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Ritualistic use of the holly *Ilex guayusa* by Amazonian Jívaro Indians

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In Amazonian Peru and Ecuador leaf decoctions of the rainforest holly *Ilex guayusa* with high caffeine concentrations are used as a morning stimulant. After daily ingestion, ritualistic vomiting by male Achuar Indians, better known as Jivaros, reduces excessive caffeine intake, so that blood levels of caffeine and biotransformed dimethylxanthines do not cause undesirable CNS and other effects. Emesis is learned and apparently not due to emetic compounds.

Key words: emesis; Jívaro; upper Amazon; holly; stimulant.

Introduction

Known almost exclusively from cultivation, *Ilex guayusa* Loes. (Aquifoliaceae) grows as a shrub or small tree from southern Colombia to northern Peru (Fig. 1). It is planted by Jívaro (Achuar sub-through a 0.45-μm nitrocellulose filter. Extract aliquots (to 20 μl) were loaded onto a C18 reverse-phase column, 100 mm × 4.6 mm with a 3-μm the Andes Mountains. In 1857 Spruce (Wallace, 1908) found a grove of trees near Baños, Ecuador, which was supposed to date from before the Conquest. Leaves of this holly have been uncovered in the tomb of a medicine man in highland Bolivia dating from the 5th century (Schultes, 1972), and Schultes and Raffauf (1990) report their use in Ecuador for numerous medicinal purposes. Today, Achuar men in Peru and Ecuador drink large amounts of holly leaf decoctions (*wayus*) before daybreak for an hour or less, and then vomit. They conduct this daily ritual for stimulating effects.

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Recent data have shown caffeine in leaf samples (1.8%, Holmstedt and Lindgren, 1972; 1.7%, Lewis et al., 1987) at sufficient levels to account for the stimulating properties of *I. guayusa*. However, this does not explain why the Achuar conduct an emetic ceremony when stimulation is at least a major desired physiological effect. This paper attempts to explain this apparent anomaly.

Materials and Methods

Leaves of *I. guayusa* were dried in the field and brought to St. Louis for extraction. Voucher herbarium specimens were prepared, identified, and deposited at the Missouri Botanical Garden Herbarium (MO) (Table 1). The Achuar probably elicit maximum extraction of methylxanthines (MXs) and particularly caffeine by bringing whole dried leaves to boil for about 1 h, and by sometimes simmering the decoction for some time longer. Using dried leaves from one shrub (No. 13967), we tested the native procedure: boiling 1 h = caffeine 3.33% ± 0.01%, theobromine 0.02% ± 0.003%, other DMX trace (Table 1); boiling only 10 min = caffeine 2.59% ± 0.01%, theobromine 0.01% ±

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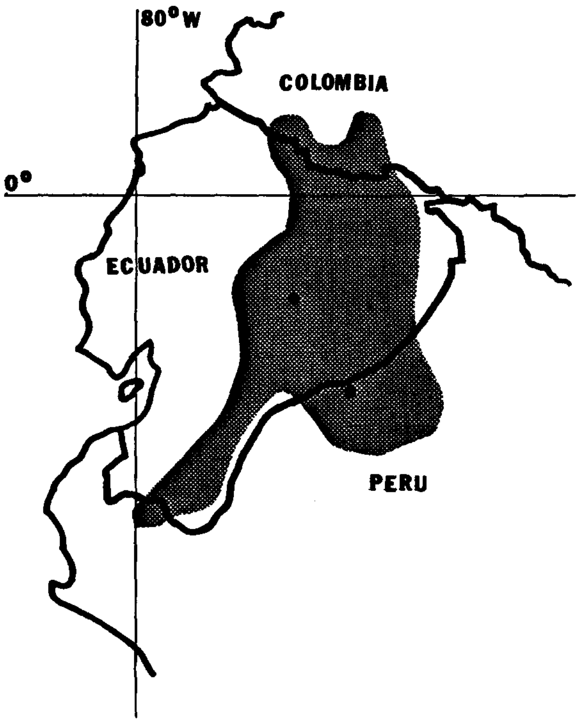

Fig. 1. Distribution of *I. guayusa* in Colombia, Ecuador and Peru based on historic records (Patino, 1968; Schultes, 1979) and herbarium specimens at F, GH, K, MO, US, almost exclusively from cultivated plants. Circles show localities of the only two known collections of these rare rainforest canopy trees in Ecuador at 500 m (Shemluck 327) and Peru at 220 m (Lewis et al. 13132). The species may also occur in lowland Bolivia, but this specimen needs verification (Schultes, 1972).

