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# PHYSICOCHEMICAL AND CYTOTOXIC STUDY OF AROGYAVARDHINI VATI AND LIVAMRIT ADVANCE, THE POPULARLY USED AYURVEDIC FORMULATIONS AGAINST LIVER DISEASES

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**ABSTRACT**

Arogyavardhini Vati & Livamrit Advance (Livamrit A) is a Polyherbal formulation that is being used for centuries with claimed efficacy and safety in the treatment of Jaundice, liver disease, and various skin conditions**.** Considering their use by a larger population in India they must be assessed for their efficacy and cytotoxic effects, if caused any therefore the present study was aimed to evaluate the physicochemical and cytotoxicity effect of Arogyavardhini Vati and Livamrit tablets.Both Ayurvedic formulations were evaluated for their physicochemical properties by AAS, phase analysis through XRD, and cytotoxicity through MTT assay on HepG2 cell line in five different concentrations (62.5µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml) of the drugs.The Physicochemical analysis resulted in a very low concentration, below the permissible limit of targeted heavy metals i.e. Cadmium, Lead, Zinc, and Copper in the tested samples. The phase analysis revealed crystalline structure in Arogyavardhini Vati in two different phases of their active ingredients only namely Metacinnabar (HgS) and Hematite ($Fe_2O_3$) while Livamrit A did not show any crystalline structure. Also, not any significant change in cell viability was observed in the exposure to higher dose concentrations (1000 µg/ml) of both formulations.In our study both Ayurvedic formulations (Arogyavardhini Vati & Livamrit A) are found safe having no heavy metals in higher concentrations than the permissible limit and any considerable cytotoxic effects on the liver.

**Keywords; HepG2, Ayurvedic Formulations, Cytotoxicity assessment of Ayurvedic formulations, Heavy metals toxicity, MTT Assay, Arogyavardhini Vati, Livamrit A**

## INTRODUCTION

Ayurveda is the ancient Indian medical system that has always been practiced for good health and wellbeing. It is traditionally skilled and has been used for treating liver disease, When compared to conventional medicine, drug toxicity appears to be lower. About 2 million people around the world die each year from liver diseases like liver cirrhosis, viral hepatitis, and hepatocellular cancer **[1]**. Liver diseases are also the most prevalent type of iatrogenic disease, which may be caused by excessive use of several medications like paracetamol **[2-4]** and some organic and inorganic substances like naturally occurring plant toxins, arsenic, phosphorus, copper, iron **[5]**. For liver problems, more than half of the population in our country rely on Ayurveda and herbal medicine **[6]**. Long-term use of modern allopathy therapies for the treatment of liver disease causes systemic toxicity, for this reason, the physician does not recommend the long-term use of these medicines **[7]**. There are more than 300 Ayurvedic preparations available in the Indian Ayurvedic system of medicine for the treatment of liver diseases such as jaundice and other liver-related chronic disorders. Many plants like *Amaranthus tricolor*, *Phyllanthus emblica, Phyllanthus reticulatus,* and their extracts have been found to have hepatoprotective properties **[8-10]**. *In-vitro* and *in-vivo* research have shown that Ayurvedic herbs and products with a biochemical active component can protect the liver from oxidative stress, enhance virus elimination, block fibrogenesis, reduce inflammation, modulate the immune system, help in liver regeneration, and inhibit the cancer growth **[11]**.

Arogyavardhini Vati & Livamrit A both are Polyherbal formulation which is being used for centuries with claimed efficacy and safety in the treatment of jaundice, liver disease, and various skin conditions **[12]**.

Arogyavardhini vati consists of Bibitaka(*Terminalia bellirica*), Amalaki (*Emblica officinalis*), Silajatu-Suddha (Asphaltum), Haritaki (*Terminalia chebula*), Guggulu Shuddha(*Commiphora wightii*), Katuka (*Picrorhiza kurroa*), leaf juice of Neem (*Azadirachta indica*), Eranda (*Ricinus communis*), and metals including Shuddha Rasa (purified mercury), Lauha Bhasma Shuddha Gandhaka (purified sulfur), Abhraka and Tamra Bhasma[6]. Livamrit A is also a Polyherbal formulation used for the treatment of fatty liver, hepatitis, anemia, jaundice, and lack of appetite. It consists of Bhringraj (*Eclipta alba*), Bhumi Amla (*Phyllanthus niruri*), Giloy (*Tinospora cordifolia*), Kalmegh (*Andrographis paniculata*), Kutki (*Picrorhiza kurroa*), Makoy (*Solanum*

*nigrum*), Arjun (*Terminalia arjuna*), Punarnava (*Boerhaavia diffusa*), Daru Haldi (*Berberis aristata*) **[13]**.

