Neurotoxicity of Polyherbal Formulations: Challenges and Potential Solutions

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Abstract Herbal products with potential therapeutic and nutritional values are gaining importance among people around the world. Herbal products are generally considered safe for human applications. Extracts from various herbal products or purified bioactive compounds are prepared and marketed in various forms.

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Polyherbal products are formulations with more than two herbal extracts. They are considered prophylactically or therapeutically effective on many occasions due to their complementary and/or potentiation activities due to each other's benefits. In most of the incidents, these herbal products have not been scientifically validated. Hence, patients and/or consumers are at risk for various adverse effects, leading to acute and chronic toxicity. General toxicity tests are generally conducted for herbal products; however, the neurotoxicity tests are not routinely conducted. Heavy metals such as lead, mercury, and arsenic present in the polyherbal products often cause severe neurotoxicity. The major challenge of using polyherbal formulations products for prophylactic and/or therapeutic purposes is the nonavailability of valid scientific information on the complete metabolite profile, human equivalent dose, potential adverse effects, and possible antidotes. In this chapter, we look into the potentially toxic substances present in the polyherbal products, and various general toxicity and neurotoxicity tests of the herbal products are discussed.

Keywords Herbal products · Neurotoxicity · Neurotoxin · Polyherbal formulation

Abbreviations

AAS	Atomic absorption spectroscopy
CNS	Central nervous system
GABA	γ-aminobutyric acid
GMP	Good manufacturing practice
HED	Human equivalent dose
JNK	C-jun-N-terminal kinase
MAPK	matrix metalloproteinase kinase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RT-PCR	Reverse transcription-polymerase chain reaction

1 Introduction

World Health Organization (WHO) estimated that approximately 80% of the global population relies on herbal-based medicines to treat various ailments. The current global market for the herbal formulation is UD$1.5 billion, and it is expected to grow exponentially due to the ever-increasing demand for natural remedies for preventing and treating various diseases. Herbal medicines are accepted as complementary alternative medicine worldwide and are generally considered safe (Yuan et al. 2016). Herbs/plants with ethnomedicinal values are continuously explored and well documented in the literature. Either single herbal or polyherbal formulations are used for the treatment of various diseases in various traditional treatment systems

such as Ayurveda, Unani, Siddha, and other traditional systems of medicine around the world (Pole 2006). Herbs/plants produce various secondary metabolites such as alkaloids, phenolic compounds, terpenes, catechols, flavonoids, stilbenoids, puerarin, baicalein, ginsenosides, saponin, phytosterols, tannin, and so on, to protect themselves from external adversaries/stimuli such as insects, microbes, and other living creatures. Medicinal plants contain significant quantities of some of these compounds belonging to flavonoids, alkaloids, isoflavanols, catechols, polyphenols, lignans, monoterpenes, triterpenes, quinones, coumarins, and lectins are responsible for exerting a beneficial role in herbal extracts (Song et al. 2012; Aslam et al. 2016; Konieczynski et al. 2018; Anand et al. 2019). The therapeutic efficacy of the herbal formulations depends on the quality and quantity of the bioactive compounds. Various advanced analytical methods like chromatography, spectrophotometry, and molecular fingerprinting are employed to determine these phytoconstituents. Apart from prophylactic and/or therapeutic properties, compounds present in certain herbs/plants also possess toxic substances that affect the various cells, tissues, or organs of the human body. The brain, spinal cord, and peripheral nervous system control the whole body. Some of the phytochemicals from herbal extracts cause potential structural and/or functional nerve/neuronal damages and are called neurotoxins. If the herbal extracts are not purified to remove the toxins and tested for its presence through valid and sensitive tests, there is a great possibility for the production of herbal medicines with potential toxins, including neurotoxins. Although regulations with respect to toxicity testing of herbal formulations are currently present, they are not strictly enforced by regulatory authorities and practiced by the manufactures. Hence, most of the currently available herbal formulations in the market are sold without complete testing details. Although a polyherbal formulation is therapeutically efficient unless scientific data on its safety is evaluated, it cannot be recommended for human or animal applications. Most of the polyherbal formulations are nontoxic at low concentration but at higher concentrations, there might be potential neurotoxicity.

