

Herbal extracts differentially inhibit oxidative effects caused by the biotransformation of nifurtimox, nitrofurantoin and acetaminophen on rat liver microsomes

[Extractos herbales inhiben diferencialmente los efectos oxidativos causados por la biotransformación de nifurtimox, nitrofurantoína y acetaminofeno en microsomas hepáticos de rata]

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Abstract: Inflammation is a cellular defensive mechanism associated to oxidative stress. The administration of nitrofurantoin, nifurtimox and acetaminophen generates oxidative stress by their biotransformation through CYP450 system. The main adverse effect described for the first two drugs is gastrointestinal inflammation and that of the last, hepatitis. Therefore, standardised dry extracts from *Rosmarinus officinalis*, *Buddleja globosa* Hope, *Cynara scolymus* L., *Echinacea purpurea* and *Hedera helix* were tested to evaluate their capacity to decrease drug-induced oxidative stress. For that, rat liver microsomes were incubated with drugs in the presence of NADPH (specific CYP450 system cofactor) to test oxidative damage on microsomal lipids, thiols, and GST activity. All drugs tested induced oxidation of microsomal lipids and thiols, and inhibition of GST activity. Herbal extracts prevented these phenomena in different extension. These results show that antioxidant phytoextracts previously evaluated could alleviate drugs adverse effects associated to oxidative stress.

Keywords: Antioxidants; Herbal extracts; Polyphenols; Herbal thiols; Oxidation inhibition; GST.

Resumen: Inflamación es un mecanismo de defensa el cual está asociado a estrés oxidativo. La administración de nitrofurantoína, nifurtimox y paracetamol genera estrés oxidativo al metabolizarse a través del sistema CYP450. El principal efecto adverso de los dos primeros fármacos es inflamación gastrointestinal y del tercero, hepatitis. Por lo tanto, utilizamos diversos extractos herbales para disminuir el estrés oxidativo inducido por estos fármacos. Para esto se incubaron microsomas hepáticos de rata con dichos fármacos en presencia de NADPH (cofactor específico del sistema CYP450) y se evaluó el daño oxidativo generado sobre los lípidos, los tioles y la actividad GST microsómica. Todos los fármacos indujeron oxidación de los lípidos y los tioles microsómicos e inhibieron la actividad GST. Los extractos herbales previnieron estos fenómenos oxidativos en diferente extensión. Estos resultados indican que fitofármacos antioxidantes previamente evaluados, podrían aliviar los efectos adversos asociados a estrés oxidativo de los fármacos.

Palabras clave: Antioxidantes; Extractos herbales; Polifenoles; Tioles herbales; Inhibición de la oxidación; GST.

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ABBREVIATIONS

CYP450: Cytochrome P450

GST: Glutathione S-Transferase

NAPQI: *N*-Acetyl-*p*-Benzoquinone Imine

INTRODUCTION

Nitrofurantoin (antimicrobial used in urinary tract infections) and nifurtimox (drug used in the treatment of Chagas disease) induce oxidative stress by nitroreduction. This reaction generates a nitro anion radical intermediate and it is catalysed by different reductases, between them the CYP450 reductase (Olea-Azar *et al.*, 2003). This intermediate can: 1) react with molecular oxygen generating superoxide anion and consequently oxidative stress, and 2) in a futile cycle, be reduced to nitrous compound, hydroxylamine and amine (Olea-Azar *et al.*, 2003). Likewise, acetaminophen, an analgesic and antipyretic drug, is biotransformed in the liver by conjugation with glucuronic acid (60%) and sulphate (35%), reactions catalysed by UDP-glucuronyltransferase and sulphotransferase, respectively. A small proportion of acetaminophen undergoes *N*-hydroxylation mediated by CYP450 system (~5%) to form a highly reactive electrophilic intermediary called *N*-acetyl-*p*-benzoquinone imine (NAPQI). This metabolite is then conjugated with GSH, reaction catalysed by GSH-transferase (GST) (James, 2003). High doses of acetaminophen increase NAPQI concentration, which may saturate GST increasing the risk to develop acute liver failure which is a severe toxic effect of this drug (Jenkins *et al.*, 2008). The administration of nitrofurantoin and nifurtimox generate gastrointestinal inflammation and acetaminophen, hepatitis, processes closely associated with oxidative stress (Dröge, 2002; Masella & Mazza, 2009).