0.002%, other DMX 0; cold infusion soaking 24 h = caffeine 2.78% ± 0%, theobromine 0.03% ± 0.01%, other DMX 0. Therefore, caffeine was only 77.8% (boiled 10 min) and 83.5% (cold infusion 24 h) extracted when compared to the 1-h boiling used by the Achuar. We followed their procedure.

For thin layer chromatography (TLC) analysis to test possible emetic effects, 200 mg of ground *I. guayusa* leaves were extracted for 24 h in 5 ml of chloroform made basic with a drop of KOH, filtered, and the filtrate spotted on a silica gel TLC plate (Merck), and developed in chloroform/methanol (85:15); emetine (Sigma) in methanol was also spotted on the same plate. The plate was exposed to iodine vapor, heated, and spots observed under UV (emetine and related ipecacuanha alkaloids fluoresce at 365 nm).

To determine MX concentration, high performance liquid chromatography (HPLC) analyses were conducted. Dried leaf collections were boiled for 1 h in water, cooled and extracts filtered through a 0.45-μm nitrocellulose filter. Extract aliquots (20 μl) were loaded onto a C18 reverse-phase column, 100 mm × 4.6 mm with a 3-μm particle size (Rainin, Microsorb), and eluted with citrate phosphate buffer, pH 7, 6.5 ml 0.1 M citric acid and 43.6 ml 0.2 M dibasic sodium phosphate to 100 ml of water, and methanol (90:10), at a flow rate of 1 ml/min. MXs were detected at 254 nm for up to 15 min, theobromine at about 2.5 min, caffeine at about 7.5 min, and no more than trace amounts of dimethylxanthines, either paraxanthine or theophylline which we could not separate, at about 4 min (Grgurinovich, 1986). Standards included caffeine, paraxanthine, theophylline and theobromine (Sigma).

Blood samples were taken from one participant of a *wayus* ceremony at Puranchim, Peru, in December 1988, before drinking and 55 min later just following emesis. Two groups of men drank a total of 20.3 l of decoction using 164.2 g dried leaves. Assuming that their drinking was more or less linear and given that absorption of MXs was essentially complete in 45 min (Bonati et al., 1982, 47 ± 5 min; Blanchard and Sawers, 1983, 29.8 ± 8.1 min), only 51% of MXs in the ingested beverage were retained when emesis occurred. Calculations of μg/ml of MXs in our participant's blood were based on a male weighing 70 kg with total body water (TBW) as 61% of body weight or 42.7 l TBW, age group 18—40 (Ganong, 1985), who drank a 2.2-l decoction prepared from 17.8 g of dried leaves, this being his portion of the total volume drunk and the dried leaves used to prepare the decoction.

Methylxanthines were extracted from whole human blood by adding 0.5 ml water to 0.5 ml whole blood, vortexing, and adding 0.5 ml methylene chloride. This was vortexed repeatedly for 1 min, stored at 4°C overnight, centrifuged at 12,000 rev./min for 5 min, and the non-aqueous phase removed and evaporated to dryness. Samples were resuspended in water to 200 μl.

TABLE 1

METHYLXANTHINE CONCENTRATIONS OF DRIED LEAF DECOCTIONS OF *ILEX GUAYUSA* FROM ECUADOR AND PERU

Lewis et al. Coll. No.[a]	% Caffeine	% Theobromine	% Other DMXs (paraxanthine, theophylline)
11687	1.73 ± 0.04[b]	0.02 ± 0.004	trace[c]
14084	1.92 ± 0.03	trace[c]	trace
12264	2.07 ± 0.03	0.02 ± 0.003	trace
11492	3.11 ± 0.05	0.12 ± 0	trace
13967	3.33 ± 0.01	0.02 ± 0.003	trace
12263	3.48 ± 0.06	0.04 ± 0.003	0
12265	3.95 ± 0.06	0.02 ± 0.003	trace
13132	7.57 ± 0.12	trace	trace

[a]Collection numbers refer to the following respective localities: Huagromona, Peru (No. 11687); Puyo, Ecuador (No. 14084); Puranchim, Peru (No. 12264); Washintsa, Peru (No. 11492); Kapawi, Ecuador (No. 13967); Puranchim, Peru (No. 12263); Puranchim, Peru (No. 12265); north of Puranchim, Peru (No. 13132). All vouchers are at MO (Missouri Botanical Garden Herbarium). Collection No. 13132 represents the only wild tree leaves sampled; all others are from cultivated plants, one plant of each collection was sampled.
[b]All data represent mean values ± S.E.M., dry weight/weight.
[c]Trace = < 0.005%.

