROL group: untreated rats with standard diet.
- TAM group: rats on standard diet that were orally administered 1.54 mg of tamoxifen/kg body weight daily during a period of 21 days.

- TAM+APPLE group: rats supplemented diet with apple snack without mandarin juice during 28 days and TAM orally administered (1.54 mg of tamoxifen/kg body weight daily) during 21 days.
- TAM+APPLE+VIM group: rats supplemented diet with apple snack impregnated of mandarin juice by VI during 28 days and TAM orally administered (1.54 mg of tamoxifen/kg body weight daily) during 21 days.
- APPLE group: rats supplemented diet with apple snack during 28 days.
- APPLE+VIM group: rats supplemented diet with apple snack impregnated of mandarin juice by VI.

Twenty four hours after the last treatment, animals were subjected to anesthesia with halothane and quickly the intracardiac blood sample and liver were obtained following the procedure described above.

2.7 Analytical methods.

The liver injury was evaluated by the measurement of the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma by means of the kits from Linear Chemicals, S.L. (Barcelona, Spain).

To evaluate protein oxidation, the carbonyl groups (CG) released during the incubation with 2,4-dinitrophenylhydrazine of plasma and liver homogenate were measured using the method reported by Levine et al. (1990) with some modifications introduced by Tian et al. (1998).The samples were centrifuged at 13000 rpm for 10 min. Then, 20 μL of this plasma or liver homogenate were placed in a 1.5 mL eppendorf, and 400 μL of 10 mM 2,4 dinitrophenylhydrazine / 2.5 M ClH and 400 μL of 2.5 M ClH to were added. This mixture was incubated for 1 hour at room temperature. Protein precipitation was performed using with 1mL of 100% of TCA, washed twice with ethanol/ethyl acetate (1/1, v/v), and centrifugation at 12600 rpm for 3 min. Finally, 1.5 mL of 6N guanidine, pH 2.3, was added and the samples were incubated in a 37ºC water bath for 30 min and were centrifuged at 12600 rpm for 3 min. The carbonyl content was calculated from peak absorption (373 nm) using an absorption coefficient of 22.000 M-1cm-1 and was expressed as nmol/mg protein. The protein content was determined by the Lowry method using bovine serum albumin as standard.

The concentration of 8-hydroxydeoxyguanosine (8OHdG), an oxidized nucleoside of DNA, was used to assess the damage to DNA. DNA was extracted from the samples by proteinase K digestion in combination with DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany). Briefly, 0.5 μg DNA/μL of samples were incubated with 100 units of DNase I in 40 μL 10 mM Tris–ClH at 37 °C for 1 h. The pH of the reaction mixture was then lowered with 15 μL of 0.5 M sodium acetate (pH: 5.1), 10 μL of nuclease P1 (five units) and incubated for 1 h. Readjustment of pH with 100 μL of 0.4 M Tris–ClH (pH: 7.8) was followed by the addition of three units of alkaline phosphatase; samples were incubated for longer than an hour. Analysis of 8OHdG concentration in liver homogenate was determined by means of

a commercially available competitive enzyme linked immunosorbent assay kit (8OHdG EIA of Cayman Chemical Company, Ann Arbor, MI, USA).

Levels of α-tocopherol, an antioxidant defense marker, were determined by HPLC following the methodology proposed by Arnaud et al. (1991). 200 µL of plasma or liver homogenate with 100 µL of ethanol and 500 µL of hexane were centrifuged at 2000 rpm for 5 minutes, and were reflected the organic phase, which was dried with dry N2; and, finally, it was resuspended with 200 µL of mobile phase. Chromatographic conditions consisted of a column Kromasil 100 C18 (250 mm x 4.6 mm, 5 µm particle size) that was obtained from Teknokroma (Teknokroma LTd., Barcelona, Spain). We used an isocratic mode with a mixture of acetonitrile/ dichloromethane/ methanol (70/20/10, v/v/v). The flow rate was of 1.2 mL/min at room temperature. The chromatograms were followed at 291 nm.

As inflammation marker, we determined the interleukin 6 levels in plasma using the ELISA kit de Diaclone Research (Besançon, France) following manufacturer instructions.

2.8 Statistical analysis.

SPSS v 17.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. To compare the values between the different groups of animals an analysis of variance (ANOVA) test was carried out with Bonferroni post hoc test to identify which groups are significantly different from which other groups. In the study of bioavailability, a Friedman's test that measures analysis of variance by ranks was used to compare observations repeated on the same subjects. Results are presented as mean ± standard error of the mean (SEM), and $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Bioavailability of the snack antioxidants.