Data exist about the effects of co-therapy with herbal preparations in the treatment of cardiovascular and degenerative diseases, pathologies associated to oxidative stress and inflammation processes (Akhtar & Haqqi, 2012; Lin *et al.*, 2001). Therefore, we postulate that adverse effects of nitrofurantoin, nifurtimox and acetaminophen are associated to oxidative stress and therefore, they could be decreased by antioxidant herbal preparations. To prove this postulate, we isolated rat liver microsomes, an enriched preparation of endoplasmic reticulum. In this subcellular organelle are mainly located the biotransformation enzymes, especially the CYP450 system that is the enzymatic system through nitrofurantoin; nifurtimox and acetaminophen are metabolized generating oxidative stress. On the other hand, standardized dry herbal

extracts from *Rosmarinus officinalis*, *Buddleja globosa* Hope, *Cynara scolymus*, *Echinacea purpurea* and *Hedera helix* were tested. These plants have been widely characterized. *R. officinalis*, mainly used as condiment for food, contains monoterpene hydrocarbons (limonene and α -pinene), flavonoids (apigenin and luteonin), hydrocinamic acids mainly represented by caffeic acid and rosmarinic acid, and coline (Letelier *et al.*, 2015). *B. globosa*, traditionally used as a wound healing plant, contains diverse triterpenoids, sesquiterpens flavonoids as kaempferol, and glycosides as verbacoside (Backhouse *et al.*, 2008; Vogel *et al.*, 2011). *H. helix*, commonly prepared as expectorant and antitussive, contains diverse flavonoids as rutin and kaempferol, and saponins (hederacosids and hederagenine) (Demirci *et al.*, 2004; Woldemichael & Wink, 2001). *E. purpurea*, widely used in the USA for its immunological properties, contains alkamides, caffeic acid derivatives represented mainly by chlorogenic acid and cichoric acid, polysaccharides, and glycoproteins (Saeidnia *et al.*, 2015; Wagner *et al.*, 1988). *C. scolymus*, commonly known as artichoke, contains polyphenols, mainly represented by glycoside forms of flavonoids such as apigenin and luteolin and hydroxycinnamic derivatives mainly represented by mono and di-caffeoylquinic acids (Di Venere *et al.*, 2005; Miccadei *et al.*, 2008).

To evaluate the oxidative stress, rat liver microsomes were incubated with nitrofurantoin, nifurtimox and acetaminophen in the presence of NADPH, specific cofactor to CYP450 system. As a manner to observe changes in the enzymatic activities, microsomal GSH transferase (GST) activity was also evaluated. GSTs (EC 2.5.1.18) are represented by a family of enzymes widely distributed in the body. Hepatic GSTs comprise soluble and membrane-associated isoenzymes, which represented the highest concentration of these proteins in the organism (Aniya & Anders, 1989; Kaplowitz, 1980). These enzymes have a wide specificity for lipophilic and electrophilic substrates (Masella & Mazza, 2009). A dimer or trimer of identical subunits bound by their cysteine residues are the active forms of rat liver microsomal GST (Lengqvist, 2004; Strange *et al.*, 2001). On the other hand, the microsomal GST and the alpha-class cytosolic GST exhibit also glutathione peroxidase activity, suggesting that these enzymes might be of particular importance as a defence mechanism against lipid peroxidation. Data presented show the importance of GSTs in the detoxication processes and the toxicological risk represented by their saturation, either

by reversible or irreversible binding to electrophilic metabolites such as NAPQI.

In the biotransformation condition, nitrofurantoin, nifurtimox and acetaminophen provoked microsomal lipid peroxidation, decreased thiol content and inhibited microsomal GST activity. Standardized dry herbal extracts prepared from *Rosmarinus officinalis*, *Buddleja globosa* Hope, *Cynara scolymus*, *Echinacea purpurea* and *Hedera helix* prevented these oxidative phenomena in different extension. These results seem to indicate that antioxidant phytochemicals could decrease oxidative stress so alleviating the adverse effects of drugs whose metabolism is associated to oxidative stress. New *in vivo* experiments must be conducted however, to determine the efficacy and safety of herbal preparations. Thus, we can evaluate with certainty the pharmacokinetic and pharmacodynamic significance of these results.

MATERIALS AND METHODS

Chemicals

BSA (Fraction IV), Folin Ciocalteu's reagent, 5,5'-dithio-bis (2-nitrobenzoic) acid (DTNB, Ellman's reagent), 1-chloro-2,4-dinitrobenzene, GSH, thiobarbituric acid (TBA), β -NADP, Glucose-6-phosphate (G-6-P), G-6-P dehydrogenase and catechin ((2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol) were imported from Sigma Aldrich USA. Trichloroacetic acid (TCA) was obtained from Merck Santiago-Acetaminophen (*N*-(4-hydroxyphenyl)-ethanamide), nitrofurantoin ((E)-1-[(5-nitro-2-furyl) methylidene-amino]-imidazolidine-2,4-dione) and nifurtimox (4-(5-nitro-furfurylidene)-amino-3-methylmorpholine-1,1-dioxide) were obtained from Bayer Santiago-Chile S.A.

Plant extracts

R. officinalis, *B. Globosa*, *H. helix*, *E. purpurea*, and *C. scolymus* dried herbal extracts were graciously donated by Laboratorios Ximena Polanco (Santiago, Chile). Hydroalcoholic extraction and extracts concentration processes are private property of Laboratorios Ximena Polanco. Vegetal drugs (leaves) were provided by suppliers who grow medicinal plants through organic farming. For each assay, 1mg/mL of each extract was dissolved in a mixture ethanol-water (1:1).

