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Evaluation of Some Energetic and Nutritional Supplements in Brazil: Can They Originate a Positive Case for Stimulants in Doping Control?

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Introduction

In the last few years, the use of nutritional supplements, energetic drinks and ground Amazon Guaraná seeds, not only in Brazil, but also by young people world-wide who attend fitness gyms or nightclubs, aiming to reduce fatigue and increase the state of alertness[1,2,3], has dramatically increased.

Due to the stimulating properties and the combat of physical breakdown, the regular use of ground Amazon Guaraná seeds, especially in association with northern/northeastern fruit juices, has been increasing in Brazil. The use of these natural energetics in association with the usual intake of caffeine (coffee, tea, sodas, chocolate...), may become a problem to athletes during antidoping control.

In this study, a nutritional supplement containing Ma Huang and Guaraná extracts, an energetic drink containing caffeine, and ground Guaraná seeds were separately administered to healthy volunteers, according to their habitual dietary intake. Concentrations of caffeine and ephedrines detected in urine after each study are presented. Quantitation of caffeine and ephedrines were performed by HPLC and GC/NPD, respectively.

Experimental

Study Design:

Nutritional Supplement and Ground Guaraná Seeds

- Volunteers: 2 healthy volunteers (males).

- Administration:
  - Nutritional Supplement: 2 x 3 capsules / day over a 6 day period (each capsule contains 5mg Ephedra alkaloids and 227.5mg Guaraná extract).
  - Ground Guaraná Seeds: 2 x 1.5g / day over a 6 day period.
- Urine collection: pre-dose, Day 1: 0-2, 2-4 and 4-8h; Day 6: 0-2, 2-4, 4-8, 8-12, 12-24, 24-36 and 36-48h (0h corresponds to 8:00 a.m.).

Energetic Drink:

- Volunteers: 3 healthy volunteers (2 males, 1 female).
- Administration: 1 can containing 250ml (32 mg/100ml caffeine).
- Urine collection: pre-dose, 0-2, 2-4, 4-8, 8-12, 12-24, 24-36 and 36-48h.

During the study, all volunteers were on diet from caffeine or ephedrine containing foods and beverages.

Instrumentation:

GC/NPD analysis:

Instrument:	HP 5890 GC with NPD detector
Capillary column:	5% crosslinked phenyl methylsiloxane (15m x 0.2mm i.d., 0.33µm)
Injection mode:	split (1:10)
Carrier gas:	helium (linear velocity 40cm/s, 100°C)
Injector temp.:	280°C
Detector temp:	290°C
Sample volume:	2µl
Oven temperatures:	60°C (1min) - 20°C/min - 280°C (6min)

GC/HPLC analysis:

Instrument:	Waters 600 Quaternary Pump
Column:	Waters Symmetry C-18
Column temperature:	40°C
Mobile phase:	H_2O/ACN 90:10, pH 3; Flow: 1.5 ml/min
Injection volume:	20µl
Detection:	DAD, 273 nm

Results and Discussion

Urines were submitted both to the procedure for quantitation of ephedrines using GC/NPD detection and to a procedure for quantitation of caffeine by reverse phase HPLC. Standard calibration curves were obtained spiking blank urine samples with the analytes under investigation (Table 1).

Table 1: Standard calibration curves for caffeine, ephedrine and norephedrine.

Products	Compound	Calibration curve	r^2
Nutritional Supplement	Caffeine	y = 0.084 + 0.073x	0.9979
	Ephedrine	y = 0.005 + 0.129x	0.9990
	Norephedrine	y = -0.040 + 0.090x	0.9987
Energetic Drink	Caffeine	y = 0.030 + 0.287x	0.9997
Ground Guaraná Seeds	Caffeine	y = 0.048 + 0.289x	0.9992

Concerning the nutritional supplement, the results of caffeine quantitation showed that maximum urine concentrations did not attain values above 4.5μg/ml (Table 2).

Table 2. Estimated amounts of caffeine from nutritional supplement.

	Caffeine (μg/ml)	
Time (h)	Vol 1	Vol 2
	Day 1	
Pre-dose	-*	-*
0 - 2	1.6	2.7
2 - 4	1.9	2.9
4 - 8	2.9	3.4
	Day 6	
0 - 2	2.6	3.2
2 - 4	4.5	2.5
4 - 8	3.4	3.9
8 - 12	2.5	2.7
12 - 24	1.1	1.6
24 - 36	0.6	0.4
36 - 48	1.0	0.2

* Not detected

Maximum ephedrine concentrations, on the other hand, were 30.9μg/ml and 37.7μg/ml for the volunteers submitted to the nutritional supplement experiment (Table 3).

The metabolite norephedrine was also observed in almost all collected fractions in concentrations, not exceeding 5.3μg/ml (Table 3).

Table 3. Estimated amounts of ephedrine and norephedrine.

Time (h)	Ephedrine (μg/ml) Vol 1	Ephedrine (μg/ml) Vol 2	Norephedrine (μg/ml) Vol 1	Norephedrine (μg/ml) Vol 2
		Day 1		
Pre-dose	-*	-*	-*	-*
0 - 2	2.6	0.5	0.5	0.5
2 - 4	26.9	35.3	5.2	3.6
4 - 8	18.4	17.6	2.7	2.4
		Day 6		
0 - 2	13.3	15.8	2.5	2.0
2 - 4	6.4	23.2	3.5	1.1
4 - 8	30.9	37.7	5.3	4.7
8 - 12	22.4	25.5	3.7	3.5
12 - 24	5.9	15.2	2.2	1.0
24 - 36	1.1	1.6	-*	-*
36 - 48	-*	0.6	-*	-*

* Not detected

For the energetic drink (Table 4) and Guaraná seeds (Table 5), urinary caffeine concentrations were inferior to 2.5μg/ml for the volunteers submitted to the experiment. Similar results were found in other studies[4,5].

Table 4. Estimated amounts of caffeine from energetic drink.

Time (h)	Caffeine (μg/ml) Vol 1	Vol 2	Vol 3
		Day 1	
Pre-dose	-*	-*	-*
0 - 2	1.3	1.3	1.0
2 - 4	1.5	1.9	0.4
4 - 8	0.9	2.0	0.8
8 - 12	0.4	0.9	0.4
12 - 24	0.6	0.5	-*
		Day 2	
24 – 36	-*	-*	0.5
36 - 48	-*	0.4	-*

* Not detected

Table 5. Estimated amounts of caffeine from Guaraná seeds.

Time (h)	Caffeine (μg/ml) Vol 1	Vol 2
	Day 1	
Pre-dose	-*	-*
0 - 2	n.c.	0.7
2 - 4	1.3	0.8
4 - 8	1.0	0.8
	Day 6	
0 - 2	0.8	0.7
2 - 4	-*	1.1
4 - 8	0.5	2.2
8 - 12	0.9	0.8
12 - 24	-*	0.3
24 - 36	0.3	-*
36 - 48	-*	-*

* Not detected
n.c. Not colected

Conclusion

Considering typical daily intakes and actual cut-off values defined by the IOC, only the nutritional supplement studied could provide positive cases of doping (ephedrine). However, as caffeine is present in many daily-consumed products, the simultaneous ingestion of caffeine based nutritional supplements could considerably increase urinary caffeine concentrations to near threshold levels.

References

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