Figure 1 shows the antioxidant capacity of plasma at 30,60 and 90 minutes after the intake of snacks. We observe an increase in the antiradical activity after 30 minutes that it is significant by the ABTS method. At 60 minutes the maximum antiradical activity values were achieved, being significant statistically by both ABTS and FRAP methods. The values decrease until normal levels after 90 minutes.

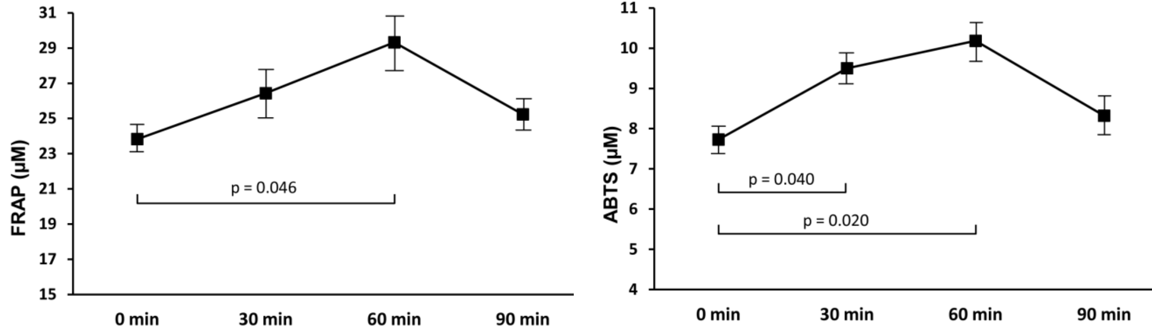

Figure 1. Serum antioxidant capacity of rats after ingestion of the apple snack impregnated with mandarin juice (n = 5).

3.2 Effect of TAM on the parameters of liver injury and oxidative/antioxidative status in liver and plasma.

The administration of TAM increased significantly the activity of both enzymes when compared with control group (Figure 2). In addition, the marker of protein oxidation, CG, and the interleukine 6, a multifunctional proinflammatory cytokine were increased in plasma. On the contrary, the α-tocopherol, indicative of antioxidant defense, was decreased in the animals treated with TAM (Figure 3). Similar results were observed in liver (Figure 4). Furthermore, the marker of DNA damage, 8OHdG, that was additionally determined in liver, was significantly increased. All these data suggest an important liver toxicity and inflammation induced by reactive oxygen species in the animals treated with TAM.

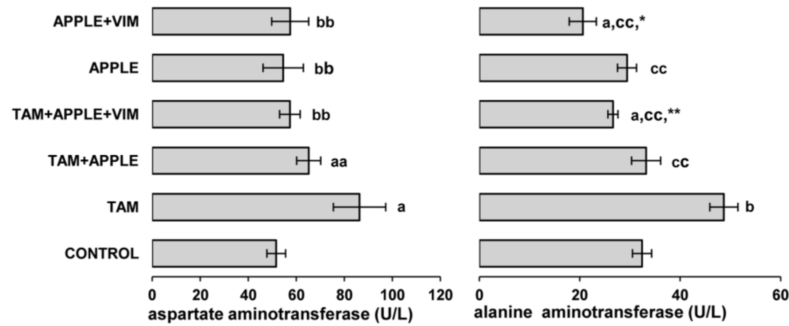

Figure 2. Aspartate aminotransferase and alanine aminotransferase values in plasma of rats (n = 10 rats per group). $^{a}p < 0.001$, $^{b}p < 0.0001$, vs CONTROL. $^{aa}p < 0.05$, $^{bb}p < 0.01$, $^{cc}p < 0.0001$ *vs* TAM group. $^{*}p < 0.05$ *vs* APPLE group; $^{**}p < 0.05$ *vs* TAM+APPLE group

