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Review

Recent advances on *Ilex paraguariensis* research: Minireview

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ABSTRACT

Ilex paraguariensis dried and minced leaves are made into a brewed tea, prepared in a sui generis manner by large populations in South America, having evolved from a tea drunk by the Guarani ethnic group to a beverage that has a social and almost ritualistic role in some South American modern societies. It is used both as a source of caffeine, in lieu or in parallel with tea and coffee, but also as a therapeutic agent for its alleged pharmacological properties. Although with some exceptions, research on biomedical properties of this herb has had a late start and strongly lags behind the impressive amount of literature on green tea and coffee. However, in the past 15 years, there was a several-fold increase in the literature studying *Ilex paraguariensis* properties showing effects such as antioxidant properties in chemical models and *ex vivo* lipoprotein studies, vaso-dilating and lipid reduction properties, antimutagenic effects, controversial association with oropharyngeal cancer, anti-glycation effects and weight reduction properties. Lately, promising results from human intervention studies have surfaced and the literature offers several developments on this area. The aim of this review is to provide a concise summary of the research published in the past three years, with an emphasis on translational studies, inflammation and lipid metabolism. *Ilex paraguariensis* reduces LDL-cholesterol levels in humans with *Ilex paraguariensis* dyslipoproteinemia and the effect is synergic with that of statins. Plasma antioxidant capacity as well as expression of antioxidant enzymes is positively modulated by intervention with *Ilex paraguariensis* in human cohorts. A review on the evidence implicating *Ilex paraguariensis* heavy consumption with some neoplasias show data that are inconclusive but indicate that contamination with alkylating agents during the drying process of the leaves should be avoided. On the other hand, several new studies confirm the antimutagenic effects of *Ilex paraguariensis* in different models, from DNA double breaks in cell culture models to mice studies. Novel interesting work has emerged showing significant effect on weight reduction both in mice and in rat models. Some mechanisms involved are inhibition of pancreatic lipase, activation of AMPK and uncoupling of electron transport. Intervention studies in animals have provided strong evidence of anti-inflammatory effects of *Ilex paraguariensis*, notably protecting cigarette-induced lung inflammation acting on macrophage migration and inactivating matrix-metalloproteinase. Research on the effects of *Ilex paraguariensis* in health and disease has confirmed its antioxidant, anti-inflammatory, antimutagenic and lipid-lowering activities. Although we are still waiting for the double-blind, randomized prospective clinical trial, the evidence seems to provide support for beneficial effects of mate drinking on chronic diseases with inflammatory component and lipid metabolism disorders.

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1. Introduction

Ilex paraguariensis extracts constitute an interesting example of ethno-pharmacology. *Ilex paraguariensis* dried and minced leaves are made into a brewed tea, prepared in a very sui generis way by large populations in South America, having evolved from a tea drunk by the Guarani ethnic group to a beverage that has a social and almost ritualistic role in some South American modern societies. It is used both as a source of caffeine, in lieu or in parallel with tea and coffee, but also as a therapeutic agent for its alleged pharmacological properties.

"Maté", *Ilex paraguariensis* St. Hil. var. *paraguariensis* (Aquifoliaceae) has been popular for centuries, and was adopted from the native inhabitants (Guaraníes) of a region that comprises Paraguay, Uruguay, North Eastern Argentine and Southern Brazil, for the preparation of stimulant beverages or for its medicinal properties. The beverage is known as yerba mate, hierba mate, maté, té del Paraguay, kaĩhá, erva maté, chimarrão, and tereré. After the Jesuits colonizers decided to promote the cultivation of maté as an industry, the beverage was later adopted by white people who named it "chimarrão" in Brazil, "maté" in Argentina and Uruguay and "tereré" in Paraguay where it is prepared with cold water in the summer.

Morphologically, two varieties of *Ilex paraguariensis* can be identified: *Ilex paraguariensis* St. Hil. var. *paraguariensis* and *Ilex paraguariensis* var. *vestita* (Reisseck) Loes (which is densely pubescent and not used industrially). Both of these species coexist in some regions of Northeastern Argentina and Brazil (Filip et al., 2010a).