Using HPLC, aliquots to 20 μl were loaded onto a C18 reverse-phase column with 17% acetonitrile in 0.1 M monopotassium phosphate, pH 3.8, as the mobile phase at a flow rate of 1 ml/min, and MXs were detected at 254 nm for up to 10 min (Evenson and Warren, 1976). The original blood sample was re-extracted and run as before, and only traces of caffeine and no DMXs were found. Standards as above.

Results

The Achuar state that following puberty, boys join the men in the *wayus* ceremony already knowing how to vomit, accomplished in prepuberty by use of a feather or finger to learn throat muscle control, and thereby effecting emesis with relative ease. Mothers may even teach young boys how to initiate vomiting with facility. One of us (W.H.L.) drank about 600 ml of the decoction on several occasions without emetic effects. Leaf samples from three collections (Nos. 11492, 12264, 13967) were tested for emetine by using TLC. Neither emetine nor allied ipecacuanha compounds were found in *I. guayusa* leaf extracts.

Using HPLC we found in six samples a range of 1.73—3.48% caffeine (trimethylxanthine, TMX) with small amounts of theobromine and traces of other dimethylxanthines (DMXs) (first six, Table 1). These plants were frequently used by the Achuar. However, the Indians held no enthusiasm for one discarded cultivar of *wayus*, sample No. 12265 (Table 1). They described how decoctions from its leaves having nearly 4% MXs could give them severe headaches, blood-shot eyes and unsettling perceptions (e.g., sticks on the forest floor seemed to move when approached, resembling snakes). Left unharvested, this plant had already grown to 12 m, a height more than twice that of commonly used plants. One, and apparently the only, wild tree of *I. guayusa* found by us in northern Peru and Ecuador, No. 13132, (Table 1) contained even higher amounts of caffeine (7.6%) and traces of DMXs.

Using the mean frequency of 2.64 ± 0.73% MXs of the six commonly used cultivated collections (first six, Table 1), we calculated that in 45 min our subject ingested 469.9 mg of MXs, almost entirely caffeine, an amount found in 5.5 cups of coffee based on Graham's (1978) standard average caffeine content per cup of roasted and ground coffee of 85 mg. Because he eliminated 49% of MXs at emesis, we estimated that only 239.7 mg were absorbed, equivalent to 2.8 cups of coffee. Values of caffeine above 250 mg can produce nervousness, tremors and other signs of excessive CNS stimulation (Rall, 1985). Therefore, we agree with the Achuar that when they do not vomit they are likely to be highly agitated and irritable, and to have a generally miserable day following the *wayus* ritual. If, however, they do vomit and absorb only 250 mg or less caffeine, mild stimulation, less drowsiness, less fatigue and more rapid and clearer flow of thought are the desired effects (Rall, 1985), as our subject experienced.

Analysis of our participant's blood after emesis confirmed our estimate of caffeine ingested. A total of 5.24 μg/ml MXs, namely, 3.18 μg/ml caffeine and 2.06 μg/ml of DMXs, was observed. Based upon the distribution of MXs (Rall, 1985), we estimated that he had absorbed 88.0 mg of DMXs and 135.8 mg of caffeine, for a total of 223.8 mg of MXs, 55 min after drinking began, or the equivalent of 2.6 cups of coffee. This value would have been 438.8 mg if the whole decoction had been retained (5.2 cups of coffee), or a blood level of 10.27 μg/ml. Transformation of caffeine (TMX) to DMXs was 39.3% in 55 min, probably metabolized to paraxanthine and/or theophylline (Tang-Liu et al., 1983). No trace of MXs was found in his blood just prior to drinking, even though he habitually drank ± 2.2 l of caffeine-containing decoction daily. Levels of caffeine and DMXs from this blood sample were only 7% less than from the average TMX and DMX of plants used by the Achuar in several communities and by us as an estimate of MX ingestion by our subject. This close relation between the calculated absorption of MXs and our estimate of ingested MXs indicates the validity of our linear absorption model.