2 Polyherbal Formulations

Herbal-based therapy is one of the oldest treatments for many diseases across the world. Formulations with more than two herbal ingredients are called polyherbal formulations (Parasuraman et al. 2014). More than one herbal bioactive compounds are used to prepare herbal formulation to increase the prophylactic and/or therapeutic efficiency. Most of the time, it has been noted that bioactive compounds from a single herb do not successfully show an effective therapeutic index (Petchi et al. 2014). When more than two herbal ingredients are added, better therapeutic efficacy is recorded due to the synergistic effect for most of the diseases (Spinella 2002). A combination of herbal extracts in a particular ratio provides an enhanced therapeutic effect than the individual herbal extracts (Sachdeva et al. 2013; Ghelani et al. 2014). Furthermore, when more than two herbal extracts are added, either therapeutic efficiency is increased, or toxicity due to any one of the herb is decreased

Table 1 Advantages and disadvantages of the polyherbal formulations

Polyherbal Formulations	
Advantages	Disadvantages
✓ Combination of herbal active ingredients may bind to more than one target to produce faster relief. ✓ Enhanced pharmacology actions ✓ The presence of multiple active compounds, hence higher therapeutic efficacy ✓ Reduce adverse effects due to a lower dose of the polyherbal formulation ✓ Helping patients to take a single dose of polyherbal formulation rather than taking many single herbal formulations to treat various ailments	• Incompatibility between herbal extracts in a polyherbal formulation may lead to precipitation of ingredients and instability during storage • Toxicity due to ingredients present in the herbal plant extracts affect the polyherbal formulation as a whole

(Parasuraman et al. 2010). Some of the herbal extracts used for treating central nervous system disorders are *Convolvulus pluricaulis, Ginkgo biloba, Bacopa monniera* (Kumar 2006). Neurodegenerative diseases are caused due to damage to the neurons in the central nervous system.

"Sarangdhar Samhita" is the concept of using two or more herbal extracts in an appropriate ratio for treatment. It has been well documented in the ancient Ayurvedic literature way back to 1300 A.D. When the polyherbal formulations are used for treatment due to their synergistic activity, pharmacokinetic (absorption, distribution, metabolism, and elimination) and/or pharmacodynamic (possible interaction between herbal drug) parameters are altered (Bhope et al. 2011; Karole et al. 2019; Srivastava et al. 2012). Many studies were conducted to study the herb–drug interactions and reported the safety of polyherbal formulation (Undale et al. 2014; Pandit et al. 2017); however, still, on certain occasions, the interaction between drug and herbal extracts showed adverse effects (Chavez et al. 2006). For example, turmeric (active ingredient is curcumin) is taken with black pepper and asafoetida to get the maximum immunity-boosting effect. Major advantages and disadvantages of polyherbal formulations over single herbal formulations are listed in Table 1, and some of the polyherbal formulations tested for various diseases are given in Table 2.

3 Toxicity of Polyherbal Formulation

Plants/herbs produce secondary metabolites as a natural defense mechanism (Wink 2003). When herbal extracts are used for prophylactic/therapeutic purposes with partial purification, these toxic metabolites cause adverse effects (Fatima and Nayeem 2016). Some of the secondary metabolites are synthesized by plants to kill the insects since few receptors are conserved across animals; the toxic effect is also shown in humans. Apart from bioactive compounds responsible for therapeutic potential, herbal extracts used for formulation contains other constituents, among

Table 2 Polyherbal formulations currently used for the treatment of various diseases