Ilex paraguariensis is a dioecious evergreen tree, which can grow to an elevation of up to 8–15 m. The 8 cm long olive-green leaves are perennial, alternate, coriaceous, obovate with slightly crenate dentate margins and obtuse apex, and have a wedge shaped base. The petioles are up to 15 mm long. The flowering stage occurs during spring season, producing small, unisexual flowers which have 4 white petals. In some tropical or subtropical species, the number of petals may be 5, 6 or 7. These may be clustered in groups of 1–15 flowers that appear in the axil of the leaves. The fruits are red-colored berries containing 4–5 seeds.

The term "maté" actually refers to a gourd made from the dry and hollowed fruit of *Lagenaria vulgaris* Ser. (*Cucurbitaceae*), a plant of widespread use across the globe. In Uruguay and Southern Brazil it is frequent to see gourds averaging 12–15 cm wide with openings of 10 cm. In Argentina the gourds commonly used are smaller: approximately 7–10 cm wide and have an opening of 2.5–3.0 cm at the top which is used to fill it with maté leaves and water in order to prepare the infusion. Once the infusion is ready, a special drinking straw, a narrow tube which has a flattened open end which serves as a mouth piece and finishes in a closed perforated bulb-like filter is inserted into the maté (Filip et al., 2010a). This bulb is approximately the size of a teaspoon and the perforations, which are the size of a pinhole, avoid the aspiration of fine solids – powdered maté leaves – when the infusion is sucked up through it. This straw-like device, about 20 cm long and 6–8 mm diameter, which is known as "bombilla" (literally: small pump) is usually made of metals such as stainless steel (Bracesco, 2003). This peculiar method of brewing allows for a continuous extraction of the compounds in the dried leaves, indeed, a portion of the compacted tea is left dry on top. About 20–40 ml of freshly extracted concoction is drunk each time, and many consumers drink 1–2 l per day. The drinking process is continuous and usually accompanies daily activities in such a way that 1 l is drunk over periods averaging 1 h. Instead of the quick peaks and decays of circulating absorbed components that are seen when 200 ml of coffee are drunk in 10 min, the continuous manner in which maté is drunk may produce sustained (albeit lower) plasma concentrations of compounds that may thus afford antioxidant and other effects in a more constant way. This deserves exploration. An illustration of gourd and strainer can be found in the central area of Figs. 2 and 3 in this review.

Instead of using hot water, maté can also be prepared using cold water; this beverage is known as "tereré" and is consumed mainly in Paraguay. In South Eastern Brazil, *Ilex paraguariensis* leaves are roasted in a manner reminiscent of that used for tea and the resulting "cha matte" is brewed as tea or made into sweetened drinks, very popular in So Paulo and Rio de Janeiro.

Along with the ongoing traditional usage of maté beverages, *Ilex paraguariensis* has made its way to beers, creams, candy, and other non-traditional uses in the last decade (Vieira et al., 2008). Interestingly it has reached supermarkets in the form of energy drinks in California and is been sold in Europe in combination with other herbs as an energy tea or as a weight reduction aid.

Maté has a very important social role and the act of offering it and sharing has connotations similar to those of the tea ceremony for some oriental cultures. Argentina is the first exporter of mate while Uruguay has the highest *per capita* consumption: 6–8 kg/person/year. Argentina follows with 5 kg/person/year. Only 3 states in Brazil have maté drinkers in their population (lowering the *per capita* intake), but up to 70% of the male population in the states of Rio Grande do Sul, Santa Catarina and Parana drinks chimarrão daily.

Although with some exceptions, research on biomedical properties of this herb has had a late start and strongly lags behind the impressive amount of literature on green tea and coffee, also a reflection of the different economic development and sizes of the populations consuming the latter (Heck and de Mejia, 2007).

However, in the past 15 years, there was a several-fold increase in the literature studying *Ilex paraguariensis* properties, that have been extensively reviewed earlier (Bastos et al., 2007; Heck and de Mejia, 2007; Filip et al., 2010a), showing effects such as:

1. antioxidant properties using chemical models and *ex vivo* lipoprotein studies
2. vaso-dilating and lipid reduction properties
3. mutagenic vs. antimutagenic effects depending on the model
4. controversial association with oropharyngeal cancer
5. anti-glycation effects
6. weight reduction properties

Originally stemming from the same area where it is drunk, research on maté has extended to countries across the globe such as Korea, Japan, China, USA and this, in turn has motivated the establishment of an international society that nucleates most of these researchers as well as the hosting of a first international symposium on *Ilex paraguariensis* where more than 40 scientists presented their latest research on the topic. In the past, most of the investigations on this natural product have consisted of *in vitro* studies on model proteins or lipoproteins, cell culture studies and some animal studies. Lately, promising results from human intervention studies have surfaced and the literature offers several developments on this area.