Of the remaining two collections, the rarely used cultivar No. 12265 with 3.97% MXs (Table 1) would include 708.4 mg in 2.2 l of leaf decoction ingested, and even the post-emetic level of 361.3 mg (4.3 cups of coffee) could produce symptoms known by the Achuar as unpleasant, reason enough to avoid using the plant. The final collection of Table 1 is the wild tree (No. 13132) whose leaves have a very high TMX content. If the usual 2.2 l of decoction were drunk, it would include 1237.5 mg caffeine (15.9 cups of coffee) or 687.2 mg caffeine (8.1 cups) after emesis. When leaves were rarely used as a source of beverage, we were told only small amounts were drunk.

Discussion

Populations of plant species growing in different localities throughout their geographic ranges may be subject to different selections and are usually genetically as well as physiologically, morphologically and chemically different (Lewis, 1984). Such inherent diversity may be selected by humans if species prove useful. This has been the case of *I. guayusa* which has long been cultivated in the Upper Amazon Basin by Indians who select cultivars of diverse chemical constituents which give varying physiological effects. Thus, as a stimulating beverage they will use plants having a range of caffeine concentration of about 1.5—3.5%, because they know desired effects will be experienced, if as we have described, emesis occurs about 45 min to 1 h after ingestion begins. They avoid specific cultivars when they learn that unsettling symptoms follow drinking decoctions made from their leaves; we have shown that these are higher in caffeine, from about 4% to as high as 7.6%. The plant having 7.6% MXs in the leaves was far too powerful to be used in the *wayus* ceremony, and cultivars from the tree growing near an Achuar village were never propagated.

To our knowledge this wild tree of *I. guayusa* (No. 13132, Table 1) has the highest concentration of caffeine or methylxanthines from any plant. The allied South American *I. paraguariensis* St.-Hil. was reported by Baltassat et al. (1984) to yield 0.78—1.25% caffeine, 0.34—0.43% theobromine and no theophylline. Using commercial maté leaf tea we confirmed the species' relatively low contents of caffeine at 1.16% ± 0.12%, theobromine at 0.18 ± 0.03% and the absence of theophylline (Indiana Botanic Gardens, Inc., Hammond, IN, sample No. 000108). In North America, leaves of *I. vomitoria* Aiton from Stone Co., Mississippi (Croom No. 1000) had very low levels of MXs (caffeine 0.09%, theobromine 0.04%, no theophylline). These values were lower than those reported by Power and Chesnut (1919) who found caffeine ranging from 0.16% to 1.65% (X = 0.88% ± 0.03%), using much less accurate techniques in 1919 than the HPLC available to us today. Only one additional *Ilex* has been reported to contain caffeine, namely, *I. ambigua* (Michx.) Chapm., but no values were given (Bohinc et al., 1977). Theobromine is more widespread in the genus, and is known at low levels from *I. aquifolium* L., *I. argentina* Lillo, *I. caroliniana* (Lam.) Loes., *I. cassine* L., *I. crenata* Thunb., *I. perado* Aiton and *I. ambigua* (Alikaridis, 1987).

Bark of the South American *Paullinia cupana* Kunth (Uphof, 1968) and *P. yoco* Schultes and Killip (Stout and Schultes, 1973) are reputed to contain up to 6% caffeine, but most xanthine-containing plants range from 1—3% caffeine, occasionally to 4.5% in Asian tea, *Camellia sinensis* (L.) Kuntze (Lewis and Elvin-Lewis, 1977).

Initial ingestion of high levels of caffeine by Achuar Indians is invariably followed by emesis, apparently to eliminate excessive intake of caffeine before absorption and bioconversion are complete, thereby avoiding unwanted symptoms of trimethyl- and dimethylxanthines. By invoking emesis, the men have learned to attain and not to exceed desired stimulating effects of caffeine use. Similar physiological effects could also be obtained by drinking less, as women do, but large volumes are drunk because men enjoy the drink per se and participation in a cultural ritual reinforces male bonding.

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References

Alikaridis, F. (1987) Natural constituents of *Ilex* species. *Journal of Ethnopharmacology* 20, 121—144.