There are a lot of other Ayurvedic preparations available in the market, but their efficiency has been questioned by many authors due to a lack of data and proper medication. Even the excessive use of some Ayurvedic herbs and medications such as *Withania somnifera* (Ashwagandha) **[14]**, *Cassia angustifolia* (Indian senna) **[15]**, *Aloe barbadensis miller* (aloe vera) **[16]**, Giloy kwath primarily composed of *Tinospora cordifolia*, Manjishthadi Kwatham (contain 52 individual plant extract), Aragwadhi Kwatham(which contained 10 individual plant extracts), Kanchnar Guggulu, (containing 10 individual plant extracts) **[17]** have been reported to be hepatotoxic. Therefore, the current study was conducted to analyze the physicochemical and cytotoxic effect of Arogyavardhini vati and Livamrit A on the HepG2 cell line to scientifically validate and authenticate the therapeutic claims of Ayurvedic preparations.

## METHODS

### Test Samples

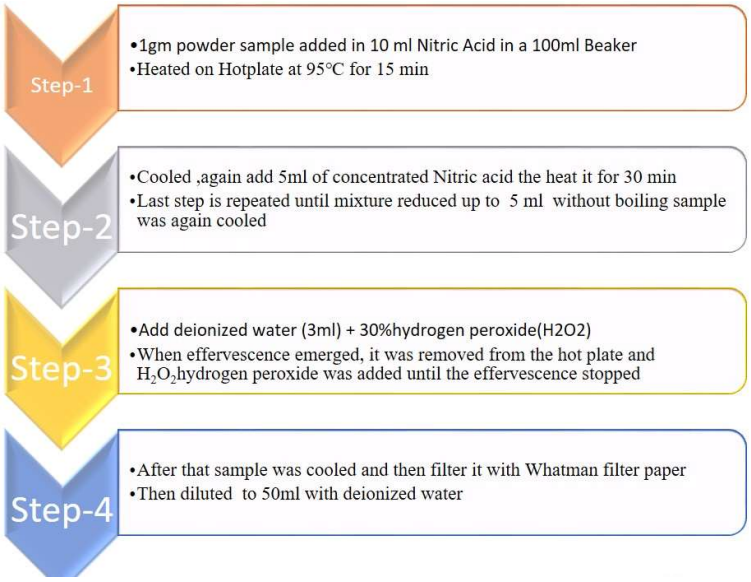
Both Ayurvedic preparation namely Arogyavardhini vati (Batch No.#BAVV210018) and Livamrit A (Batch No.#ALAT210028) were taken as the test samples of the study. The preparations were purchased from the local market of Jhansi, Uttar Pradesh, India in September 2021.

### Phase Analysis of Sample

The phase analysis of the samples was confirmed by the X-ray diffraction (XRD) technique using an X-ray powder diffractometer (Rigaku Corporation Japan, Smart Lab 3kW). The powder samples were scanned with $CuK_{\alpha}$ radiation ($\lambda$ = 1.5405Å) in slow scan in the 2θ range of 5°-80°.

### Elemental analysis (Metallic)

The Arogyavardhini vati and Livamrit A samples were tested for the qualitative and quantitative estimation of metallic elements like Iron (Fe), Copper (Cu), Manganese (Mn), Chromium (Cr), Nikle (Ni) Lead (Pb), and Cadmium (Cd) on the Atomic Absorption Spectrophotometer (Model AA-6880F, Shimadzu) at the Innovation Centre, Bundelkhand University, Jhansi.




**Method of metal analysis [18]**

**Cell Culture**

HepG2, a human liver cancer cell line, procured from the National Centre for Cell line, Pune, India (Passage Number: 32) was used in our study for the cytotoxicity assessment. The HEPG2 cells were cultured under DMEM-HG (DULBECCO'S MODIFIED EAGLE'S MEDIUM with High Glucose) supplemented with 10% FBS (fetal bovine serum), 1% penicillin-streptomycin (w/v). Cells were kept in a humidified chamber at 37°C with 5% constant $CO_2$ supply for 48 hours before the treatment.