Animals

Adult male Sprague Dawley rats (200 – 250 g), maintained at the vivarium of Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile

(Santiago, Chile) were used. Rats were allowed to free access to pellet food, and maintained with controlled temperature ($22 \pm 1^\circ \text{C}$) and constant photoperiods (lights on from 07:00 h to 19:00 h). All procedures were performed using the protocols approved by the Institutional Ethical Committee of Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, and according to the guidelines of the Guide for the Care and Use of Laboratory Animals [Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research (National Research Council), 2003].

Microsomal fraction isolation

Microsomal fraction was prepared from rat livers as previously reported (Letelier et al., 2005). Groups of 10 animals were fasted for 15h with water *ad libitum*, and sacrificed by decapitation. Four volumes of 25 mL 0.9% w/v NaCl were used to perfuse the livers *in situ*; then, they were excised and placed on ice (4°C). All homogenization and fractionation procedures were performed at 4°C using either a Suprafuge 22 Heraeus centrifuge or an XL-90 Beckman ultracentrifuge. Liver tissue devoid of connective and vascular tissue was homogenized with five volumes of 0.154 M KCl, with eight strokes in a Dounce Wheaton B homogenizer. Homogenate was centrifuged at 2,000 g for 10 min; the sediment was discarded and the supernatant centrifuged at 10,000 g for 10 min; the sediment obtained was discarded and the supernatant centrifuged at 105,000 g for 60 min. The sediment of this centrifugation corresponds to microsomal fraction. All collected sediments were stored at -80°C until use. Microsomal protein was determined according to Lowry et al. (1951) using BSA fraction IV as standard. The reproducibility of this preparation was checked measuring the concentration of monooxygenase CYP450/mg of microsomal protein; this corresponded to $0.67 \text{ nmol} \pm 0.005$. Also, the yield of microsomal protein of each preparation (mg of microsomal protein/g of rat liver) was determined.

Polyphenols content of herbal extracts

Polyphenols were determined as previously described (Letelier et al., 2008), In a final volume of 5 mL, 50 μL of herbal extract, 250 μL of Folin Ciocalteu's reagent, 750 μL of 20% w/v sodium carbonate and 3950 μL of distilled water were mixed. Blanks contained all the reagents with the exception of herbal extracts. Then, blanks and mixtures were incubated for 2 h under darkness. At the end of this period, the absorbance of

the samples was determined at 760 nm in a UV3 Unicam UV-VIS spectrophotometer, using their respective blanks as reference. Catechin was used as reference standard.

Microsomal lipid peroxidation

The extent of microsomal lipid peroxidation following incubation of microsomes with drugs and the NADPH generating system was estimated assaying thiobarbituric acid reactive substances (TBARS), as previously described (Aracena *et al.*, 2014). Microsomes (1mg/mL) were incubated with a NADPH-generating system comprising 6 mM glucose-6-phosphate, 0.6 mM NADP, and 0.15 U/mL glucose-6-phosphate dehydrogenase in 50 mM phosphate buffer (pH 7.4) for 30 min at 37° C. The extent of microsomal lipid peroxidation was estimated assaying TBARS. In brief, lipid peroxidation reaction was stopped with 0.5 mL of 20% TCA and then supernatant was separated by centrifugation. The samples were then incubated with TBA 1% for 1h and absorbance was read at 532 nm. The results are expressed as nmole of TBARS per minute per mg of microsomal protein and were calculated using the extinction coefficient of formed conjugated, 156 mM⁻¹cm⁻¹.

Microsomal thiol content

Thiol content was titrated in microsomes previously incubated with drugs and the NADPH generating system, using DTNB as previously reported (Letelier *et al.*, 2005). Experimental absorbance values were transformed to nmole of thiol/mg of microsomal protein using the extinction coefficient of formed conjugated, 13,600 M⁻¹cm⁻¹.

Oxidative conditions

Microsomes (1 mg of protein/mL) suspended in 50mM phosphate buffer, pH 7.4 were preincubated with or without herbal extracts (20 µg of extract/mg of microsomal protein) for 15min at 37° C before adding nitrofurantoin (10 µM), nifurtimox (10 µM) or acetaminophen (1mM) and NADPH generating system (10mM G-6-P, 1mM NADP and G-6-P dehydrogenase (5U). Then all mixtures were again incubated for 30min at 37° C before to determine TBARS and GST activity, and 60min to determine microsomal thiol content.

