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HEAVY METAL ANALYSIS AND AFLATOXIN ASSAY OF SIDDHA FORMULATION PEENISA CHOORANAM

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Abstract

The aim of the study was to analyse the presence of heavy metal and aflatoxin of the siddha formulation Peenisa Chooranam, which is indicated for the respiratory related conditions. The study medicine was prepared as per the literature and both analytical studies were conducted at Regional Research Institute Of Unani Medicine (Drug Standardisation Research Unit), Chennai, according to PLIM guidelines of AYUSH to evaluate its safety profile. The result of heavy metal analysis show that the Peenisa Chooranam has no traces of lead, arsenic and shows the presence of cadmium and mercury below the WHO permissible limit. The result of aflatoxin assay show the presence of B1,B2,G1,G2 below detection limit using afla-test fluorometer. The results that have been provided will help with future study. This ensures that the Siddha medication under research, Peenisa Chooranam, was safe to use therapeutically.

Keywords: Peenisa Chooranam, Heavy metal, Aflatoxin, Siddha, PLIM

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Introduction

In order to meet the medical demands of the general population, generally accepted evidence-based medications must be used due to the shift in public opinion towards complementary and alternative medicine. There is also a contrary that anything that comes from traditional medicine may be harmful to one's health and are not always safe, despite popular belief [1]. Nowadays, standardization is required for proving the chemical composition and safety of every medication. This could contribute to the global acceptability of Siddha medicine [2]. The Pharmacopoeia Laboratory for Indian Medicine by the Department of AYUSH has developed methods to evaluate ASU medications [1]. *Peenisa Chooranam* [PC] is a Siddha herbal formulation indicated for *Peenisangal, Nasi naatram and Nasi raththam* mentioned in the literature *Dhanvandhri Sootchama Vaithiyam 200 Visha Bedhi Vaithiyam* [3]. The present study deals with the Heavy metal analysis and Aflatoxin assay of Siddha formulation *Peenisa Chooranam* [PC] in a preliminary attempt to demonstrate the safety and quality of Siddha medicine rather than its controversy to the scientific world.

Materials and Methods

Medicine Preparation

The study medicine *Peenisa Chooranam* [PC] is poly herbal formulation consisting of *Chukku, Milagu, Thippili, Chenkathari, Kodiveli, Kandangkathiri, Sangam ver, Karunjeeragam, Seeragam.* The herbal raw drugs were properly purified and pounded into fine powder using pounder and sieved well individually, mixed together as per the literature [3].

Study Place

The heavy metal analysis and aflatoxin assay of Siddha formulation *Peenisa Chooranam* [PC] were conducted at Regional Research Institute of Unani Medicine (Drug Standardisation Research Unit), Chennai.

Heavy Metal Analysis

Methodology

The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series, extraction solvent HCl and HNO3 in

order to determine the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item. Standard: Hg, As, Pb and Cd –Sigma.
Sample Digestion

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. The sample was digested with 1mol/L of HNO3 for the detection of lead and cadmium.

Standard preparation
As & Hg-100 ppm sample in 1mol/L HCl
Cd & Pb-100 ppm sample in 1mol/L HNO3

Aflatoxin Test Using Afla-Test Fluorometer

Aflatoxins are a group of naturally occurring toxins produced by Aspergillus flavus and Aspergillus parasiticus, two common mold species. AflaTest is a quantitative method for the detection of aflatoxin in B1, B2, G1, G2, M1, and M2. One gram of *Peenisa Chooranam* and 0.4 g of sodium chloride was mixed with methanol: 2% Tween 20 or phosphate buffer (60:40 v/v). Vortex the mixture of extract on high speed 3 minutes. The extract was filtered through fluted filter paper. 10 ml of filtered extract wasadded in measuring cylinder, in that 20 mL purified water was added and vortex on high for 1 minute. Then the diluted extract was filtered through a pre-wet glass microfiber filter (1.5µm). 10 mL of diluted extract was passed through AflaTest WB column. Pressure was applied to get 1-2 drops per second. Then the column was washed with with 10 mL 2% Tween 20. and then washed the column with 10 mL purified water twice. Elute AflaTest WB columns by passing 1 mL HPLC-grade methanol (100%) through column, and pressure was applied to get 1 drop per second. After collecting eluate in sterile VICAM cuvette, added 1.0 mL of AflaTest Developer and mixed well, placed immediately in fluorometer (VICAM fluorometer-sereies 4EX). Concentration after 60 seconds was read in the fluorometer [4,5,6].

Result

Heavy Metal Analysis of PC

Table 01: Test report of heavy metal analysis of *Peenisa Chooranam*

Heavy/Toxic metals	Result	WHO Permissible limit (ppm)
Lead	Nil	10
Cadmium	0.0029 mg/L	0.3
Arsenic	Nil	3
Mercury	1.5305 µg/L	1

Aflatoxin assay of PC

Table 02: Test report of aflatoxin assay of *Peenisa Chooranam*

S.No.	Parameters	Method / Reference	Results
1.	Total Aflatoxin B1+B2+G1+G2	Vicam Aflatest Fluorometer Instruction Manual	Below detection limit

(Note: Detection Limit - 1ppb)

Discussion

If a medication contains more heavy metals than permitted, it might have negative impacts on the kidney, brain, growing foetus, vascular system, and immune system [7]. In the present study, the heavy metal analysis of Siddha medicine *Peenisa Chooranam* shows no presence of metals lead and arsenic. It also shows presence of 0.0029mg/L of cadmium and 1.5305 µg/L of mercury which is under WHO permissible limit in parts per million. Also, the presence of more Aflatoxin, which is a toxic substance, in the medicine may have serious adverse effects like hepatotoxicity, carcinogenicity and immune system suppression [7,8]. In the present study, total aflatoxin B1, B2, G1, G2 using afla-test fluorometer show its presence below detection limit of 1 parts per billion. Both the present analysis of *Peenisa Chooranam* ensures the safety and quality of the medicine with respect to its toxicity.

Conclusion

Based on the current investigation, it was determined that the *Peenisa Chooranam* (PC) sample was safe because it contained no lead or arsenic and only trace amounts of aflatoxins and heavy metals cadmium and mercury, all of which were below the detection level. This validates PC's quality profile with regard to toxicity and biological chain contamination.With the fundamental quality maintained, this preliminary standardisation study would support future research and clinical studies.

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Conflict of Interest

Author has declared that no competing interest exists.

Inform Consent and Ethical Statement

Not applicable

Author Contribution

Sure! Here's the revised version as a single paragraph: Sujatha R. and Saranya N. conducted the study and prepared the manuscript, while Sudhamathi Pushparaj K. guided the study and approved the manuscript. U. Chitra and R. Menaka supported and guided the study.

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