

## Review Article



## Toxicological Analysis of Herbal Drugs – A Review

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### ABSTRACT

This article is a review of the Analyses of the toxicity of herbal drugs based on the researches and journals published on this. 80% of the world's population uses herbal medicine for some aspect of primary health care. The medicinal and cultural acceptance of herbal drugs has been established since ancient time but often without any toxicological assessment. Phytochemical analysis revealed the presence of alkaloids, tannins, glycosides and flavonoids in most of the drugs. Inorganic analysis of drugs, physical, chemical and biological analysis of herbal products are done before validation. Adulteration and its adverse effects. Some elements are essential for plants but have harmful effects at excessive concentrations. quality of a herbal product must be assessed and the herbal drug must be accepted and validated by recognised organisations for the product to be sold in the market as a drug.

**Keywords:** Analysis of drugs, photochemical analysis, adulteration, herbal drugs and adverse effects.

### INTRODUCTION

Herbal drug preparations are obtained by subjecting herbal drugs to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal drugs, tinctures, extracts, essential oils, expressed juices and processed exudates. Physicians are comfortable with herbal therapies such as using aloe on burns or eating cranberries for urinary tract infections.

There are important similarities and differences between herbal medicine and conventional pharmaceutical-based medicine. Many of our pharmaceutical drugs still come from plant sources such as morphine.

Pharmaceutical drugs however, generally contain a single refined chemical that is either synthesised in a laboratory or extracted from plants whereas herbal remedies contain hundreds of compounds that are found in whole plant parts. Plant compounds include well known nutrients like vitamins and essential fatty acids, however they also contain beneficial compounds such as antioxidants, antimicrobials or anti-inflammatories.

#### Ancient uses of herbal drugs

The first written records detailing the use of herbs in the treatment of illness are in the form of Mesopotamian clay tablet writings and Egyptian papyrus.<sup>1</sup> Traditional medicine evolved over centuries, depending on local flora, culture, and religion. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants.<sup>2</sup> Due to poverty and limited access to modern medicine, about four billion people, 80% of the world's population, living in developing countries use herbal medicine as their source of primary health care.<sup>3</sup> Herbal medicine has evolved over millenia with human culture, however in the 20th century we saw a decline in

the use of herbs particularly in many industrialised countries as new pharmaceuticals were synthesised such as antibiotics and steroids. Even animals seem to know that certain kinds of herbs are good for them. When a chimpanzee has a parasite or stomach ache, for instance, he may dig up a plant called an asplia and chew on the root until he feels better. Use of plants for medicinal purposes is as old as human civilization and continuous efforts<sup>4,5</sup> are being made towards its improvement. Despite the growing market demand for herbal medicines, there are still concerns associated with not only their use, but their safety. Less than 10% of herbal products in the world market are truly standardized to known active components and strict quality control measures are not always diligently adhered to.<sup>6</sup>

#### Toxicity of herbs

Toxicity testing can reveal some of the risks that may be associated with use of herbs, therefore avoiding potential harmful effects when used as medicine. In addition, many plants produce toxic secondary metabolites as natural defence from adverse conditions. In some toxicologically and medicinally relevant plant species like *Digitalis purpurea*, *Hyoscyamus niger*, *Atropa belladonna*, *Physostigma venenosum*, *Podophyllum peltatum* and *Solanum nigrum*, these toxic substances are not distinguished from therapeutically active ingredients.

Being stationary autotrophs, plants have evolved different means of adaptation to challenging environments and co-existence with herbivores and pathogenic microorganisms. Thus, they synthesize an array of metabolites characterized as 'phytoanticipins' or as general 'phytoprotectants' that are stored in specialized cellular compartments and released in response to specific environmental stimuli like damage due to herbivores, pathogens or nutrient depletion.<sup>7</sup> Some of the phytochemicals produced by plants against



herbivorous insects also end up being harmful to humans, because highly conserved biological similarities are shared between both taxa as seen in most pathways involving protein, nucleic acid, 64 new insights into Toxicity and Drug Testing carbohydrate and lipid metabolism.<sup>8</sup> Human neurochemicals, often with similar biological functions are also reportedly present in insects.<sup>9</sup> These include signalling molecules, neuropeptides, hormones and neurotransmitters.<sup>10</sup>