Assay of GST activity

Conjugation of 1-chloro-2,4-dinitrobenzene with GSH, reaction catalysed by GST, was assayed as previously described (Letelier *et al.*, 2010). Using 10 µg of

cytosolic protein, 1-chloro-2,4-dinitrobenzene as substrate, and GSH (1 mM and 4 mM final concentration, respectively), in 100mM sodium phosphate buffer, pH 6.5. Conjugated-substrate apparition was continuously recorded for 2 min at 25° C, at 340 nm ($\epsilon_{340} = 9.6 \text{ mM}^{-1}\text{cm}^{-1}$) in a UV3 Unicam UV-VIS spectrophotometer. All GST activity assays were realized in conditions of linearity respect to incubation time and protein concentration. Linearity conditions respect to microsomal protein and incubation time were previously determined.

Statistical analyses

Data are presented as the mean of at least four independent experiments \pm SD. Analyses of the significance of the differences in means were performed using *t*-Student test. Data were considered significantly different when $p < 0.05$. All statistical analyses were performed using GraphPad Prism, version 5.0.

RESULTS

Polyphenol content of herbal extract tested

As observed in Table 1, *R. officinalis* extract presented the highest polyphenol content (0.148 \pm 0.005 mg of catechin/mg of extract) and the *E. purpurea* extract, the lowest (0.019 \pm 0.004 mg of catechin/mg of extract).

Microsomal lipid peroxidation induced by drug biotransformation

CYP450 system present in liver microsomes metabolizes lipophilic drugs in the presence of NADPH as the only specific cofactor. In this condition, nitrofurantoin, nifurtimox and acetaminophen generate lipid peroxidation indicating the development of oxidative stress (Figure 1). Acetaminophen (1 mM) provoked higher production of TBARS than nifurtimox and nitrofurantoin (10 µM).

Inhibition of microsomal Lipid peroxidation induced by drug biotransformation: effects of herbal extracts

Microsomes were preincubated with herbal extracts (20 µg of extract/mg of microsomal protein/mL) for 15 min, before adding NADPH generator system. Microsomal lipid peroxidation induced by nifurtimox was reduced by *B. globosa* and *R. officinalis* extracts about 65.0%; *H. helix*, *C. scolymus* and *E. purpurea* extracts reduced it in 38.3%, 22.0% and 15.2%, respectively (Figure 2). Lipid peroxidation induced by nitrofurantoin biotransformation was reduced by *B. globosa*, *R. officinalis* and *H. helix* extracts approximately 50%; *C. scolymus* extract reduced this phenomenon 17.9% and

E. purpurea extract did not alter it significantly (Figure 2). Likewise, *B. globosa*, *R. officinalis*, *H. helix* and *C. scolymus* extracts reduced the microsomal lipid peroxidation induced by oxidative metabolism of acetaminophen 77.3, 53.1, 41.6 and 26.5%, respectively and *E. purpurea* extract did not significantly alter it.

Correlation studies between the amount of polyphenols present in the herbal extracts and the inhibition percentage of microsomal lipid peroxidation

induced by biotransformation of nifurtimox, nitrofurantoin and acetaminophen are shown in Figure 2 (right graphs). The inhibition of lipid peroxidation induced by herbal extracts correlated linearly with the polyphenol concentration of each one of them. The correlation coefficients (r) calculated from lipid peroxidation assays of nitrofurantoin, nifurtimox and acetaminophen were 0.9311, 0.9706 and 0.9657, respectively.

Table 1
Polyphenol Content of Herbal Extracts

Herbal Extract	mg equivalent of Catechin/ mg of Herbal Extract
<i>E. purpurea</i>	0.019 ± 0.004
<i>C. scolymus</i>	0.060 ± 0.002
<i>H. helix</i>	0.116 ± 0.004
<i>B. globosa</i>	0.129 ± 0.005
<i>R. officinalis</i>	0.148 ± 0.005

Polyphenol content was determined according Methods. Values represent the mean of at least 4 independent experiments ± S.D.

Table 2
Effect of herbal extracts on inhibition of microsomal GST activity caused by drug biotransformation

Inhibition of GST activity (%)			
Condition	Nitrofurantoin	Nifurtimox	Acetaminophen
+NADPH	*47.1 ± 3.9	*48.6 ± 3.7	*47.5 ± 3.2
RO	18.0 ± 0.9	26.2 ± 1.6	15.7 ± 1.1
CS	38.9 ± 1.5	40.7 ± 2.2	36.7 ± 0.8
HH	19.4 ± 0.9	21.2 ± 1.1	23.0 ± 1.1
BG	42.4 ± 1.9	44.2 ± 1.6	32.3 ± 1.6
EP	37.9 ± 0.8	**48.1 ± 0.9	38.1 ± 1.3

Inhibition (%) was calculated considering as 100% the GST activity measured in the absence of NADPH generator system and presence of extracts (20µg/0.1mg of microsomal protein/mL).

Microsomal GST activity was measured as described in Methods. RO: *R. officinalis*, CS: *C. scolymus* L, HH: *H. helix*, BG: *B. globosa* Hope, and EP: *E. purpurea*. All values represent the mean of at least 4 independent experiments ± S.D. (*) Values significantly different to those obtained from treatment of the samples with extracts (p< 0.05). **Value not significantly different to nifurtimox plus EP (p=0.076).

