

Inadvertent doping with the use of herbal preparations: is it a reality?

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Abstract

The use of performance enhancing drugs in sports is prohibited by World Anti-doping Agency (WADA).⁽¹⁾ Herbal preparations are usually considered safe due to least side effects but may contain substances which are prohibited in sports. Athletes often take these preparations for therapeutic purpose without considering the risks of an inadvertent doping. A study on the screening of various labelled and unlabelled Indian Ayurvedic herbal preparations to identify the substance of abuse was conducted. A total of hundred and two labelled and sixteen unlabelled herbal Ayurvedic preparations used for different therapeutic indications were tested for screening of approximately one twenty drugs prohibited by WADA. Out of hundred and two labelled herbal preparations, sixteen showed presence of Strychnine. However, out of these sixteen herbal preparations, only four preparations declared presence of strychnine on the label. Strychnine is a stimulant which is prohibited by WADA during in competition testing. Out of sixteen unlabelled preparations, two showed presence of Nicotine. The study shows that the labelled/unlabelled herbal preparations may contain undeclared substances prohibited by WADA. The excretion study showed levels of strychnine above WADA Minimum Required Performance Levels (MRPL) thereby leading to positive dope test.

Keywords: Sports, Doping, WADA, Prohibited List, Herbal Preparations, Adulteration.

Introduction

The use of herbal preparations is generally considered safe on the basis of the claimed facts of less number of side effects associated. Consequently, sportspersons may consume these preparations to keep themselves energetic, full of vitality and vigour.⁽²⁾ Herbal preparations available in India and globally may contain substances prohibited by WADA^(3,4) which may be due to adulteration with undeclared drugs or contamination or deliberate addition in the preparation. Athlete taking these preparations for therapeutic purpose may be caught for inadvertent doping.⁽⁵⁾ The risk associated with such labelled/unlabelled herbal preparations should be considered by all athletes prior to use.

In many countries, the manufacture of herbal drugs is not regulated appropriately, which means that the ingredients of the herbal drugs may not match those listed on the label of the container. The contamination of herbal preparations with the WADA prohibited drugs has been reported.⁽⁶⁻⁸⁾

Another important concern is the labeling of herbal preparations with the trivial names of ingredients which athletes may not be aware of while using such preparations. A common example being Strychnine- a natural alkaloid of *Nux vomica* plant prohibited in sports, is present in many herbal drugs with different names like Shuddha Kapilu, Kuchla Shudd, Ramyaphal and Strychnous nux vomica.

In view of the availability and use of various natural and herbal remedies in India by both athletic and non-athletic population, the present study was

planned to detect Stimulants, Narcotics and Anabolic Steroid classes of prohibited drugs in such preparations.

Material and Methods

Reagents & Chemicals: All the reagents and chemicals used were of Analytical and HPLC grade.

Reference Standards: Certified reference standards of Stimulants, Narcotics, Anabolic Steroids and Internal Standards- Diphenylamine (DPA), 10-Methyl Phenothiazine (NMPZ) and Methyltestosterone from various sources *viz.* Sigma Aldrich (USA), NMI Australia and Cerilliant were used. Stock solution of each standard was prepared at the concentration of 1.0 mg/ml in ethanol. Further dilutions were done at appropriate concentrations.

Study Protocol: A total of one hundred and two labelled and sixteen unlabelled herbal Ayurvedic preparations of different brands based on different therapeutic indications were procured from local market for screening of WADA prohibited drugs. The collected herbal preparations were screened for one twenty drugs approximately which included stimulants, narcotics and anabolic steroids. Excretion study samples were collected post administration of the herbal preparations upto 28 hours. The research work is duly passed by the ethical committee of National Dope Testing Laboratory (NDTL).

*Weight loss, Arthritis, Neurological/Psychological stress, Inflammation, Analgesia, Muscular growth.

Sample Preparation: The herbal preparations were in the form of capsules/ tablets. One capsule/ tablet was crushed and dissolved in methanol and kept overnight. The herbal preparations were centrifuged and the

supernatants were taken. Three aliquots of 1ml/2ml was taken for extraction of stimulants/narcotics and anabolic steroids.