***In-vitro* Cytotoxicity assessment (MTT)**

The cell toxicity was measured by using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay **[19]**. The cell growth was determined by the cell count after 24 hours exposure of to the testing samples in different concentrations (62.5µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml). The cells were cultured in a 96-well microtitre plate at a concentration of 10000 cells per well. After 24 hours of cell attachment, the spent medium was aspirated and 200µl of different test concentrations of testing drugs were added to the respective wells. The plate was then incubated at 37°C and 5% $CO_2$ atmosphere for 24h. After 24h drug-containing media was aspirated. 200µl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/ml and incubated for 3 hours at 37°Cand 5% $CO_2$ atmosphere. The culture medium was removed completely without disturbing the crystals formed. Then 100µl of solubilization solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured

using a microplate reader at a wavelength of 570 nm and also at 630 nm.

**Data Analysis**

The results are shown as the mean ± SD of three replicates for each treatment group. One-way analysis of variance was used in the statistical analysis, which was carried out using Microsoft Excel 2013.A *P* value < 0.05 was concidesred as statistically significant.

**RESULTS**

**XRD analysis**

X-ray diffraction (XRD) patterns were analyzed to confirm the phase formation and lattice parameters of both samples by using PDXL2 (ICDD (PDF-2/Release 2013)) software (Shown in **Figure 1 & 2**). The diffraction peaks indicated good homogeneity. The formation of two phases (Metacinnabar HgS and Hematite $Fe_2O_3$) was confirmed in the Arogyavardhini vati sample **(Figure 1)**. All the results carried out with the help of XRD are tabulated in **Tables 1 and 2**. No crystalline structures were found in Livamrit A **(Figure 2)**.

**Metal analysis by AAS**

All the targeted metallic elements were found in very lesser quantities in both Arogyavardhini vati and Livamrit A **(Table 3)**. The quantity of them was determined by using the Environmental Protection Agency (EPA 200.8) estimation method and values (in ppm) were found within the AYUSH permissible limit.

**In vitro Cytotoxicity assay (MTT)**

HepG2 cells were exposed to Arogyavardhini vati and Livamrit A in 5 different concentrations. Cells did not show any significant reduction in their viability in a dose-dependent manner for 24 hours. As compared to untreated cells, treated cells are reduced up to 5 to 10% and the viability of cells is 85 to 90% **(Figures 3 and 4)**.