Decrease of microsomal thiol content induced by drug biotransformation and the effect of herbal extracts

Under biotransformation conditions, nifurtimox and nitrofurantoin (10 μ M) decreased microsomal thiols about 50%. The preincubation of microsomes for 15min with herbal extracts partially prevented the loss of microsomal thiols induced by biotransformation of these drugs. In the presence of *B. globosa*, *R. officinalis* and *H. helix* extracts the loss of microsomal thiols provoked by nitrofurantoin reached only to 15.8%, 18.4% and 25%, respectively. In the same conditions, but in the presence of nifurtimox, *B. globosa*, *R. officinalis* and *H. helix* extracts loss of microsomal thiols reached only to 21.4%, 29.7% and 43.4%, respectively. The *C. scolymus* and *E. purpurea* extracts did not modify the loss of microsomal thiols provoked

by nitrofurantoin and nifurtimox (Figure 3). On the other hand, 1mM acetaminophen in the presence of NADPH generator system decreases microsomal thiols 35%. The addition of *B. globosa* and *R. officinalis* extracts to reaction mixture reduced totally the loss of microsomal thiols; the addition of *H. helix* extract, reduced this loss to 12.9% and *C. scolymus* and *E. purpurea* extracts, approximately to 24% (Figure 3). Interestingly, this antioxidant effect was correlated linearly with the polyphenol concentration of herbal extracts (Figure 3). The correlation coefficients (r) calculated from assays of microsomal thiols in the presence of nitrofurantoin, nifurtimox and acetaminophen were 0.9850, 0.9421 and 0.9405, respectively. All values of p were < 0.02 .

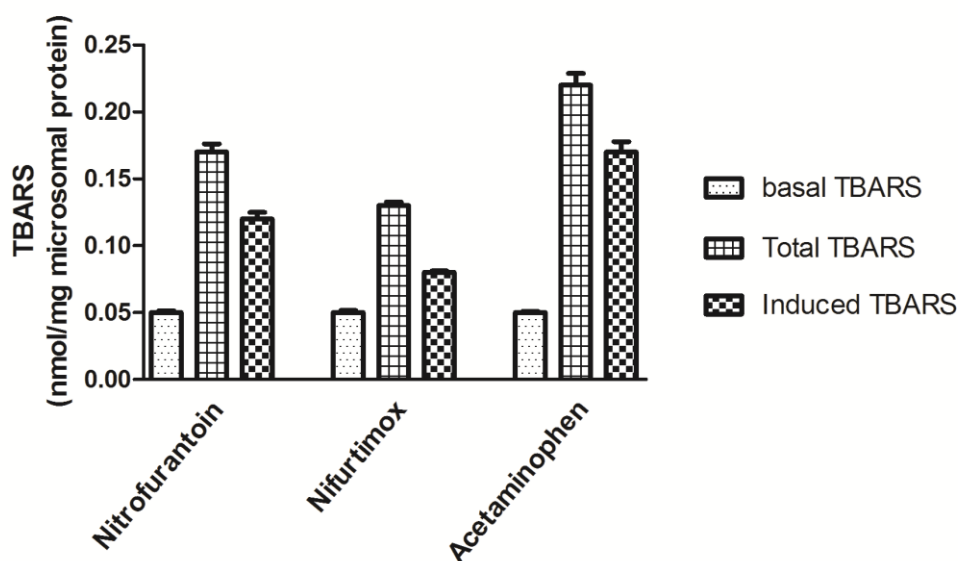


Figure 1

Microsomal lipid peroxidation induced by drug biotransformation. Microsomes were incubated with 10 μ M nitrofurantoin, 10 μ M nifurtimox or 1 mM acetaminophen and the NADPH generator system according to Material and Methods. **Basal TBARS:** microsomes incubated in the absence of drugs. **Total TBARS:** microsomes incubated in the presence of drugs and NADPH generator system. **Induced TBARS:** correspond to difference between Total TBARS and Basal TBARS. Values represent the mean of at least four independent experiments \pm S.D.

Microsomal GST activity measured in the presence of nitrofurantoin, nifurtimox and acetaminophen in their biotransformation condition

Considering that the antioxidant agents present in the herbal extracts could inhibit the GST activity reducing the disulphide bond of the active dimer form of GST,

concentration-response curves were developed. All extracts inhibited the microsomal GST activity as a concentration-response manner (Figure 4). Therefore, a mixture containing herbal extracts (20 μ g) but not NADPH generator system was used as blank to measure the oxidative damage provoked by biotransformation of

drugs on microsomal GST. Likewise, nitrofurantoin (10 μ M), nifurtimox (10 μ M), and acetaminophen (1 mM) were also tested. In the absence of NADPH all drugs,

did not modify the microsomal GST activity (data not shown).