The aim of this review is to provide a concise summary of the research presented at the first international symposium on mate, that have been published since, as well as other interesting articles published in the last three years, with an emphasis on the main focus of this issue of the Journal of Ethnopharmacology, and on translational studies.

2. Main bioactive components of *Ilex paraguariensis* extracts (Fig. 1)

Ilex paraguariensis green (non-roasted) extracts contain purine alkaloids (methyl xanthines), flavonoids, vitamins such as vitamin A, B complex, C and E, tannins, chlorogenic acid and its

derivatives, and numerous triterpenic saponins derived from ursolic acid, known as matesaponins (Bastos et al., 2007; Heck and de Mejia, 2007; Menini et al., 2007; Martinet et al., 2001). Though the presence of methyl xanthines account for many of the pharmacological activities of yerba mate, many other very interesting and important properties have been found to be independent of the presence of these compounds.

Ilex paraguariensis extracts polyphenol levels are higher than those of green tea and parallel to those of red wines (Gugliucci et al., 2009a,b). As shown in Fig. 1, *Ilex paraguariensis* extracts are especially rich in chlorogenic acids (Bastos et al., 2007; Heck and de Mejia, 2007; Menini et al., 2007; De Morais et al., 2009; Filip et al., 2010a). Chlorogenic acids are a family of esters formed between certain *trans* cinnamic acids and (−)-quinic acid and are also major phenolics compounds in coffee, strawberries, pineapples, apples, sunflower and blueberries. 5 caffeoylquinic acid (5-CQA) is the only chlorogenic acid commercially available and has been extensively studied due to its antioxidant activity. Chlorogenic acids are free radical and metal scavengers; may interfere with glucose absorption and has been shown to modulate gene expression of antioxidant enzymes, among other biological activities (Clifford, 1999, 2004; Olthof et al., 2001; Jaiswal et al., 2010).

Mate also contains saponins that are known to bind bile salts (Bastos et al., 2007; Gnoatto, 2008).

Roasted extracts of *Ilex paraguariensis* (as consumed in Sao Paulo and Rio de Janeiro in Brazil) keep essentially the same components with the addition of melanoidins, which have some bioactive properties of their own (Bastos et al., 2007).

3. Effects of *Ilex paraguariensis* extracts on lipid metabolism, obesity and oxidation (Fig. 2)

Earlier studies from some of us and other authors had uncovered a strong protection of *ex vivo* human low density lipoprotein (LDL) from oxidation as well as protection of paraoxonase activity on high density lipoprotein (HDL) (Gugliucci, 1996; Gugliucci and Menini, 2002). Paraoxonase 1 (PON1) is an antioxidant enzyme carried by HDL which has a protective effect vis-à-vis atheroma plaque formation as evidenced by epidemiological and mechanistic studies in animal and humans (Schinella et al., 2005; Menini et al., 2007; Gugliucci et al., 2009a,b).

More recently, reduction in cholesterol content and size of aortic lesions was shown in rabbits fed a cholesterol-enriched diet when they also ingested *Ilex paraguariensis* extracts (Mosimann et al., 2006; Lee, 2007). These results gave the first *in vivo* support to the initial findings of a strong protection of LDL from oxidation, afforded by *Ilex paraguariensis* extracts both *in vitro* and *ex vivo* in humans (Gugliucci and Stahl, 1995).

The first translational study in humans, conducted by the same group, has recently shown that consumption of *Ilex paraguariensis* improves serum lipid parameters in healthy dyslipidemic subjects. LDL-cholesterol was significantly reduced, in parallel with an increase in HDL-cholesterol in a cohort of dyslipidemic subjects with no treatment that drank mate for 2 months as compared to controls that did not (De Morais et al., 2009). The study standardized the ingestion doses by using mate brewed in the classic manner of tea, to avoid the confounder that may result from the ingestion in the traditional manner. The authors show that *Ilex paraguariensis* provides an additional LDL-cholesterol reduction in individuals on statin therapy, suggesting that the beverage may be used to reduce the doses of statins and therefore the side-effects (De Morais et al., 2009).