For screening of stimulants and narcotics, the supernatant was processed using acidic extraction followed by alkaline extraction⁽⁹⁾ and further injected into Gas Chromatography coupled with Nitrogen Phosphorus Detector and Mass Spectrometric detector (GC-NPD/MSD). For screening of anabolic agents, the herbal preparations were processed using alkaline extraction followed by derivatization (Fig. 1) and injected into GC/MSD. The methanolic extracts were dried and reconstituted using 1% formic acid and acetonitrile for screening of stimulants, narcotics by LC/MS/MS. Single therapeutic dose of three drugs containing declared strychnine (RHEM-50mg, RM8-25mg, PVTM-17.8mg) from different brands of Ayurveda were given to three healthy volunteers of the age group of 25 -32 years. Sample Preparation for excretion study samples was done by using liquid-liquid extraction procedure after alkalinising the samples (Fig. 2). The extracts were then injected on to GC-NPD/MSD. The instrument analytical conditions of GCNPD/MSD, GC-MS and LC-MS/MS are summarized (Table 1).

Instrumental Analysis: The analysis was performed by comparing (Retention Time) RT and full scan mass spectra of the suspicious drug with that positive quality control sample. For quantitation, multiple point calibration curve (50ng/ml-200 ng/ml) was prepared. The method employed for analysis was duly validated for screening of WADA prohibited substances which are being used in routine in the laboratory.

Results

The herbal preparations were analysed using different chromatographic and mass spectrometric approaches. Out of one hundred and two labelled herbal preparations, sixteen showed presence of Strychnine (Table 2). However, out of these sixteen herbal preparations, four indicated presence of strychnine on the label by the names of Shuddha Kapilu, Kuchla Shudd, Ramyaphal and Strychnous nux vomica and the remaining twelve did not declare strychnine on their labels. Strychnine could be confirmed in the range of 2.10ng/ml to 479ng/ml in three herbal drugs found suspicious for presence of strychnine (Fig. 3, Table 3). Highest concentration of strychnine was found in RHEM (declared), lowest concentration was found in T1(undeclared) which can be inferred that the herbal preparations containing the lowest concentration is due to contamination and the highest concentration is due to the natural addition of the drug for the purpose of treatment.

Excretion Study of the herbal Ayurvedic preparations containing declared Strychnine showed the doping may be caused as the concentration recovered exceeded WADA MRPL(Fig. 4, 5, 6).

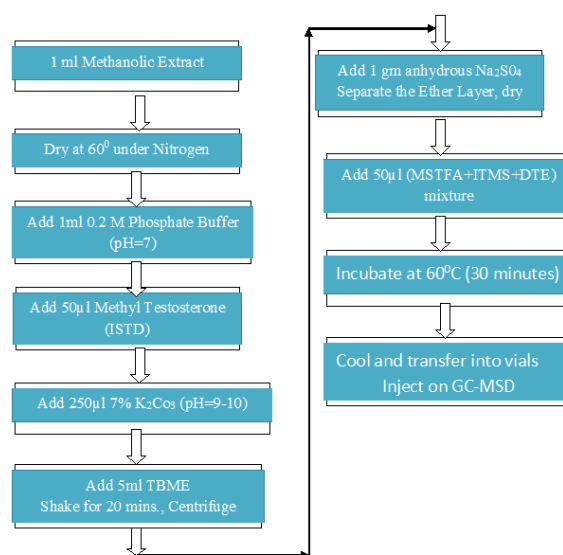


Fig. 1: Sample pre-treatment scheme for extraction of androgenic anabolic steroid from herbal preparations

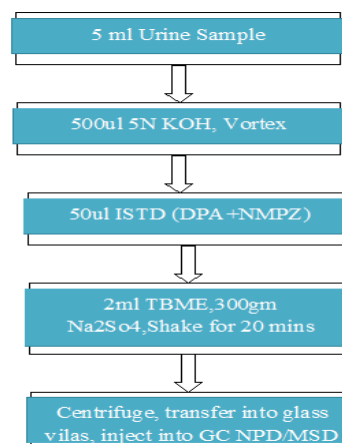


Fig. 2: Sample pre-treatment scheme for extraction of stimulants from human urine sample

Table 1: Instrumental Analytical Conditions

GC NPD/MSD Analysis	
Oven Temperature	Initial-100°C, Final-300°C
Injector Temperature	280°C, Split Ratio:5:1
Carrier Flow	Carrier Gas: Helium, Constant Pressure(150KPA)
MS Parameters	Temperature: Ion Source:230°C, Analyzer:150°C, Interface: 300°C
GC MSD Analysis	
Oven Temperature	Initial-180°C, Final-300°C
Injector	280°C, Split Ratio:11:1