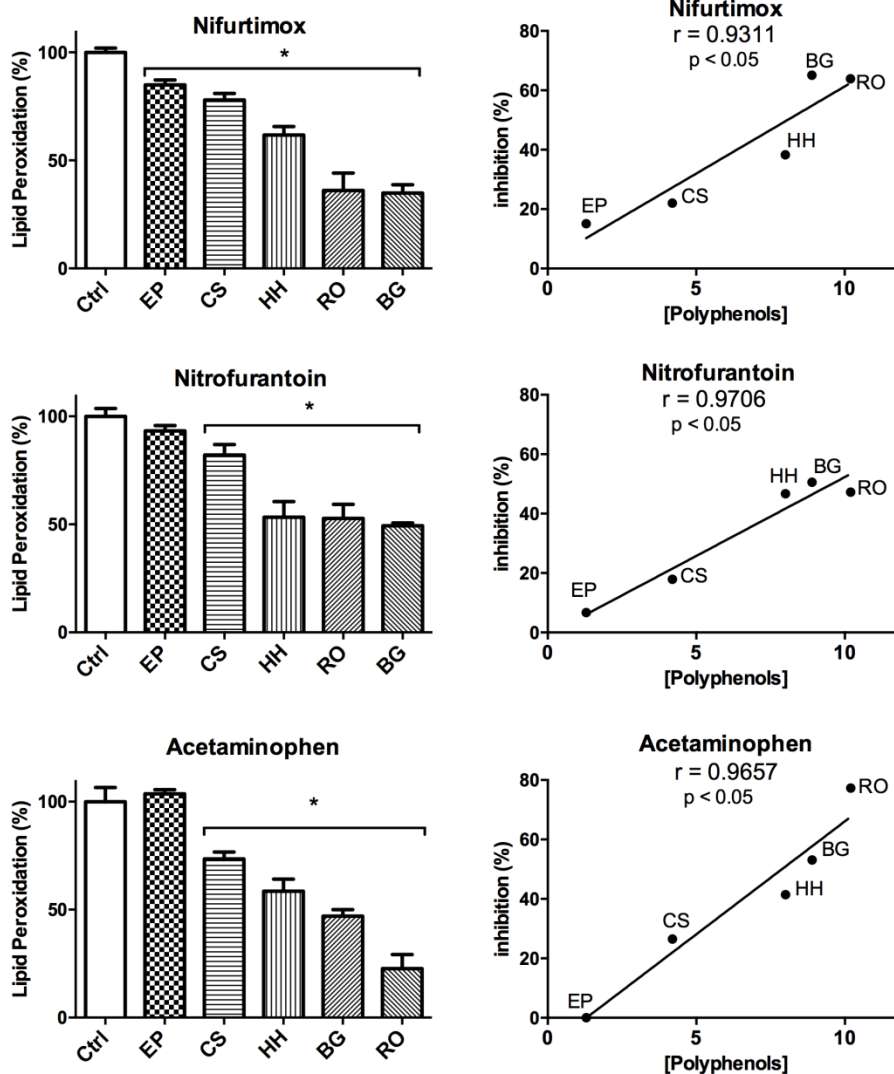


Figure 2

Effect of herbal extracts on Lipid peroxidation induced by drug biotransformation and its correlation with their polyphenol content

Left graphs show the lipid peroxidation induced by the biotransformation of drug in the presence of herbal extracts. Right graphs show the correlation between the extract polyphenol concentrations contained in 20 μ g of each extract (nmol equivalent of catechin/20 μ g) respect to the inhibition (%) of microsomal lipid peroxidation induced by biotransformation of drugs considered as 100%. RO: *R. officinalis*, CS: *C. scolymus* L, HH: *H. helix*, BG: *B. globosa* Hope, and EP: *E. purpurea*. **Control (Ctrl)**: lipid peroxidation induced by drug biotransformation (100%), according Methods. **[Herbal extracts]**: 20 μ g/mg of microsomal protein/mL. Lipid peroxidation was determined according Methods All values represent the mean of at least four independent experiments \pm S.D. (*) Values statistically different to control ($p < 0.05$).

Nitrofurantoin, nifurtimox and acetaminophen in the presence of NADPH (biotransformation conditions) inhibited the microsomal GST activity 47.1, 48.6 and 47.5%, respectively (Table 2). The inhibition of microsomal GST activity induced by nitrofurantoin (47.1%) was decreased to 18.0, 38.9, 19.4, 42.4 and 37.9% by *R. officinalis*, *C. scolyumus*, *H. helix*, *B. globosa* and *E. purpurea* extracts, respectively. Likewise, the inhibition of microsomal GST activity induced by nifurtimox (48.6%) was decreased to 26.2, 40.7, 21.2, and 44.2% in the presence of *R. officinalis*,

C. scolyumus, *H. helix* and *B. globosa*, respectively; the only exception was the *E. purpurea* extract, which inhibition percentage (48.1%) was not significantly different ($p = 0.076$) to that obtained in the presence of nifurtimox and in the absence of *E. purpurea* extract (48.6%). In the same way, the inhibition of GST activity induced by acetaminophen in its biotransformation condition (47.5%) was decreased to 15.7, 36.7, 23.0, 32.3, and 38.1% by *R. officinalis*, *C. scolyumus*, *H. helix*, *B. globosa* and *E. purpurea* extracts, respectively.