HDL carries a antioxidant enzyme, paraoxonase 1 (PON1) which protects LDL from oxidation and reduces macrophage oxidation and is thus thought to be atheroprotective. PON1 activity can be significantly reduced by the HDL oxidation by hypochloric acid (HOCl). Hypochloric acid is released by neutrophyls and macrophages which abound in inflammatory lesions. In a recent study we have shown that chlorogenic acid, the main phenolic in maté, protects PON1 in human HDL from the inactivation caused by physiological concentrations of hypochlorite (Gugliucci et al., 2009a,b).

Obesity is another key metabolic and atherogenic risk factor. One of the purported effects of *Ilex paraguariensis* extracts claimed by European herbal therapists is weight reduction (Andersen and Fogh, 2001; Pittler et al., 2005; Dickel, 2007). In this regard in the past few years several studies have addressed this claim using *in vitro* and animal studies. Pancreatic lipase inhibition is an interesting target to decrease lipid absorption and therefore caloric intake (Sugimoto et al., 2009; Martins et al., 2010). A recent article has shown that indeed *Ilex paraguariensis* extracts are capable of inhibiting pancreatic lipase activity *in vitro*. Moreover, some studies have shown that mice fed with *Ilex paraguariensis* extracts and diets rich in lipids gain less weight than controls. Data show that yerba maté extract has potent anti-obesity activity *in vivo* in obese mice (Arcari et al., 2009). Additionally, the treatment had a modulating effect on the expression of several genes related to obesity (Martinet et al., 1999; Oliveira et al., 2008; Matsumoto et al., 2009).

Independently similar results were obtained when a rat model was employed and the authors dissect some of the mechanisms involved. *Ilex paraguariensis* extract can have a protective effect against high fat diet-induced obesity in rats through an enhanced expression of uncoupling proteins and elevated Adenosine monophosphate (AMP) dependent kinase (AMPK) phosphorylation (and thereby activity of the enzyme) in the visceral adipose tissue (Pang et al., 2008). AMPK is a crossroad regulator and sensor of metabolism which is implicated in the metabolic syndrome, obesity and diabetes. Up regulation of AMPK is the subject of intense research in the pharmaceutical industry, and several active compounds are on the development pipeline. Stimulation of AMPK by natural products constitutes a therapeutic alternative and indeed resveratrol, a phenolic substance found in wine, possesses this activity (Actis-Goretta et al., 2002). The fact that *Ilex paraguariensis* extracts possess a strong AMPK activation activity is very promising, given the central role that this allosteric enzyme plays in energy metabolism.

Recently, several laboratories have expanded the original findings regarding the antioxidant properties of *Ilex paraguariensis* to animal models and human intervention studies (Paganini Stein et al., 2005).

Consumption of *Ilex paraguariensis* extracts decreases the oxidation of unsaturated fatty acids in the liver of mice; and chlorogenic acid is involved in the process (Martins et al., 2009).

In a small intervention study in humans, the authors evaluated the effects of *Ilex paraguariensis* dietary supplementation on plasma susceptibility to oxidation and on antioxidant enzyme gene expression. After a supplementation period with maté tea, lipid peroxidation was acutely lowered, an effect that was maintained after prolonged administration (Matsumoto et al., 2009). Total plasma antioxidant levels increased as did antioxidant enzyme gene expression. These results suggest that regular consumption of maté tea may improve antioxidant defenses by multiple mechanisms, not only by increasing circulating active compounds but by upregulation of the cellular enzymatic machinery to counter oxidative stress.

4. Effects of *Ilex paraguariensis* extracts on inflammation (Fig. 3)

The anti-inflammatory and immuno-modulatory effects of herbs containing polyphenols have been the object of many studies (Zuin et al., 2005; Schubert et al., 2007); they describe direct