Temperature	
Carrier Flow	Carrier Gas: Helium, Constant Pressure(122KPA)
MS Parameters	x-fer line 300°C
LC MS/MS Analysis	
Flow	0.7ml/min (Slit Less)
Solvents	B: 1% Formic Acid, D:Acetonitrile
Gradient	15%B to 100%B in 7.00 minute and then 15% in 11 minute
Ion Source	ESI

Table 2: List of herbal preparations showing presence of Strychnine with therapeutic indications

S. No	Herbal preparation (codified)	Therapeutic indications
1	T2	Anti Rheumatic, Anti Arthritic, Nerve
2	RM-1	Weight Loss
3	RM -6	Anti Arthritic
4	RM-8	Anti Arthritic
5	RM-9	Analgesic
6	RM-13	Analgesic, Anti inflammatory
7	RM-20	Analgesic, Anti Arthritic
8	RM-33	Analgesic
9	RM-57	Anti Stress, Increases strength
10	RM-60	Anti Rheumatic
11	RHEM	Analgesic, Anti Arthritic
12	PVTM	Analgesic, Anti Arthritic
13	A2	Nervine
14	T1	Weight loss
15	USFM	Weight Loss
16	RG	Anti Arthritic

Table 3: Levels of Strychnine Estimated in various Herbal Preparations

S. No	Herbal preparations	Status on labels	Concentration (ng/ml)
1	T2	UD	20
2	RM-1	“	41.8
3	RM -6	D	25.2
4	RM-8	D	442
5	RM-9	UD	5.65
6	RM-13	“	3.5
7	RM-20	“	124
8	RM-33	“	70.3
9	RM-57	“	17.2
10	RM-60	“	110

11	RHEM	D	479
12	PVTM	D	104
13	A2	UD	4.63
14	T1	“	2.10
15	USFM	“	3.12
16	RG	“	2.40

- UD=Undeclared
- D=Declared

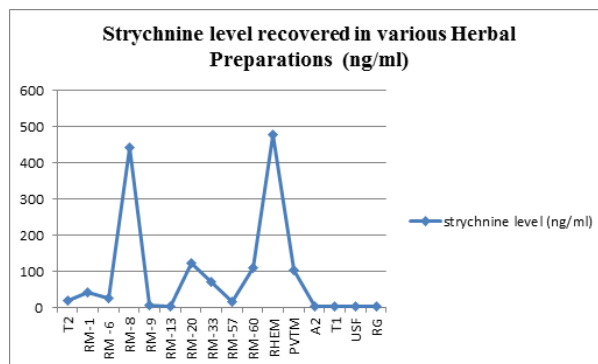


Fig. 3: Graphical presentation of Strychnine levels estimated in various Ayurvedic herbal preparations