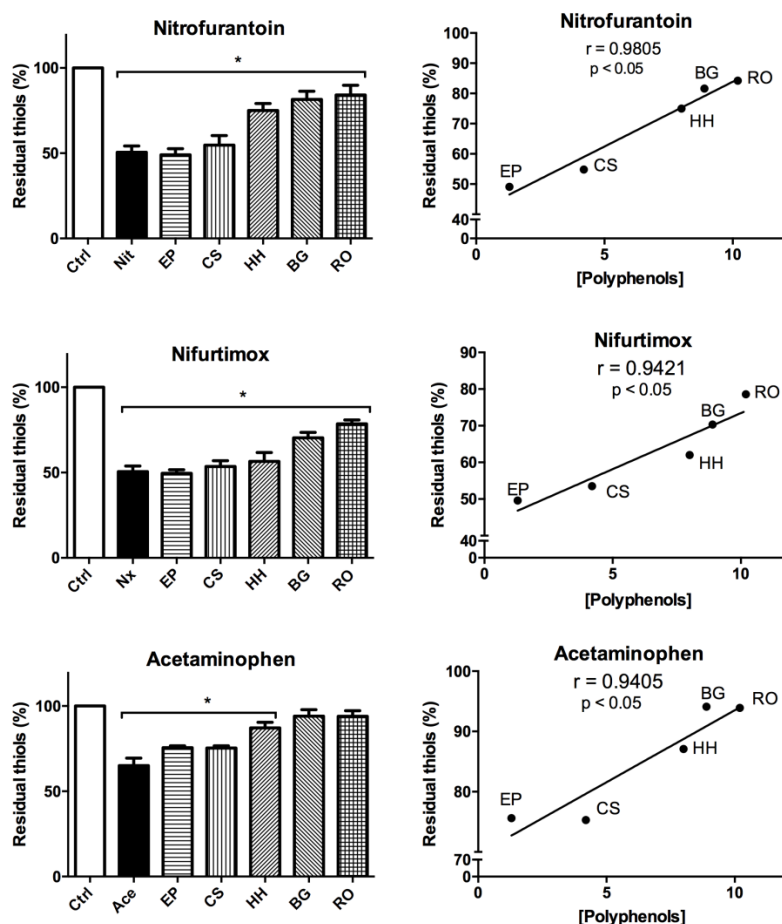


Figure 3

Effect of herbal extracts on the decrease of microsomal thiols induced by drug biotransformation

The left graphs shown these results and the right graphs, the correlation between the polyphenol concentrations contained in 20 µg of each extract assayed and the residual (%) microsomal thiols. RO: *R. officinalis*, CS: *C. scolyumus* L, HH: *H. helix*, BG: *B. globosa* Hope, and EP: *E. purpurea*. **Control (Ctrl)**: microsomal thiol in the absence of drugs, NADPH and extracts. **[Drugs]**: Nitrofurantoin (10 µM), Nifurtimox (10 µM) and Acetaminophen (1 mM). **[Herbal extracts]**: 20 µg/mg of microsomal protein/mL. All values represent the mean of at least four independent experiments ± S.D. (*) Values statistically different to control ($p < 0.05$).

DISCUSSION

There are numerous publications regarding the adverse effects caused by nitrofurantoin (antimicrobial agent), nifurtimox (trypanocidal drug) and acetaminophen (analgesic and antipyretic drug) (Bartel *et al.*, 2009; Huttner *et al.*, 2015; James, 2003). Inflammatory

processes are associated to their adverse effects. Inflammation is a phenomenon associated to oxidative stress (Rodrigo *et al.*, 2013). The generation of ROS by nitroreduction of nitrofurantoin and nifurtimox have been demonstrated (Aracena *et al.*, 2014; Letelier *et al.*, 2004).