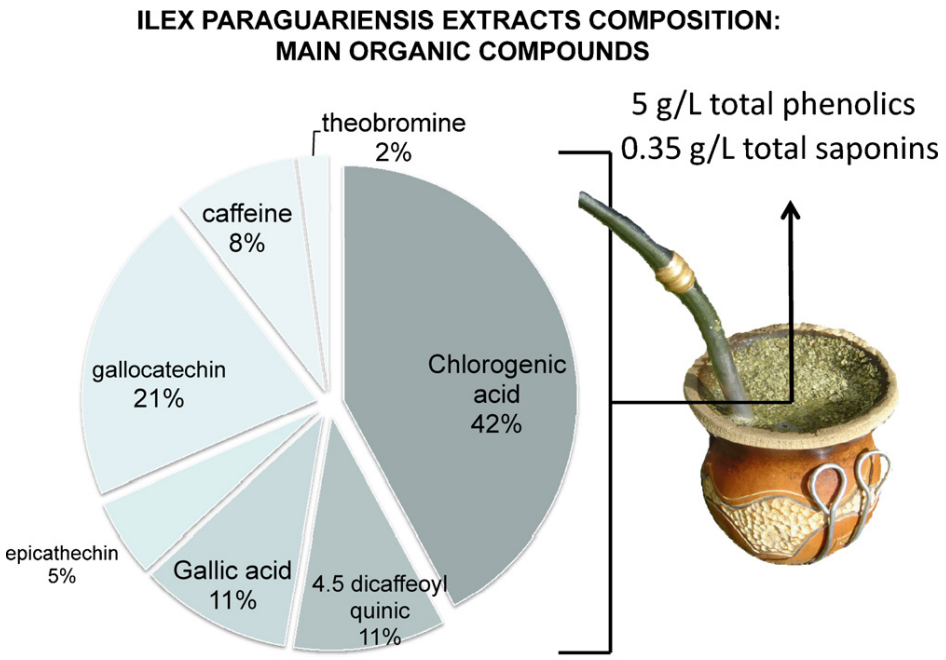

Fig. 1. Main organic components of *Ilex paraguariensis* extracts.

antioxidant effects or effects mediated by cell-signalling pathways like NF-κB and others.

The study of human macrophages activated by lipopolysaccharide (LPS) in the presence of crude extracts of *Ilex paraguariensis* or the individual components, demonstrated that quercetin was the most potent inhibitor of pro-inflammatory responses as measured by blocking of COX-2 (Puangpraphant and de Mejia, 2009). The combination of quercetin/maté saponins resulted in synergistic interaction inhibiting both nitric oxide (NO) and prostaglandin 2 (PGE 2) production. It also suppressed interleukin-6 (IL-6) and interleukin-1 β (IL-1 beta) production and resulted in reduction of LPS-induced nuclear translocation of nuclear factor-kappa B subunits. Conversely, the crude extract did not have a potent anti-inflammatory effect possibly as a result of the antagonistic effect of some of its compounds.

Recently, chemical constituents from the leaves of *Ilex paraguariensis* have been shown to inhibit human neutrophil elastase *in vitro* (Xu et al., 2009). Dicaffeoylquinic acids (chlorogenic acids) and flavonoids showed the strongest effect (Zanoelo and Beninca, 2009). Along the same lines, a recent study in an animal model has shown that maté tea reduced acute lung inflammation in mice exposed to cigarette smoke. Short-term cigarette smoke exposure