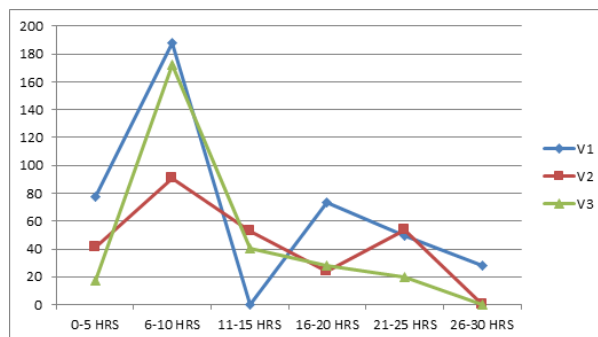


Fig. 4: Excretion profile of Strychnine in Drug 1 (RHEM)=50 mg declared

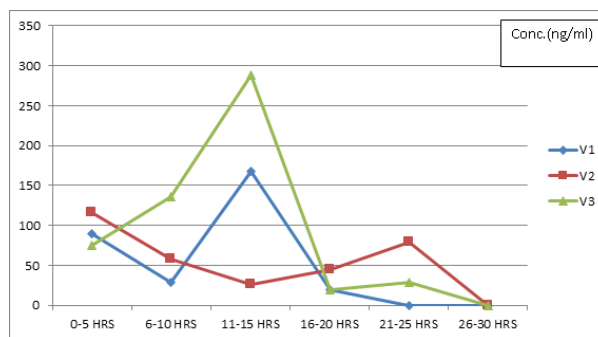


Fig. 5: Excretion profile of Strychnine in Drug 2 (RM-8)=25 mg declared

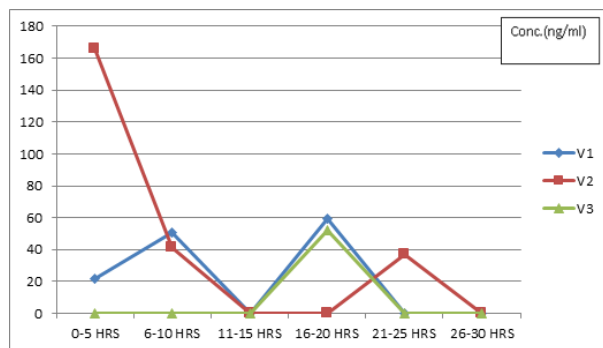


Fig. 6: Excretion profile of strychnine in drug 3 (PVTM)=17.8 mg declared

V1= Volunteer 1

V2= Volunteer 2

V3= Volunteer 3

Discussion

The inadvertent doping may occur due to the use of herbal preparations based on the following possibilities:⁽⁵⁾

- When a prohibited substance is declared on the label but the athlete is not aware that it is a banned substance.
- Prohibited substances are declared on the label, but under different names which the sportsperson is unfamiliar with. E.g. strychnine may be declared on the label by different names like kuchla, Kuchala, Balewa, Kajra.
- Prohibited substances are not declared on the label. These substances could have been added deliberately or could be the result of possible contamination during synthesis, capsulation or packaging.

WADA states that the athlete is ultimately responsible for what is found in his/her body fluids irrespective of its origin. So, the risk of inadvertent doping after taking herbal preparations remains the athletes' responsibility. Inadvertent doping can be prevented by:

- Following GMP by the manufacturing companies⁽¹⁰⁾
- Screening all the supplements before marketing⁽¹¹⁾
- Organizing health awareness program about supplements for athletes⁽¹²⁾
- Avoiding unlabelled preparations⁽¹³⁾

Conclusion

The study shows that the labelled herbal preparations may contain undeclared potential drugs of abuse prohibited by WADA. Such studies should be performed so that a database could be created to generate awareness amongst the sports fraternity which will help to prevent inadvertent doping.

Acknowledgement

The financial support of Ministry of Sports and Youth Affairs is duly acknowledged.

References

1. World Anti-Doping Code: WADA International Standard for laboratories. Version 9. www.wada-ama.org/rtecontent/document/International_standard_for_laboratories.
2. Beotra, A. "Drug Education handbook on drug abuse in sports". Seventh Edition, 2010-2011.
3. Longo Donatella, Colamonici Cristiana, Molaioni Francesco, Botre Francesco. "Synephrine (Oxedrine): Analytical and Pharmacological issues. Recent advances in doping analysis (10)", 2002.
4. George Q.Lii, Colin C.Duke, Basil D. "The quality and safety of Traditional Chinese Medicine". Australian Prescriber (26), 2003.
5. P J Van Der Merwe, Grobbelaar E. "Inadvertent doping through nutritional supplements is a reality". Sports Medicine (16), No.2, 2004.
6. Maria Kristina Parr, Geyer Hans, Sigmund Gerd, Kohler Karsten, Schanzer Wilhelm. "Screening of Nutritional Supplements for Stimulants and other drugs". Recent Advances in doping analysis (11), 2003, 67-76.
7. Savaliya, Akash A. Prasad, Bhagwat. Rajjada, Dhara K. Singh, Saranjit. "Detection and characterization of synthetic steroidal and non-steroidal anti-inflammatory drugs in Indian Ayurvedic/herbal products using LC-MS/TOF". Drug Testing & Analysis (08), 2009.
8. Bogusz Maciej J., Hassan Huda, Eid Al-Enazi, Ibrahim Zuhour, Tufail Mohammed Al-. "Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies". Journal of Pharmaceutical and Biomedical Analysis (41), 2006, 554-564.
9. Maria Kristina Parr, Hans Geyer, Gerd Sigmund, Karsten Kohler, Wilhelm Schanzer. "Screening of Nutritional Supplements for Stimulants and other drugs". Recent Advances in doping analysis (11), 2003,67-76.
10. WHO good manufacturing practices for pharmaceuticals products: main principles WHO Technical Report Series No. 986, 2014.
11. Nutritional supplement and ingredient testing services. LGC in sport and specialised analytical services. Available online at www.informed-sport.com.
12. Program for Education and Awareness on Anti-Doping in Sports (PEADS). Available online at www.mygov.in/sites/default/files/master-image/PEADS.pdf.
13. Olivier de Hon, Bart Coumans. The continuing story of nutritional supplements and doping infarctions. British Journal of Sports Medicine. Volume 41, issue 11.