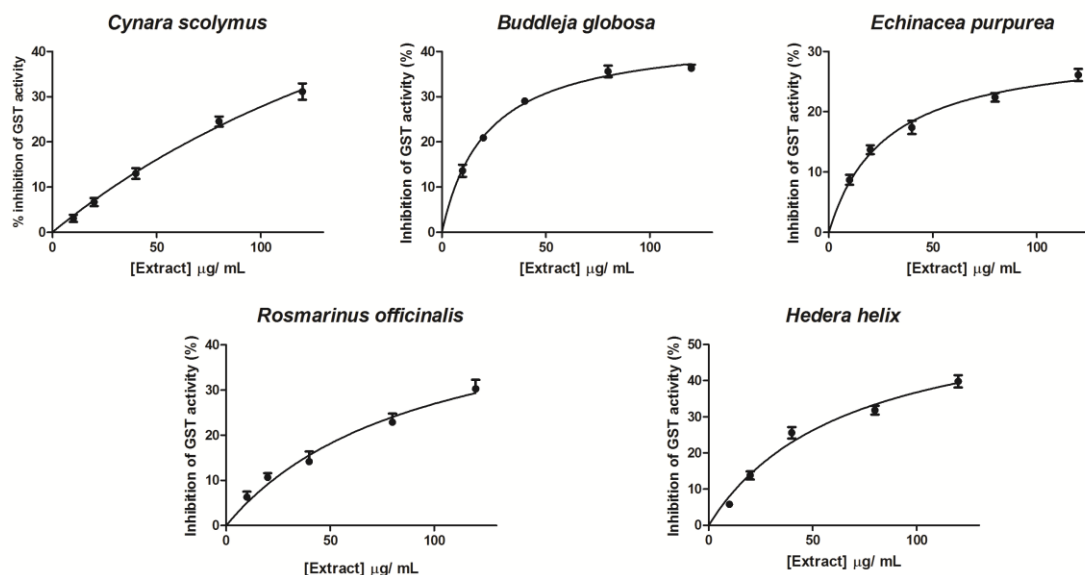


Figure 4

Effect of herbal extracts on microsomal GST activity

GST activity was measured according Methods. All values represent the mean of at least four independent experiments \pm S.D.

In vitro experiments have also shown that the metabolism of acetaminophen induces oxidative stress, which would be caused by the generation of reactive oxygen species.

The authors determined the presence of hydroxyl radicals ($\text{HO}\cdot$) in the reaction mixture (James, 2003) but the mechanism through which these reactive oxygen species are generated has not yet been determined. These oxidative phenomena were confirmed to incubate liver microsomes with these drugs in the presence of NADPH, specific cofactor of CYP450 system; all of them generated oxidation of microsomal lipids and thiol groups (Figures 1, 2, 3) and also inhibited the GST activity (Table 2). Moreover, these oxidative phenomena were prevented by herbal extracts tested which contained different polyphenol concentration. The oxidation of microsomal lipids and thiol groups was polyphenol-concentration dependent (Figures 1, 2, 3) but not the inhibition on microsomal GST activity (Table 2). *B. globosa* and *E. purpurea*

were the herbal extracts that developed the minor preventive antioxidant effect on microsomal GST activity and *Rosmarinus officinalis*, the highest; the polyphenol content of *B. globosa* and *R. officinalis* however, were very similar, 0.129 ± 0.005 and 0.148 ± 0.005 mg equivalent of catechin/mg of herbal extract, respectively. This difference may be related to: 1) the presence in the extracts not only of polyphenols but other principles such as thiol and terpenoids compounds which also behave as antioxidant compounds, 2) the quantity and proportion of the different antioxidant compounds in the extracts, 3) differences in the redox potential of the biomolecules tested. The above indicate that assays *in vitro* of antioxidant activity of herbal extract should be always checked by test *in vivo* such as antioxidant capacity of plasma and plasmatic concentration of malondialdehyde (MDA): only these tests can show a real increase in cellular antioxidant capacity provoked by the administration of herbal preparation.

Liver endoplasmic reticulum is the main cellular organelle involved in drug biotransformation. Therefore, it plays an important role in the pharmacokinetics and pharmacodynamics of drugs. The main biotransformation enzymes localized in this organelle are UDP-glucuronyltransferase, GST isoform and CYP450 oxidative system. Cysteine residues of these enzymes are involved in their enzymatic activities. Low concentrations of hydrogen peroxide (< 1 mM) activates microsomal GST and UDP-glucuronyltransferase but inhibit the CYP450 system activity (Letelier *et al.*, 2010). Under these conditions lipid peroxidation is negligible. When lipid peroxidation occurs, all biotransformation enzymes are inhibited. Nitrofurantoin, nifurtimox and acetaminophen in biotransformation condition provoked microsomal lipid peroxidation, decreased the microsomal thiol content and inhibited GST activity. Therefore, these drugs could alter hepatic metabolism. Thus, prevent oxidative stress in which unbalance of oxidant and antioxidant cellular species happens, favours the efficacy and security of drugs. In other words, pharmacokinetic and pharmacodynamics parameters of these drugs can be improved if oxidative stress is prevented. The decrease of adverse effects could also lead to reduce doses of such drugs, which also could contribute to diminish their adverse effects. Studies concerning the safety, dosage, efficacy and possible drug interactions are currently underway in our laboratory. These studies will allow that herbal preparations can be register by Human Health Institutes and then can be distributed to patients, especially those prescribed in our primary health care.

CONCLUSIONS

Antioxidant phytochemicals could be evaluated to alleviate adverse effects of drugs associated to oxidative stress.

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