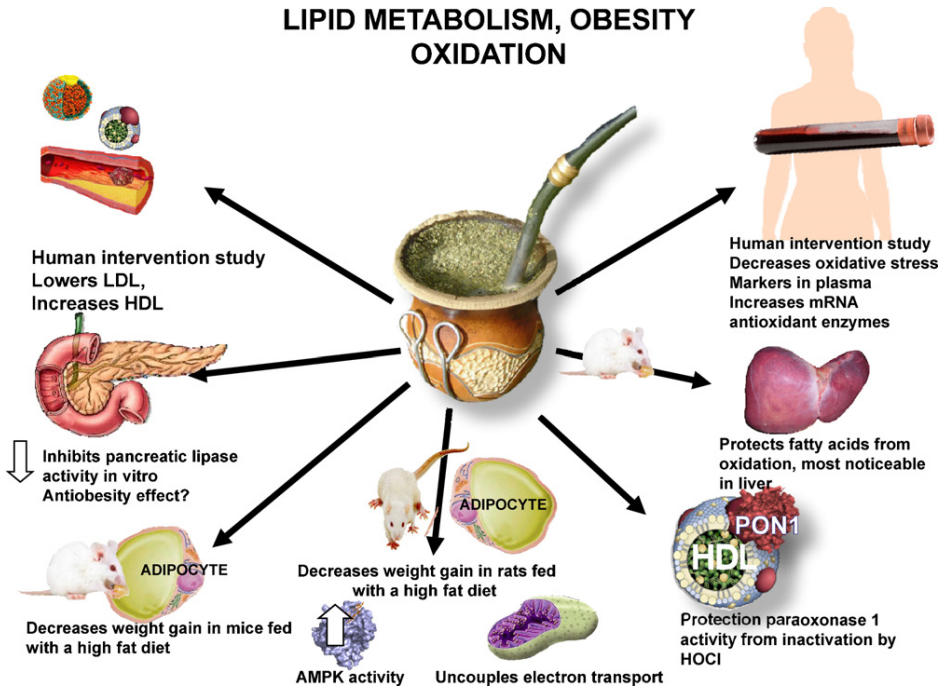

Fig. 2. Evidence supporting beneficial effects of *Ilex paraguariensis* in lipid disorders, obesity and oxidative stress. The diagram summarizes the more relevant findings reviewed in the text.

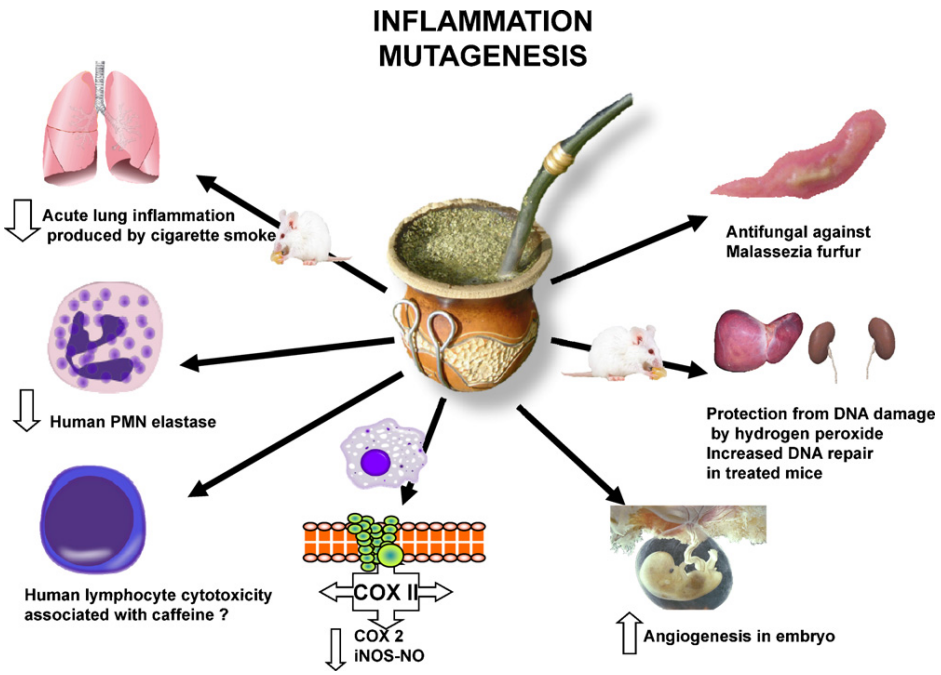

Fig. 3. Evidence supporting beneficial effects of *Ilex paraguariensis* in inflammation and mutagenesis. The diagram summarizes the more relevant findings reviewed in the text.

has been associated with acute lung inflammation and oxidative damage. In this study, mice were pretreated with *Ilex paraguariensis* extracts (intraperitoneal and oral) and then exposed to cigarette smoke. Bronchoalveolar lavages and lung tissue were studied. Number and activity of macrophages were significantly reduced in *Ilex paraguariensis* extracts-treated mice. Matrix metalloprotease-9 was one of the effectors positively modulated by the extracts. These data imply a potential antioxidant role for *Ilex paraguariensis* as an anti-inflammatory nutritional resource in lung damaged by cigarette smoke exposure (Lanzetti et al., 2008).

Ilex paraguariensis extracts display antifungal activity inhibiting the growth of *Malassezia furfur* (Filip et al., 2010b). This saprophyte fungus produces skin lesions in humans. The antifungal activity of *Ilex paraguariensis* was similar to the therapeutic ranges of the used pharmacological agent to treat the skin disorders. Topical use of *Ilex paraguariensis* extract could be then an alternative antifungal agent used for this infection. Triterpenoids from Ilex paraguariensis display inhibitory activities against Trypanosoma Cruzi (Taketa et al., 2004)

Angiogenesis plays a key role in inflammation and repair as well as in cancer biology. Treatments performed with aqueous extract of maté and caffeine in the vascular membranes of chick embryos yolk sac revealed pro-vasculo- and angiogenic properties as well as embryonic growth enhancement (Strassmann et al., 2008).

