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File: ■ Guayusa (*Ilex guayusa*, Aquifoliaceae)
■ Toxicology
■ Safety

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RE: Guayusa (*Ilex guayusa*) Extract Is Nontoxic in Rodent Studies and In Vitro Assays

Kapp RW Jr, Mendes O, Roy S, McQuate RS, Kraska R. General and genetic toxicology of guayusa concentrate (*Ilex guayusa*). *Int J Toxicol*. 2016;35(2):222-242.

Guayusa (*Ilex guayusa*, Aquifoliaceae) is a close relative of yerba maté (*Ilex paraguariensis*) and is traditionally consumed as a tea or as chewed leaves in the Amazonian region. Guayusa has been found to contain active compounds known as methylxanthines, including caffeine, known to have cellular and systemic stimulatory activity. Data on the safety and adverse effects of guayusa are limited. This study investigated its safety profile using in vitro and in vivo studies with a concentrated water extract of guayusa (procured from Runa LLC; Quito, Ecuador).

Guayusa concentrate (GC) was prepared by brewing leaves in hot water as for tea, at a per weight ratio of 1.3 to 1.6:1 for two to four hours. In vitro, the Ames test was used to gauge mutagenic activity. Briefly, this assay measures genetic mutations in *Salmonella typhimurium* and *Escherichia coli*. Water was used as a negative control and several known mutagenic compounds, such as sodium azide, were employed as positive controls. GC concentrations of 1.58, 5.0, 15.8, 50, 158, 500, 1580, and 5000 µg/plate were used, and mutation factor (MF) was calculated as the ratio of mutations observed in the test group to those in the control group (MF of 2 or 3, depending upon the strain, is considered mutagenic). A second in vitro test used to gauge GC toxicity was the chromosomal aberration assay. Human peripheral blood lymphocytes were incubated with GC for four and 20 hours, and for four hours with GC and a metabolic activation system mix; two compounds known to induce chromosomal aberration were used as positive controls, and pure caffeine was tested for comparison.

Three in vivo tests, both acute and long term, in rats also were conducted. To determine acute toxicity, female rats between eight and nine weeks old, said to be more sensitive than males, were studied. GC was administered orally by gavage at 5000 mg/kg to a single rat. When death did not occur, the same dosage was given to two other rats. As no mortality occurred, this study was stopped. Animals were observed daily for 14 days following the GC ingestion and weighed on days seven and 14; they were then sacrificed

and necropsies were conducted. To determine dosages for a 90-day study, a 14-day study was conducted in 70 rats (both male and female) at six to seven weeks old, divided into seven treatment arms, each with five males and five females. Treatments included a distilled water control, three GC dosages (1200, 2500, and 5000 mg/kg/day), and three caffeine dosages comparable to the caffeine content in the GC dosages (36, 75, and 150 mg/kg/day). Caffeine dosages were determined based on expected caffeine content of 3% in GC; all treatments were given by gavage at 10 ml/kg. Weights and food consumption were recorded at baseline and on days three, seven, 11, and 14 of the study. Animals were euthanized on day 15. During this experiment, dosing samples were assessed on days one, seven, and 14 for caffeine and chlorogenic acid content to confirm stability of the preparation.

For the 90-day in vivo experiment, GC dosages of 0, 1200, 2500, and 5000 mg/kg/day and a single caffeine dose of 150 mg/kg/day (the amount in the 5000 mg/kg/day GC dosage) were chosen. This experiment used 100 rats, both male and female, at eight weeks of age. At baseline and day 81, rats' eyes were screened. General health was assessed daily and weekly, with weights and food consumption measured throughout the experiment. At day 86 or 87, urine and blood samples were taken. At the end of the study, animals were euthanized, and necropsy and histological analysis were conducted. Dosing samples and the original GC lot were tested for stability on days one, 43, and 94.

Chemical characterization of the main lot of GC detected caffeine at 36 mg/ml, chlorogenic acids at 52 mg/ml, and small amounts of several catechins and theobromine. GC was also analyzed for the presence of a number of major plant compounds (e.g., delphinidins, beta-sitosterol, genistein) that were not found. The extract was found to be adequately stable, and actual caffeine dosages given in all studies were within 15% of the expected amounts. In the Ames test, no problems such as toxicity, precipitation, or contamination were seen. The MF did not increase significantly in any strain at any dose level, so it was determined that GC is not mutagenic. Also, neither GC nor dose-equivalent concentrations of caffeine caused chromosomal aberrations in the chromosomal aberration assay.

In the acute toxicity study, rats were hypoactive and displayed salivation, respiratory, and fecal abnormalities, and hunched posture. At day three, these adverse effects were gone, and the animals remained healthy through day 14. It was concluded that the oral median lethal dose (LD_{50}) of GC is > 5000 mg/kg body weight for female rats. In the 14-day experiment, no death was observed. Hypoactivity and salivation were seen in animals given 5000 mg/kg/day of GC and 75 or 150 mg/kg/day of caffeine. In general, weight loss and decreased food intake occurred in animals consuming either GC or caffeine. This effect disappeared during the course of the study. Necropsy did not show any abnormalities associated with GC or caffeine.

During the 90-day in vivo study, no deaths linked to GC treatment were reported. Three animals died during the study of what were believed to be unrelated causes (though exact cause of death could not be determined) and one was sacrificed due to ill health caused by a dental problem. Hypoactivity and salivation were noted in some animals taking either GC or caffeine. No animals showed any ocular changes. Across the study, weight and food efficiency declined in treated groups, but nonsignificantly. According to the histology, weights of fat pads and several organs in both males and females of the GC and caffeine groups were significantly decreased as compared to control animals, and hypertrophy of salivary glands was observed. Urine composition was unchanged in

male rats, with unimportant reduction in protein content in female rats in the high-dose GC and caffeine groups. Small, dose-dependent changes in blood measurements (hemoglobin, red blood cell distribution width, and others) in females, decreased blood triglycerides, and increased cholesterol and liver function enzymes were noted in rats on both GC and caffeine. The authors review previous literature reporting most of these biological effects to result from caffeine consumption.

Overall, this study reports that GC does not show toxicity in the assays and animals employed here. Adverse effects noted at high doses were most likely due to the caffeine content of GC, as caffeine was tested alongside the botanical and caused similar adverse effects. Clarifying the ideal dosage and adverse effects in a clinical setting is worthy of further study.

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—Amy C. Keller, PhD

The American Botanical Council has chosen not to include the original article.