Another implication in the toxicity of certain herbs is the presence of toxic minerals and heavy metals like mercury, arsenic, lead and cadmium.<sup>11</sup> Lead and mercury can cause serious neurological impairment when a herbal medicinal product contaminated with these metals is ingested. The presence of high levels of arsenic in kelp seaweed may result in toxicosis in some patients.<sup>12</sup> Herbs and herbal preparations can cause toxic adverse effects, serious allergic reactions, adverse drug interactions, and can interfere with laboratory tests.<sup>13, 14</sup>

### Quality control of herbal drugs

Quality can be defined as the status of a drug that is determined by identity, purity, content, and other chemical, physical, or biological properties, or by the manufacturing processes. Identity, content and purity are the three things taken into account before choosing a herb for manufacturing the herbal drug. Identity can be achieved by macro and microscopical examinations. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification.<sup>15</sup> Purity is closely linked with the safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. Analytical methods such as photometric analysis, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are not known. The source and quality of raw materials, good agricultural practices and manufacturing processes are certainly essential steps for the quality control of herbal medicines and play a pivotal role in guaranteeing the quality and stability of herbal preparations.<sup>16, 17, 18</sup> Good manufacturing procedures includes microscopic, physical, chemical, and biological analysis.

## PARAMETERS OF QUALITY CONTROL

### Factors affecting the quality of herbal drugs

Herbal ingredients of high quality should be free from insects, animal matter and excreta. It is usually not possible to remove completely all contaminants, hence specifications should be set in order to limit them:

**Ash values:** Incineration of a herbal ingredient produces ash which constitutes inorganic matter. Treatment of the

ash with hydrochloric acid results in acid-insoluble ash which consists mainly of silica and may be used to act as a measure of soil present. Limits may be set for ash and acid-insoluble ash of herbal ingredients.

**Foreign organic matter:** It is not possible to collect a herbal ingredient without small amounts of related parts of plant or other plants. Standards should be set in order to limit the percentage of such unwanted plant contaminants.

**Microbial contamination:** Aerobic bacteria and fungi are normally present in plant material and may increase due to faulty growing, harvesting, storage or processing. Herbal ingredients, particularly those with high starch content, may be prone to increased microbial growth. Pathogenic organisms including *Enterobacter*, *Enterococcus*, *Clostridium*, *Pseudomonas*, *Shigella* and *Streptococcus* have been shown to contaminate herbal ingredients. It is essential that limits be set for microbial contamination and the European Pharmacopoeia now gives non-mandatory guidance on acceptable limits.<sup>19</sup>

**Pesticides:** Herbal ingredients, particularly those grown as cultivated crops, may be contaminated by DDT (dichlorodiphenyltrichloroethane) or other chlorinated hydrocarbons, organophosphates, carbamates or polychlorinated biphenyls. Limit tests are necessary for acceptable levels of pesticide contamination of herbal ingredients. The European Pharmacopoeia includes details of test methods together with mandatory limits for 34 potential pesticide residues.<sup>20</sup>

**Fumigants:** Ethylene oxide, methyl bromide and phosphine have been used to control pests which contaminate herbal ingredients. The use of ethylene oxide as a fumigant with herbal drugs is no longer permitted in Europe.<sup>20</sup>

**Toxic metals:** Lead, cadmium, mercury, thallium and arsenic have been shown to be contaminants of some herbal ingredients. Limit tests for such toxic metals are essential for herbal ingredients.

**Radioactive contamination:** There are many sources of ionization radiation, including radionuclides, occurring in the environment. Hence, a certain degree of exposure is inevitable.<sup>20</sup>

**Other contaminants:** As standards increase for the quality of herbal ingredients it is possible that tests to limit other contaminants such as endotoxins and mycotoxins will be utilized to ensure high quality for medicinal purposes.<sup>19</sup>

### Screening of drugs

Phytochemical screening, which involves bioassays, extraction, purification, and characterization of the active constituents of pharmaceutical importance.<sup>21</sup> There are many pharmacological screening tests available.<sup>22</sup> Broad-based empirical screening, which is time consuming and expensive, can detect novel activities but is not suited for screening large numbers of samples.<sup>21, 22</sup>



## Phytochemical Screening of Herbal Drugs

This consisted of simple chemical test to detect the presence of the following phytochemicals: alkaloids, tannins, saponins, cardiac glycosides, anthraquinones and phlobatannins. Extracts were suction filtered until colourless filtrates were obtained necessary for colour reactions characteristic of these tests. The methods described by Harborne (1998) were used to ascertain the presence of alkaloids, cardiac glycosides and phlobatannins. The presence of saponins was detected using the screening procedures of Sofowora (1993), while tannins and anthraquinones were screened for using the method of Trease and Evans (1985).