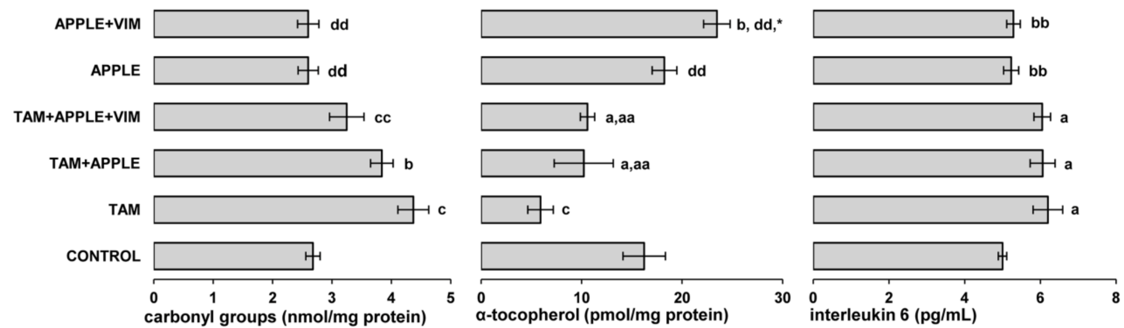

Figure 3. Carbonyl groups, α-tocopherol, and interleukin 6 values in plasma of rats (n = 10 rats per group). [a]$p < 0.01$, [b]$p < 0.001$, [c]$p < 0.0001$ *vs* CONTROL group. [aa]$p < 0.05$, [bb]$p < 0.01$, [cc]$p < 0.001$, [dd]$p < 0.0001$ *vs* TAM group. *$p < 0.01$ *vs* APPLE group.

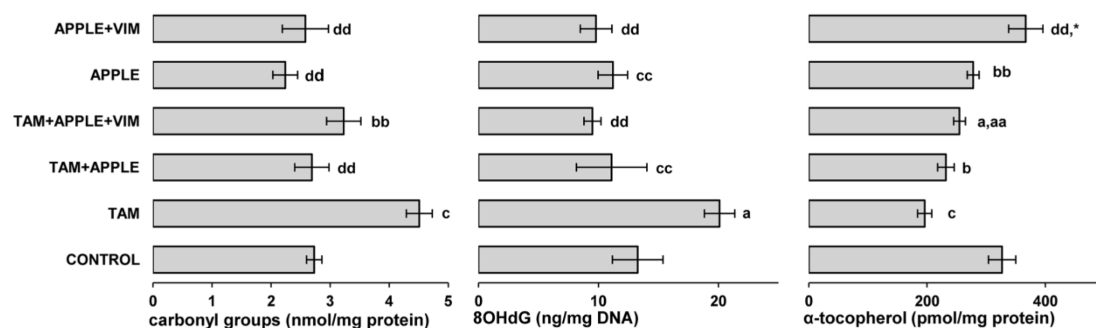

Figure 4. Carbonyl groups, 8OHdG, and α-tocopherol values in the liver of rats (n = 10 rats per group). [a]$p < 0.01$, [b]$p < 0.001$, [c]$p < 0.0001$ *vs* CONTROL group. [aa]$p < 0.05$, [bb]$p < 0.01$, [cc]$p < 0.001$, [dd]$p < 0.0001$ *vs* TAM group. *$p < 0.001$ *vs* APPLE group.

3.3 Snack supplementation effect.

The parameters related to oxidative stress, liver injury and inflammation were measured in plasma and liver of rats that had not been stress induced by TAM [Groups CONTROL, APPLE and APPLE+VIM]. The Figure 2 shows the results of liver enzymes. The plasma levels of ALT and AST from animals that have followed a standard diet and those that receive snack supplementation with and without mandarin juice did not differ. Moreover, either the supplementation with the snack impregnated or not does not modify essentially the markers of protein and DNA oxidation either in plasma or liver (Figure 3 and 4). However, the α-tocopherol levels both in plasma (Figure 3) and liver (Figure 4) were significantly increased in the rats supplemented with the snack impregnated with mandarin juice (APPLE+VIM group) with respect the rats supplemented with the non-impregnated snack (APPLE group).

3.4 Snack supplementation effect on tamoxifen toxicity.

The TAM-treated rats along with snack suplementation with and without mandarin juice showed ALT and AST levels significantly decreased in comparing to the TAM-treated group (Figure 2).The effect was more marked in the case of ALT

levels in rats supplemented with the snack impregnated with mandarin juice (TAM+APPLE+VIM group).