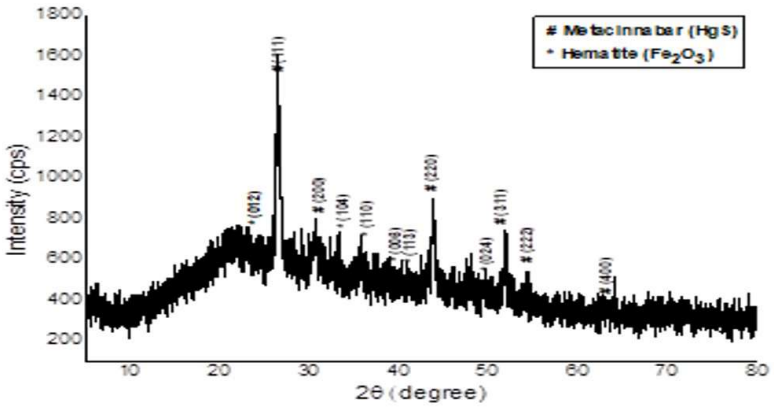



**Figure 1: X-ray diffraction pattern of Arogyavardhini Vati**

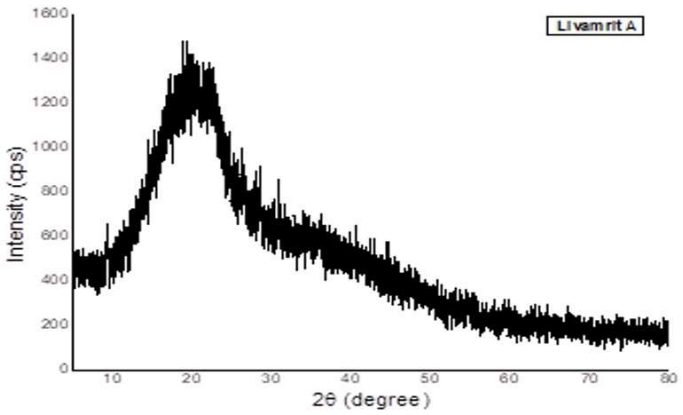



**Figure 2: X-ray diffraction pattern of Livamrit A**

**Table 1: Calculated lattice parameters, type of structure and space group of Arogyavardhini vati**

| Phase Name | Lattice parameters | | | Structure | Space group | DB card no. |
|---|---|---|---|---|---|---|
| | a(A°) | b(A°) | c(A°) | | | |
| Metacinnabar (HgS) | 5.8461 | 5.8461 | 5.8461 | Cubic | F-43m(216) | 01-071-5165 |
| Hematite ($Fe_2O_3$) | 5.0342 | 5.0342 | 13.7483 | Trigonal | R-3c(167) | 01-073-0603 |

**Table 2 : XRD phase analysis of Arogyavardhini vati**

| No | 2-theta (deg) | Intensity | Phase name (h,k,l) |
|---|---|---|---|
| 1 | 24.21 | 723 | Hematite,syn (0,1.2) |
| 2 | 26.46 | 1609 | Metacinnabar (1,1,1) |
| 3 | 30.67 | 798 | Metacinnabar (2,0,0) |
| 4 | 33.22 | 723 | Hematite,syn (1,0,4) |
| 5 | 35.70 | 723 | Hematite,syn (1,1,0) |
| 6 | 39.33 | 508 | Hematite,syn (0,0,6) |
| 7 | 40.84 | 592 | Hematite,syn (1,1,3) |
| 8 | 43.86 | 903 | Metacinnabar (2,2,0) |
| 9 | 43.63 | 710 | Hematite,syn (2,0,2) |
| 10 | 51.83 | 745 | Metacinnabar (3,1,1) |
| 11 | 54.38 | 540 | Metacinnabar (2,2,2) |
| 12 | 63.70 | 416 | Metacinnabar (4,0,0) |

**Table 3: Heavy metals concentration in Arogyavardhini vati and Livamrit A**

| S. No. | Metals | Arogyavardhini vati (ppm) | Livamrit A (ppm) | Limit value as per Ayush (ppm) |
|---|---|---|---|---|
| 1 | Cadmium (Cd) | -0.0046 | -0.0015 | 0.3 |
| 2 | Lead (Pb) | -0.0633 | -0.0034 | 10 |
| 4 | Zinc (Zn) | 0.4879 | 0.2005 | 50 |
| 5 | Copper (Cu) | 14.79 | 0.347 | 20 |