Plant species	Disease	In vitro tests	References
Glycosmis pentaphylla, Tridax procumbens, Mangifera indica	Diabetic mellitus	Acute toxicity Histopathological	Petchi et al. (2014)
Aegle marmelos, Benincasa hispida, Garcinia indica, Musa paradiasica, Rosa indica, Hibiscus rosa sinensis	Hypertension	Oral toxicity tests to the whole animal	Ghelani et al. (2014)
Adhatoda vasica, Glycyrrhiza glabra, Piper longum, Ocium sanctum, Zingiber officinale, Azadirachta indica	Anxiety	Oral toxicity and observations on elevated pulse maze, light/dark exploration test	Mathew and Babu (2011)
Curcuma longa, Ocimum sanctum, Murraya koenigii, Nyctanthes arbortristis	Hepatotoxicity	Acute, subacute, subchronic oral toxicity tests	Sachdeva et al. (2013)
Cicer arietinum, Phaseolus mungo, Mucuna pruriens, Triticum sativum, Allium cepa	Methionine supplement	Acute toxicity	Rajurker et al. (2009)
Bacopa monneiri, Evolvulus alsinoids, Acorus calamus, Saussurea lappa	Epilepsy	Acute toxicity, behavioral tests	Achliya et al. (2005)
Ginkgo biloba, *Beta vulgaris, Emblica officinalis*	Diabetic mellitus Cataract	Eye test (eye opacity)	Mahajan et al. (2018)
Withania somnifera, Nardostachys jatamansi, Rauwolfia serpentina, Evolvulus alsinoides, Asparagus racemosus, Emblica officinalis, Mucuna pruriens, *Hyoscyamus niger*	Schizophrenia/ psychosis/ bipolar disorder	Passive avoidance learning and elevated plus-maze model (EPM)	Shah and Goyal (2011)
Andrographis paniculata, Picrorhiza kurroa	Hepatotoxicity	Biochemical and histopathological tests	Okhuarobo et al. (2014)
Prunus amygdalus, *Arachis hypogaea, Citrullus lanatus*	Parkinson's disease	Assay for dopamine, lipid peroxidation, glutathione, and superoxide dismutase, histopathology of the brain tissue	Nandagopal and Ali Khan (2020)
Cichorium intybus, *Gymnema sylvestre*, *Nigella sativa*, *Trigonella foenum graecum*	Cardio-metabolic disorders	Measuring vasomodulatory effect	Malik et al. (2017)

which neurotoxins are potentially dangerous (Williamson 2017). In most cases, these neurotoxins are not correctly identified, and inadequate processing of the herbal extracts from various herbs for preparing polyherbal formulations and, at times, adulteration with other herbal extracts also introduce neurotoxins in the polyherbal formulations. Usually, more than one biological compound is present in the herbal extracts. Often, the concentration of the extract varies from one batch to the other depending on environmental factors under which the herbs are grown,

techniques of extraction, and storage conditions, which also contribute to the presence of toxic substances in the herbal formulation. Many polyherbal products are reported to possess side effects. The awareness and scientific analysis of their side effects are not studied yet.

There is a limited understanding of the interaction of herbal formulations with the drugs. Pharmacodynamics studies need to be conducted to understand the mechanism of herb-drug interactions (Ifeoma and Oluwakanyinsola 2013). WHO has formulated guidelines to be followed for herbal medicines, and this has been circulated to the manufacturers of herbal formulations. Noncompliance with these guidelines often leads to a problem of low-quality herbal formulation and subsequent adverse effects to the consumers and patients. Furthermore, WHO also formulated guidelines for the appropriate use of herbal medicines as a prophylactic and therapeutic agent. However, the Food and Drug Administration (FDA) has given strict guidelines to be followed for medications, which are not directly applicable to herbal formulations.

The general perception is that herbal formulations are safe and do not cause any adverse effects compared to allopathic chemicals. But many clinical reports revealed the toxic effects of herbal products due to the presence of various phytochemicals and heavy metals, which are present in the herbal formulations (Kabelitz 1998). Sometimes, the natural compounds from the herbal extracts are metabolized into toxic molecules after human consumption. Hence intermediate metabolic profiling is needed to identify the intermediate toxicity. The safety of herbal formulations, in general, is a big debate as continuous intake of herbal formulation has been reported with various toxicity issues. Some of the neurotoxic effects are convulsion, psychosis, encephalopathy, and neurovascular damages. Other in vitro and in vivo toxicity tests are to be conducted to identify and validate the safety of the polyherbal formulations. The testing of polyherbal formulations is challenging because of the presence of many compounds whose structure and assay procedure are not known. When the herbal extract is used as a whole, the neurotoxicity effect is not the same as purified compounds. As per the regulations, even a trace amount of heavy metals is not permitted as they are well known to cause adverse health complications. But in certain polyherbal preparations, metals are added intentionally to improve the therapeutic efficacy of the preparation (Dwivedi and Dey 2002). However, according to herbal experts, the presence of metals causes toxicities. Some of the heavy metals present in the polyherbal formulation are mercury, lead, arsenic, and cadmium, which causes neurological disturbances (Dargan et al. 2008). Certain herbal extracts contain a higher level of neurotoxic heavy metals (Ernst 2002). Therefore, polyherbal formulations are to be systematically tested for the presence of heavy metals, particularly lead and mercury (Choi 2005). Lead is assessed using sensitive techniques like AAS to detect the lowest possible level. The heavy metals cause neurotoxicity by altering the free radical production pathways to induce oxidative stress, which can induce neuronal damage. Reactive nitrogen species (RNS) and reactive species (ROS) are produced in excess and cause damage to the nervous system. Various types of toxicities caused by herbal formulations (Fig. 1).