Figure 3 shows the results obtained in the six groups of animals included in the experiment with respect the parameters related to oxidative stress and inflammation measured in plasma. Protein damage reflected in an increase in carbonyl groups levels after TAM treatment decreases considerably in the rats supplemented with the snack impregnated with mandarin juice (TAM+APPLE+VIM group). This significant decrease was not noted in the case of the non-impregnated snack (TAM+APPLE group). Moreover, the α-tocopherol levels that were decreased significantly after TAM treatment were partially restored by the addition of the snacks to the diet of animals. On the other hand, we have not found differences with respect the marker of inflammation interleukin 6 in the groups of rats supplemented with the snack either impregnated or not with mandarin juice.

In the Figure 4 are shown the results found in the six groups of animals with respect the parameters related to protein oxidation, DNA damage and α-tocopherol level measured in the liver homogenate. The protein and DNA oxidative damage that was increased significantly after TAM decrease until normal values after supplementation with the snack. There were not differences between the results obtained after supplementation with the snack impregnated or not with mandarin juice. Contrarily, the α-tocopherol levels in liver homogenate obtained from rats after supplementation with the snack impregnated with mandarin juice (TAM+APPLE+VIM group) were significantly higher than those obtained in rats feed with the snack no impregnated (TAM+APPLE group).

4. DISCUSSION

In the present study, we confirmed our previous hypothesis that the consumption of the apple snacks counteract liver toxicity produced by TAM administration in rats, probably as a result of reducing the oxidative protein and DNA status. The additional effect of the mandarin juice included into the snack development was manifested by enhancing the antioxidant defense.

As it was expected, TAM in toxic doses produces liver injury in the animals. This fact is shown by the increased activity of ALT and AST in plasma and it can be attributed to hepatic structural damage as these enzymes are normally localized in the cytoplasm (ALT) and mitochondria (AST) of hepatocytes and are released into the circulation after cellular damage has occurred. Also, oxidative stress evaluated by a combination of biomarkers that represent different aspects of oxidative damage or antioxidant capacity was present after TAM administration.

It was previously shown the presence of lipoperoxidation in the pathogenesis of TAM-induced liver toxicity (Albukhari et al., 2009). In addition, protein carbonylation is one of the reactions set into motion as a consequence of the formation of oxygen radicals in cells and tissues (Grattagliano et al., 2009). CG are formed early and circulate in the blood for longer periods, compared with the

other parameters of oxidative stress (Dalle-Donne et al., 2003).Thus, the quantification of CG could be used to measure the extent of oxidative modification to cellular proteins. In this sense, the content of CG generated by oxidation of proteins significantly increases both in plasma and liver homogenate of the animals treated with TAM indicating a deep damage to cells.

A significant increase in the modified nucleoside base 8OHdG in the liver homogenate that reflects DNA covalent modifications was also noted. This alteration in the DNA structure may be at the basis of apoptosis induced by TAM but also is responsible of liver damage (Zhao et al., 2009). All these findings suggest a liver injury promoted by TAM administration and the presence of oxidative stress.

With respect to antioxidant defense, it has been described that TAM alters the endogenous antioxidant glutathione (Albukhari et al., 2009). We have found that α-tocopherol a major exogenous antioxidant that acts as a "scavenger" capturing the reactive oxygenic species, was also decreased in the liver and at plasmatic level after TAM treatment, probably as a consequence of its consumption in the situation of enhanced oxidative stres. In general, to prevent or slow down the consequences of oxidative stress induced by free radicals, a sufficient amount of endogenous and exogenous antioxidants is needed. Exogenous antioxidants are provided by the diet. It is known that fruits and vegetables contain a wide variety of antioxidant substances such as vitamins, phenolic compounds and carotenoids that may help to protect cellular systems from oxidative damage. These particular constituents in foods might exert synergistic antioxidant actions or protect by different mechanisms. It is also important to take into account that some compounds with limited direct antioxidant activity might exert antioxidant action in vivo by upregulating endogenous antioxidant factors.

The specific characteristics of fruits have lead to an emerging area in agro-alimentary industry in which fruit based functional foods are in progress (Fito et al., 2001). Characterisation of food properties and analysis of bioactive composition are not the only requirement during food research and product development but also the evaluation of the beneficial effects that can exert those bioactive components in vivo studies to verify health content claims. In this sense, the antiradical activity of the product used in the present study (slices of dehydrated apple enriched with mandarin juice by VI) has been evaluated in a previous work; their antioxidant compounds derived from apple and from mandarin juice also have been identified (Betoret et al., in press). In the current study we have proven the effect in vivo. Our results show that after dietary supplementation with this product the antioxidant capacity increases after 30 minutes and are maximally available 60 minutes after ingestion. Hence, we can infer that the developed product has antioxidant effect in vivo. This result agrees with those obtained by other authors in which humans were supplemented with apple juice and increase their antioxidant capacity (Vieira et al., 2012).