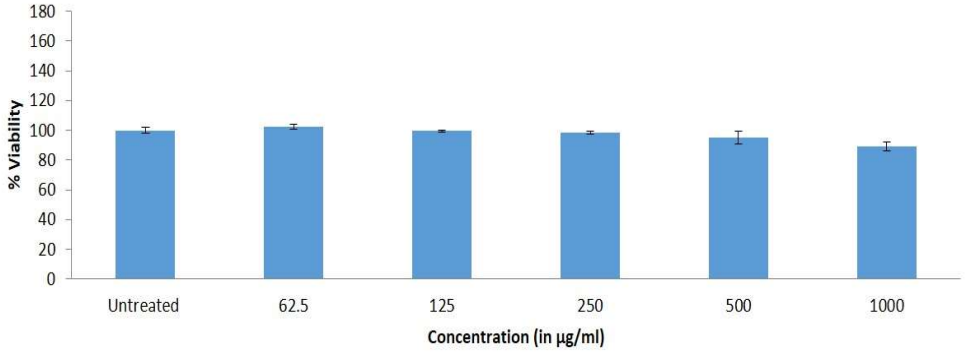



**Figure 3: Effect of Arogyavardhini vati on HepG2 Cell line**

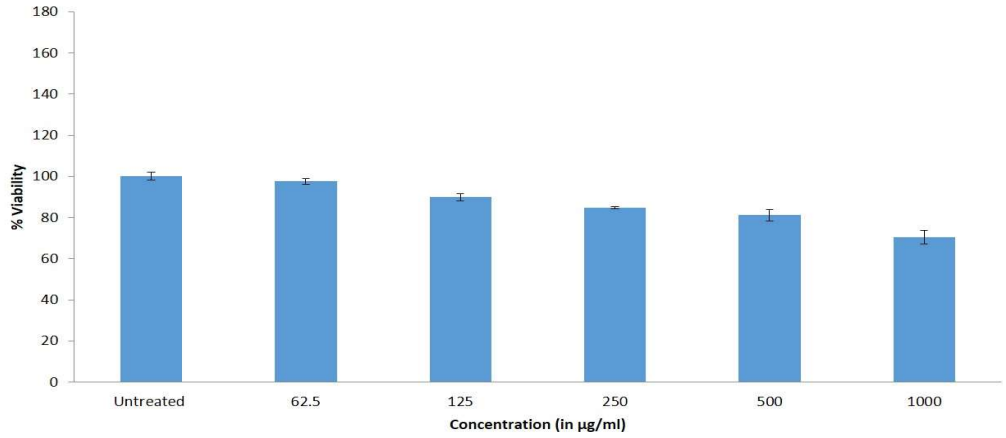



**Figure 4 : Effect of Livamrit A on HepG2 Cell line**

**DISCUSSION:**

The appearance of two crystalline phases of active ingredients namely Metacinnabar (HgS), and Hematite ($Fe_2O_3$) in Arogyavardhini Vati confirmed the presence of Shuddha Gandhaka and Lauha Bhasma in their composition as mentioned on the packets of the drugs. At the same time in Livamrit, no crystalline phases of concern are present. Singh *et al*., (2018) **[20]** have also confirmed the presence of HgS (Metacinnabar) and $Fe_2O_3$ (Hematite). HgS form of mercury is used in various ailments like nerve pain, and heart-related diseases mentioned in Ayurveda. Liu *et al*., (2008) **[21]** demonstrated that Metacinnabar has lower gastrointestinal tract reabsorption and has 1000 times less toxicity than methyl chloride. Hematite is also found safe at a small concentration for the human body **[22]**. The very less concentration of heavy metals like Lead, Cadmium, Zinc, and Copper indicates the safety of the selected Ayurvedic formulations from the metal toxicity however, this level may vary from batch to batch of the selected drugs as the metals are generally transferred from the soil, water, and environment to the plant materials. But the tested batch of the drugs is found safe in terms of metal toxicity. Agarwal *et al*., (2018) **[23]** also tested the heavy metal concentration of Arogyavardhini vati and found that the heavy metals concentration was under the permissible limit. The heavy metals concentration was tested to ensure safety aspects of the drugs from metal toxicity as a higher value of heavy metal like Cd may cause substantial toxicological consequences for human health because it can be deposited in the kidneys and lead to chronic re-absorption impairment **[24]**. Similarly, the higher levels of Lead (Pb) may cause acute toxicity and exert their impact on the nervous system, circulatory system, etc. **[25, 26]**. As long as Copper and Zinc are within the permissible limit they are utilized as essential elements for

the human body. The cytotoxicity study of both Ayurvedic preparations (Arogyavardhini vati and Livamrit A) demonstrated that both drugs considerably improve cell viability and proliferation in a dose-dependent manner. The Ayurvedic preparations used in this study are available in the market for the treatment of several liver diseases and are also used as a metabolism enhancer **[21]**. The Ayurvedic preparations (Arogyavardhini vati and Livamrit A) were found to be safe for liver or hepatic cells and did not cause cell death even at its highest tested concentration (1000µg/ml) for up to 24 hours.

## CONCLUSIONS

In our study both Arogyavardhini vati and Livamrit A have not exhibited heavy metal content higher than the permissible limit and also have not reduced the cell viability even at their highest concentration (1000ug/ml). Moreover, the appearance of two phases of HgS and Fe2O3 in XRD analysis of Arogyavardhini vati confirmed their inclusion of them in the formulation as mentioned on the pack as constituents. Very low concentrations (below permissible limits) of the tested heavy metals i.e. Cd, Pb, Zn, Cu indicated that both Ayurvedic preparations were prepared by following the standard Ayurvedic procedures. Therefore, both Arogyavardhini vati and Livamrit A Ayurvedic preparations are found safe agents in our study to be used for the said purposes. we also know that manufacturers companies also do quality control analysis but as demand is high, we have the right to check the quality of products. Also, we generate quality supporting data that may enhance their market values. However, the extensive study may further generate more data on this.

**Acknowledgement**

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