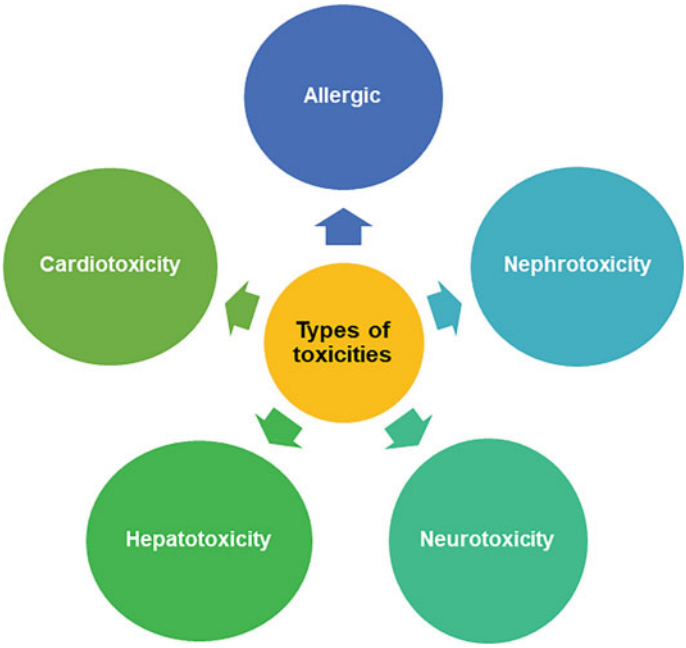

Fig. 1 Toxicities associated with medicinal herbal formulations

4 Neurotoxicity of the Polyherbal Formulations

Neurotoxicity refers to the study of anatomical (structural) and/or physiological (functional) alterations of the nerves/neurons in the central and peripheral nervous system. Due to the structural and complex network of the nervous system, toxic substances affect the various sites and cause different neurotoxicity. The various sites are the axons, dendrites, neuronal membranes, mitochondria, nucleus, and endoplasmic reticulum. The nervous system is one of the complex organs of the human body. It consists of different cell types such as neuron and gila, which makes two important parts, the central nervous system (CNS) and the peripheral nervous system (PNS). The functional molecules, neurotransmitters, such as acetylcholine, monoamines, glutamate, and GABA, play major roles in controlling nervous system activity. The cholinergic system is one of the most important neuromodulators which control the movement, cognitive, and physiological functions of the body (Kawashima et al. 2007). Any compound which binds to the acetylcholine receptor (nicotinic and muscarinic) affects the metabolism (synthesis/degradation), increases release, which can lead to an increase in the neurotransmission, which is referred to as cholinergic substances. The parasympathetic nervous system is part of the autonomous nervous system which is present in the peripheral nervous system. In the brain, there is a specific cholinergic tract, starting from the basalis meynert and extending to the cortex and hippocampus. Some of the neurotoxins present in herbal formulations are given in Table 3 and the corresponding structure in Fig. 2.

Table 3 Common neurotoxins present in medicinal herbal plants

Scientific name	Toxin	Mode of action	References
Coscinium fenestratum	Berberine	Deceases neuron density	Wattanathorn et al. (2006)
Vinca rosea	Vincristine	Inhibits anterograde and retrograde axonal transport	Nicolini et al. (2015)
Datura stramonium	Tropane alkaloid	Affects the central nervous system	Devi et al. (2011)
Atropa belladonna	Atropine (tropane alkaloid)	Affects central and peripheral nervous system	Kwakye et al. (2018)
Cannabis sativa	Delta-9-tetrahydrocannabinol	Interfere with psychomotor coordination	Rocchetti et al. (2013)
Veratrum nigrum L.	Veratridine	Toxic insult of peripheral nerves and affects MAPK pathway	Zhang et al. (2018)
Annona squamosa	Acetogenin	Causes neuronal cell death	Bonneau et al. (2017)
Ginkgo biloba	Ginkgo toxin	Inhibits glutamate decarboxylase and reduces GABA	Arenz et al. (1996)
Tanacetum vulgare	Thujone	GABA-A receptor antagonist	Zámboriné and Thi Nguyen (2020)
Digitalis purpurea	Cardiac glycosides	Inhibits Na^+/K^+-ATPase	Hauptman and Kelly (1999)
Rhododendron maximum	Andromedotoxin	Interfere with voltage-gated sodium channels	Moran et al. (1954)