## Safe dose (pre – LD50) Determination

Preliminary acute toxicity of the drug was tested in swiss albino mice using different doses. Six groups of four animals each, were given different doses of the crude extract at a range of 100 – 1400 mg/kg body weight respectively. The drugs were suspended in water and given intraperitoneally (ip) using sterile apyrogenic

disposable syringes. A control group was given normal saline (0.9% w/v NaCl) at 20ml/kg body weight. The mice were observed over 72 hours for clinical signs and mortality. Suitable doses were hence identified for each herbal drug and used in subsequent analyses.

## Physiological alterations on laboratory diagnosis in patients taking herbal drugs

Contrary to popular belief that herbal remedies are safe, severe organ damage and even death have been reported following ingestion of herbal remedies. Most common abnormality after use of herbal remedies is abnormal liver function test.

Liver damage is caused by herbal drugs like **kava kava** which is used for anxiety causes hepatotoxicity.<sup>23</sup> In addition to hepatitis and liver failure due to chronic use of kava, a recent publication reports a case of acute kava overdose resulting in altered mental status and ataxia similar to that seen with ethanol intoxication.<sup>24</sup> **Chaparral** which is available as capsules and tablets and is used as an anti-oxidant and anti-cancer herbal product. However, this product can cause severe hepatotoxicity. **Germander** has been used as a remedy for weight loss and as a general tonic. Several cases of liver toxicity have been reported in Europe due to ingestion of germander. **Mistletoe** is a parasitic evergreen plant that lives on trees such as oaks, elms, firs, pines, apple and elms. Mistletoe was used in the folk medicine as a digestive aid, heart tonic and a sedative. It was also used in treating arthritis, hysteria and other mental disturbances. It was also used in the treatment of cancer. The patient was diagnosed with a drug-induced hepatitis.<sup>25</sup>

## Kidney damage

In 1993, rapidly progressing kidney damage was reported in a group of young women who were taking pills

containing Chinese herbs while attending a weight loss clinic in Belgium. It was discovered that one prescription Chinese herb had been replaced by another Chinese herb containing aristolochic acid, a known toxin to the kidney.<sup>26</sup> There are several herbal supplements which are known to cause blood in urine (hematuria) and loss of protein in urine (proteinuria). Examples of these herbs are aloe juice from leaf, kava, saffron and many other herbs such as calamus, chaparral, horse chestnut seed and wormwood oil may cause nephrotoxicity.<sup>28</sup> Herbs such as asparagus roots, lovage root, parsley herb and root, watercress and white sandalwood should be avoided if you have any kidney problem.<sup>28</sup>

## Interaction of Warfarin with Herbal Supplements

Warfarin acts by antagonizing the cofactor function of vitamin K<sup>29</sup> supplements containing vitamin K such as green tea can antagonize the anticoagulant effect of warfarin

## TOXICOLOGICAL ANALYSIS

Heavy metal contamination, adulteration with Western pharmaceuticals and prohibited animal and plant ingredients are regularly reported in herbal remedies.<sup>30</sup>

## Microscopic examination

Primary visual evaluation, which seldom needs more than a simple magnifying lens, can be used to ensure that the plant is of the required species, and that the right part of the plant is being used. It is of prime importance, especially when different parts of the same plant are to be used for different treatments. Stinging nettle (*Urtica urens*) is a classic example where the aerial parts are used to treat rheumatism, while the roots are applied for benign prostate hyperplasia.<sup>31</sup>

## Determination of Foreign Matter

Herbal drugs should be made from the stated part of the plant and be devoid of other parts of the same plant or other plants. Animal matter such as insects and “invisible” microbial contaminants, which can produce toxins, are also among the potential contaminants of herbal medicines.<sup>17, 18</sup> TLC is often needed to detect the contaminants.<sup>32</sup>

## Determination of arsenic and toxic metals

In general, quantitative tests and limit tests accurately determine the concentrations of toxic metals in the form of impurities and contaminants. The latter are unavoidably present in the samples being tested i.e. herbal medicines and their herbal products.

## Determination of aflatoxins

Determination of aflatoxins should take place after using a suitable clean-up procedure, during which great care should be taken not to become exposed or to expose the working or general environment to these dangerous and toxic substances. Only products that have a history of aflatoxin contamination need to be tested. There are



specific sampling problems especially of aflatoxins due to the way in which contamination spreads, as described for some food commodities, such as nuts and corn. This may need to be taken into consideration when sampling, for example in terms of sample selection and sample size, and when the analysis is made.<sup>33,34</sup>

### Determination of radioactive contamination

Following a severe nuclear accident, the environment may be contaminated with airborne radioactive materials. These may deposit on the leaves of medicinal plants.