Baltassat, F., Darbour, N. and Ferry, S. (1984) Étude du contenu purique de drogues a caféine: I. — Le maté: *Ilex paraguariensis* Lamb. *Plantes Médicinales et Phytothérapie* 18, 195—203.

Blanchard, J. and Sawers, S.J.A. (1983) The absolute bioavailability of caffeine in man. *European Journal of Clinical Pharmacology* 24, 93—98.

Bohinc, P., Korbar-Smid, J. and Marinsek, A. (1977) Xanthine alkaloids in *Ilex ambigua* leaves. *Farmacevtski Vestnik* 28, 89—96.

Bonati, M., Latini, R., Galletti, F., Young, J.F., Tognoni, G. and Garattini, S. (1982) Caffeine disposition after oral doses. *Clinical Pharmacology and Therapeutics* 32, 98—106.

Evenson, M. A. and Warren, B. L. (1976) Serum theophylline analysis by high-pressure liquid chromatography. *Clinical Chemistry* 22, 851—855.

Ganong, W. F. (1985) *Review of Medical Physiology*, Edn. 12. Large Medical Publications, Los Altos, CA. p. 15.

Graham, D. M. (1978) Caffeine — its identity, dietary sources, intake and biological effects. *Nutrition Reviews* 36, 97—102.

Grgurinovich, N. (1986) Simple and sensitive method for the simultaneous determination of enprofylline, theobromine, paraxanthine, theophylline and caffeine using high-performance liquid chromatography. *Journal of Chromatography* 380, 431—436.

Holmstedt, B. and Lindgren, J.-E. (1972) Alkaloid analyses of botanical material more than a thousand years old. In: S.H. Wassen (Ed.), *A Medicine-man's Implements and Plants in the Tiahuanacoid Tomb in Highland Bolivia.* Etnologiska Studier 32, Göteborgs Etnografiska Museum, Göteborg, Sweden, pp. 139—144.

Lewis, W.H. and Elvin-Lewis, M.P.F. (1977) *Medical Botany: Plants Affecting Man's Health.* Wiley, New York, p. 383—391.

Lewis, W.H. (1984) Biosystematics and medicines. In: W.F. Grant (Ed.), *Plant Biosystematics.* Academic Press, Canada, Don Mills, Ontario, pp. 561—578.

Lewis, W.H., Elvin-Lewis, M.P.F. and Gnerre, J.C. (1987) Introduction to the ethnobotanical pharmacopeia of the Amazonian Jívaro of Peru. In: A.J.M. Leeuwenberg (Ed.), *Medicinal and Poisonous Plants of the Tropics,* Pudoc Wageningen, The Netherlands, p. 102.

Patiño, V.M. (1968) Guayusa, a neglected stimulant from eastern Andean foothills. *Economic Botany* 22, 310—316.

Power, F.B. and Chesnut, V.K. (1919) *Ilex vomitoria* as a native source of caffeine. *Journal of the American Chemical Society* 41, 1307—1312.

Rall, T.W. (1985) The Methylxanthines. In: A.G. Gilman, L.S. Goodman and A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics.* Macmillan, New York, pp. 589—603.

Schultes, R.E. (1972) *Ilex guayusa* from 500 A.D. to the present. In: S.H. Wassen (Ed.), *A Medicine-man's Implements and Plants in the Tiahuanacoid Tomb in Highland Bolvia.* Etnologiska Studier 32, Göteborgs Etnografiska Museum, Göteborg, Sweden, pp. 115—138.

Schultes, R.E. (1979) Discovery of an ancient guayusa plantation in Colombia. *Botanical Museum Leaflets* (Harvard University) 27, 143—153.

Schultes, R.E. and Raffauf, R.F. (1990) *The Healing Forest: Medicinal and Toxic Plants of the Northwest Amazonia.* Dioscorides Press, Portland, pp. 80—81.

Stout, G.H. and Schultes, R.E. (1973) In: L.P. Miller (Ed.), *Phytochemistry*, Volume 3. Van Nostrand, New York, pp. 386—388.

Tang-Liu, D.D.-S., Williams, R.L. and Riegelman, S. (1983) Disposition of caffeine and its metabolites in man. *Journal of Pharmacology and Experimental Therapeutics* 224, 180—185.

Wallace, A.R. (1908) *Notes of a Botanist on the Amazon and Andes.* Macmillan, London, pp. 453—454.