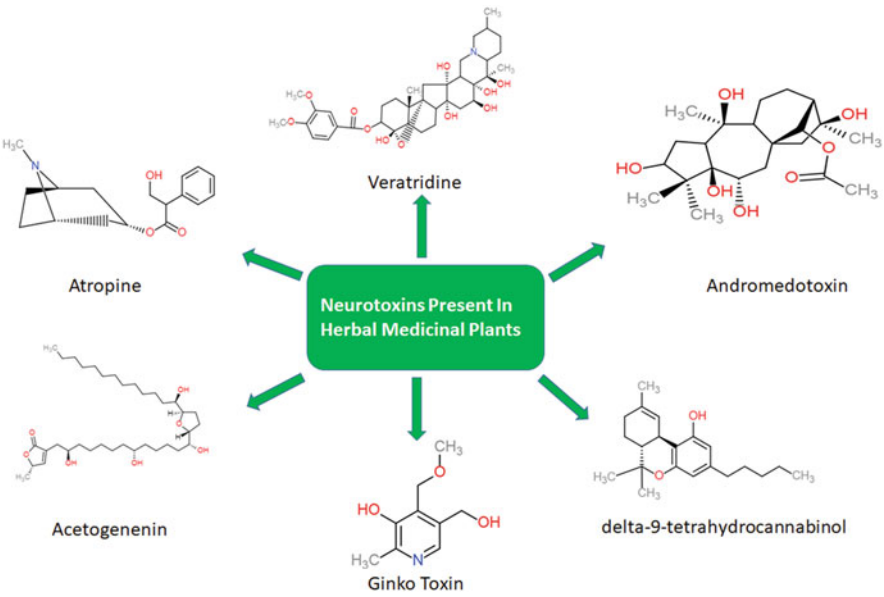

Fig. 2 Neurotoxins present in medicinal plants

Depending on the nature of the neurotoxin, it might cause acute or chronic neurotoxicity. When the polyherbal formulation is prepared with extracts having neurotoxin is consumed, typical symptoms of neurotoxicity can occur that include headache, impaired vision, fatigue, memory loss, sexual dysfunction, and numbness of limbs. Neurotoxins are those compounds that affect or damage the neurons and render them inactive. Otherwise, neurotoxins affect the brain functions and result in many psychological symptoms like anxiety, depression, headache, impaired vision, and limb weakness that occur to the persons who have taken the polyherbal formulations. Neuropeptide and neurotransmitters are inactivated because of the binding to the neurotoxins. Some of the alkaloids produced by plants are known to interfere with neurotransmitters in humans. The conserved nature of acetylcholine, an important neurotransmitter, is found in all life forms. The parameters to be measured for neurological tests for the central nervous system include motor activity, behavioral coordination, electrophysiological examinations, and sensory reflex responses.

5 Neurotoxicity Tests for the Polyherbal Formulations

5.1 In Vitro Neurotoxicity Tests

Toxicity testing of a polyherbal formulation is essential to know the presence of potentially toxic molecules and also determine the concentration of the herbal extracts that can be permitted to use as therapeutic substances. The results of the toxicity analysis at preclinical and clinical stages will help to take appropriate steps to either define the maximum limit or concentration that can be used to prepare the formulation and also chemical modification, or addition of suitable additives may be done to reduce the adverse effects. Commonly followed in vitro toxicity tests for herbal formulations are:

- Acute high-dose toxicity tests.
- Chronic low-dose toxicity tests.
- Histopathological studies.
- Cellular or organ-specific testing.
- Cytotoxicity tests.

The various in vitro toxicity tests are mentioned in Fig. 3**.**

Various factors that need to be considered while carrying the neurotoxicity tests of the polyherbal extracts are as follows: the age of the animal, relevant neurotoxicity biomarkers, a dose of the extract, and duration of the exposure. Acute toxicity effects of herbal extracts showed convulsions, whereas the subacute and chronic effects of herbal extracts showed encephalopathy and psychosis.