We have also observed a normalisation in the levels of hepatic enzymes in the rats supplemented with the snacks after the injury provoked by TAM. Thus, the action of this food product might be to down regulate ongoing oxidative stress and/or inflammatory processes induced by TAM, allowing for the recovery of function of cellular homeostasis. In the present experiment, we have demonstrated that the supplementation with apple snacks markedly attenuated hepatic protein and DNA oxidation induced by the xenobiotic, which is consistent with well-documented in vitro free radical-scavenging ability and antioxidant activity of the apple products (Kujawska et al., 2011).

Protein carbonyl content is by far the most common marker of protein oxidation. CG are relatively difficult to induce compared to other products of protein oxidation. Thus, they are reflective of more severe cases of oxidative stress. In rats treated with TAM dose used here, the concentration of serum protein carbonyls was distinctly increased. We have noted a significant protective effect with a decrease in this biomarker mostly in the animals supplemented with the apple snacks VI, suggesting the action of specific compounds present in mandarin juice and not in the apple (Choi, 2008). Because TAM contributes to increased free radical production in many ways (Nazarewicz et al., 2007) the administration of specific agents with antioxidant or free radical-scavenging activity could be important for the protection against its deleterious action.

DNA damage results from the balance between the action of xenobiotic metabolites and the power of numerous possible counteracting systems, such as endogenous antioxidant defense and repair of the occurring lesions. Both apples (Poulsen et al., 2011) and citrus (Razo-Aguilera et al., 2011) show a strong protective effect against hydrogen peroxide-induced DNA damage. Hence, a product that combines these two fruits could be effective. However, the incorporation of mandarin juice to the snacks did not offer an additional advantage.

Determination of α-tocopherols may contribute to the information on the antioxidant status at hepatic level. Moreover, it is known from previous studies that the concentration of this antioxidant in the blood decreases as a consequence of increased oxidative load (Patil et al., 2008). On that account it was expected that the concentration of α-tocopherol in plasma would decrease in TAM toxicity as occurs in the experiment. Animals from the group fed with the apple snack VI of mandarin juice presents the highest plasma α-tocopherol concentration that was statistically different from the apple snack without VI. This difference was also noted in liver. These results may be due to the high content on vitamin C of mandarin juice because, in addition to scavenging reactive oxygen species, ascorbic acid can prevent the loss of lipophilic antioxidant α-tocopherol by repairing tocopheryl radicals (Bruno et al., 2006).

A possible explanation for the greater effect of the snacks impregnated with mandarin juice in several markers of oxidative stress may be found in the synergistic activity of different natural antioxidants in the product that therefore

may be more effective than supplementation with only apple snacks. According to data in the literature apples contain more phenols (Jung et al., 2009) and mandarins more vitamin C and specific flavonoids and carotenoids (Sentandreu et al., 2007). This finding is in accordance with that of previous studies in which animals supplemented with different fruits shown different responses in the parameters of oxidative stress (Pajk et al., 2006).

In general terms, we have found that both plasma and liver markers of oxidative stress decrease significantly after snack consumption, in most of cases they even reach normal values as those achieved previous to induce the stress with TAM. The plasma CG and ALT decrease more markedly after consumption of impregnated snack with respect the non impregnated snack. The effect of the mandarin juice incorporated into the structure of apple mainly accounts for the increasing observed in α-tocopherol levels suggesting that at least some of the beneficial effects of these enriched diets in snacks VI might be exerted through to the downregulation of oxidative alteration of macromolecules.

5. CONCLUSION

In summary, the present study has shown the potential protective action of a foodstuff made of apple and mandarin by preventing damage of essential cellular macromolecules in the conditions of chemically induced oxidative stress in rat. Our results indicate that feeding of the snack impregnated with mandarin juice has an antioxidant effect in vivo and may prevent the toxicity generated after TAM supplementation in rats. The overall protective effect of the impregnated snack is probably due to a counteraction of free radicals by its antioxidant nature and/or to its ability to restore the normalcy in tissue under oxidative stress. However, the precise molecular mechanism by which the impregnated snack exerts its protective action against oxidative damage remains to be established.

6. ACKNOWLEDGEMENTS

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