## ANALYTICAL METHODS

Published monographs in a pharmacopeia are the most practical approach for quality control of herbal drugs and there are many available.<sup>17,35</sup> Valuable sources for general analytical procedures are included in the pharmacopeias, in guidelines published by the WHO.<sup>36,37</sup> Qualitative and quantitative information can be gathered concerning the presence or absence of metabolites or breakdown products.<sup>37</sup>

### Validation is also important

If the herbal products are marketed as therapeutic agents, and irrespective of whether the products really have any positive effects to cure and reduce the severity of the disease, it is necessary to ensure scientific validation and periodic monitoring of the quality and efficacy by drug control administrators. It is feasible that the introduction of scientific validation would control the production of impure or adulterated herbal products and would eventually ensure their rational use. By definition, validation is the process of proving that an analytical method is acceptable for its intended purpose for pharmaceutical methods. Validation investigations must include studies on specificity, linearity, accuracy, precision, range, detection, and quantitative limits, depending on whether the analytical method used is qualitative or quantitative.<sup>38</sup>

### Determination of microorganisms

The total viable aerobic count (TVC) of the herbal material being examined is determined, as specified in the test procedure below, using one of the following methods: membrane-filtration, plate count or serial dilution. Aerobic bacteria and fungi (moulds and yeasts) are determined by the TVC. Usually a maximum permitted level is set for certain products, but when the TVC exceeds this level then it is unnecessary to proceed with determination of specific organisms; the material should be rejected without being subjected to further testing. Enterobacteria and certain other Gram-negative bacteria *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* are included as target strains of the test. Laboratory procedures investigating microbial contaminations are laid down in the well-known pharmacopeias, as well as in the WHO guidelines.<sup>17, 39</sup> The European Pharmacopoeia also specifies that *E. coli* and *Salmonella* spp. should be absent from herbal preparations.<sup>40</sup>

### Determination of pesticide residues

Chromatography (mostly column and gas) is recommended as the principal method for the determination of pesticide residues. These methods may be coupled with mass spectrometry (MS). Determination of total chlorine and phosphorus, chlorides, phosphates, organochlorine pesticides, esters of organophosphorus compounds, desmetryn, prometryn and simazine residues, pyrethroid insecticide residues.

### List of culture media and strains

Baird–Parker agar, Brilliant green agar, Buffered sodium chloride–peptone solution pH 7.0, Casein–soybean digest agar, Cetrimide agar, Cooked-meat medium, Defibrinated sheep blood agar medium (five per cent) (blood agar medium), Deoxycholate citrate agar, Enterobacteriaceae enrichment broth (Mossel), Kligler's iron agar (KIA), Lactose broth, MacConkey agar, MacConkey broth, Sabouraud glucose agar with antibiotics, Soybean-casein-digest medium, Tetrathionate bile brilliant green broth, Triple sugar iron agar, Violet-red bile agar with glucose and lactose, Xylose, lysine, deoxycholate agar, etc

### Adulteration

Foreign matter such as other parts of the same plant with no active ingredients, sand and stones, manufactured artifacts, and synthetic inferior principles are used as substitutes.<sup>41</sup> The practice of intentional adulteration is mainly encouraged by traders who are reluctant to pay premium prices for herbs of superior quality, and hence are inclined to purchase only the cheaper products. This encourages producers and traders to sell herbs of inferior quality. Physical factors such as air (oxygen), humidity, light, and temperature can bring about deterioration directly or indirectly.<sup>41</sup>

## CONCLUSION

There are various methods of analysis of toxic substances and side effects of herbal drugs which we use. A multidisciplinary team approach with pharmacist, chemical pathologist, scientific officer and physician may be appropriate to deal with toxicity and related problems due to use of herbal medicines.<sup>42</sup> Another important issue is to establish legal requirements for quality control of herbal remedies available to public.<sup>43</sup> Quality control for efficacy and safety of herbal products is of paramount importance.<sup>44,45</sup> The possibility of herb–drug interactions is important but “under-research” is an issue. The World Health Assembly in resolutions WHA31.33 (1978), WHA40.33 (1987), and WHA42.43 (1989) has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards.<sup>46</sup> The assurance of the safety of a herbal drug requires monitoring of the quality of the finished product as well as the quality of the consumer information on the herbal remedy.



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