Along with the effects described above, a recent article suggests some deleterious effects of *Ilex paraguariensis* extracts on lymphocytes. In an *in vitro* study using human lymphocytes the authors show a cytotoxic activity of the extracts against these cells which was due mostly to caffeine and therefore it is not unique to maté beverages (Alves et al., 2008; Wnuk et al., 2009).

5. Effects of *Ilex paraguariensis* extracts on mutagenesis (Fig. 3)

A few epidemiological studies indicate that there is an association between heavy maté consumption and oropharyngeal cancer (Vassallo et al., 1985; De Stefani et al., 1988; Pintos et al., 1994; Sewram et al., 2003; Loria et al., 2009). The effect may be related to the temperature of the infusion and not to the beverage itself (Ramirez-Mares et al., 2004), and confounder factors such as heavy smoking, malnutrition and concomitant alcohol consumption complicate the finding of a clear answer to these issues. At the same time, high levels of carcinogenic polycyclic aromatic hydrocarbons have been found in maté drinks and this may be a matter of concern (Leitao and Braga, 1994; Kamangar et al., 2008). They are produced during the manufacturing process of the leaves (drying) using firewood (Hsu et al., 1995; Jacques et al., 2007; Heck et al., 2008); therefore changing production practices may remove this potential hazard (Tenorio Sanz and Torija Isasa, 1991; Vera Garcia et al., 1997; Wrobel and Urbina, 2000). Almost all epidemiological studies shared similar methodology: hospital-based, case–control studies where participants were interviewed on the main risk factors, using similar questionnaires. Size, exposure assessment, methods of analysis, and quality were different among the studies reviewed and the question remains open since there is no sound population-based case–control study on maté consumption as a risk factor for cancer (De Stefani et al., 1991). The topic has been the subject of recent reviews and the reader is directed to them (Warnakulasuriya, 2009; Loria et al., 2009).

Conversely, many studies in cell culture models as well as in animals seem to converge to show an antimutagenic and DNA protecting effect for *Ilex paraguariensis* extracts and its individual components, chlorogenic acid, rutin, quercetin (Bracesco et al., 2003; Miranda et al., 2008).

The regular ingestion of maté tea increased the resistance of DNA to hydrogen peroxide-induced DNA strand breaks and improved the DNA repair after hydrogen peroxide challenge in liver cells, irrespective of the dose ingested. These results suggest that maté tea could protect against DNA damage and enhance the DNA repair activity. Protection may be attributed to the antioxidant activity of the maté bioactive compounds chlorogenic acid, rutin, quercetin (Bracesco et al., 2003; Miranda et al., 2008).

6. Conclusion

Biomedical research has grown exponentially in the past five years. Along with many reports showing beneficial effects of these extracts *in vitro* and in animal models, several new intervention studies in humans have emerged. *Ilex paraguariensis* reduces LDL-cholesterol levels in humans with *Ilex paraguariensis* dyslipoproteinemia and the effect is synergic with that of statins. Plasma antioxidant capacity as well as expression of antioxidant enzymes is positively modulated by intervention with *Ilex paraguariensis* in human cohorts. A review on the evidence implicating *Ilex paraguariensis* heavy consumption with some neoplasias shows data that are inconclusive but indicate that contamination with alkylating agents during the drying process of the leaves should be avoided. On the other hand, several new studies confirm the antimutagenic effects of *Ilex paraguariensis* in different models, from DNA double breaks in cell culture models to protection of DNA in mouse hepatocytes. Novel, interesting work has emerged showing a significant effect on weight reduction both in mice and in rat models. Some of the mechanisms involved are inhibition of pancreatic lipase, activation of AMPK and uncoupling of electron transport. Intervention studies in animals have provided strong evidence of anti-inflammatory effects of *Ilex paraguariensis*, notably protecting cigarette-induced lung inflammation acting on macrophage migration and inactivating matrix-metalloproteinase.

Research on the effects of *Ilex paraguariensis* in health and disease has confirmed its antioxidant, anti-inflammatory, antimutagenic and lipid-lowering activities. Although we are still waiting for the double-blind, randomized prospective clinical trial, the evidence seems to provide support for beneficial effects of mate drinking on chronic diseases with inflammatory components and on lipid metabolism disorders.

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