In vivo toxicity studies are carried out using whole animals. Four major tests under in vivo toxicity studies: (a) acute (b) subacute (c) chronic and (d) subchronic.

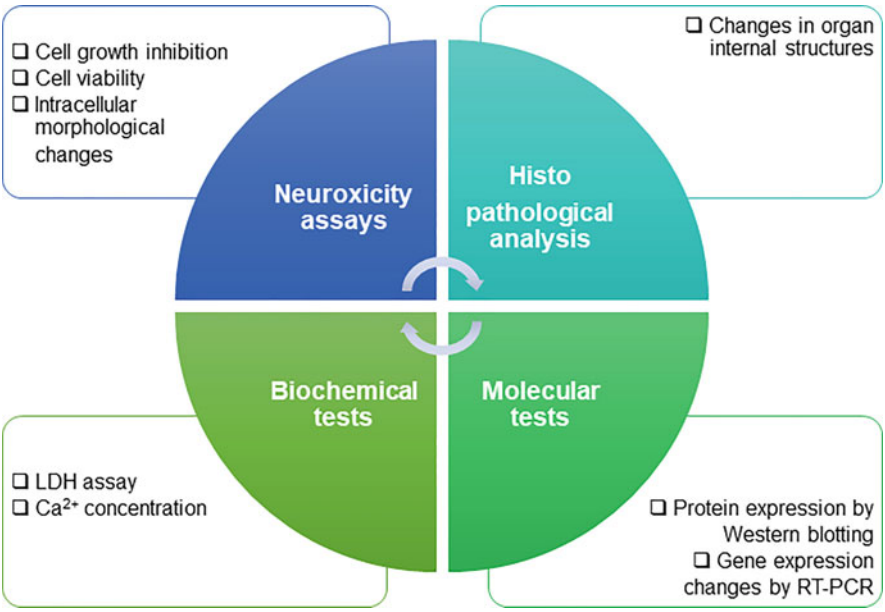

Fig. 3 Various in vitro and in vivo toxicity studies for polyherbal formulations

All these studies are performed to test the safety level of herbal formulations. At present, well-established experimental protocols are available, which are carried out in compliance with the regulatory guidelines. The findings of the in vivo experiments help to predict the possible adverse effects of herbal formulations in addition to information on safety dose for human use. Commonly used animal models are rat, mouse, and to some extent, hen and dog. The morbidity and mortality effects of herbal formulations were also investigated using in vivo studies. At the end of the experimental study period, the surviving animals are sacrificed. The vital organs such as the liver, kidney, brain, thymus, spleen, and lung are carefully removed and subjected to histopathological examination to assess the damages caused due to the herbal products (Saiyed et al. 2015; Aydın et al. 2016; Fonseca et al. 2018).

5.2 Cytotoxicity Tests

Cytotoxic tests are the first stage of testing the polyherbal formulations for any possible toxicity before proceeding to other tests such as the safety of the herbal formulation in whole animal models. Cytotoxic tests are performed with various cell types, which include regular as well as transformed cell types. The cytotoxic effect of herbal compounds is measured in terms of the viability of the cells, inhibition of cell growth detected through a change of cell membrane and intracellular structural deformities (Aslantürk 2018). The cytotoxic tests are an indispensable part of the

toxicity evaluation of any herbal formulation. Cytotoxicity analysis provides first-hand information on the safety of the polyherbal formulation and reduces animal usage, and it is easy to obtain results with high throughput screening. The most commonly used cell lines for toxicity analysis are mouse fibroblast cell lines and Syrian Hamster embryo cell lines. Some of the cell lines commonly used for neurotoxicity experiments are SH-SY5Y, PC12, and N27 (Heusinkveld and Westerink 2017).

5.3 Histopathological Tests

Neuropathology of neuronal cells provides evidence for the damage of nerve cells and their associated disruption of neural communication. Most of the time, it becomes difficult to directly associate behavioral changes with corresponding neural system damage that occur either in the CNS or PNS. Histopathological image analysis plays a major role in identifying the neurotoxicity of the toxins. To understand the neurotoxicity of the polyherbal formulation, brain tissue from the test animal after treating the formulation is collected, and hematoxylin–eosin staining is used to stain the brain samples. Different regions of the brains (nucleus accumbens, cortex, and hippocampus) are mainly analyzed as they control the memory and learning process of animals. The number of neurons is counted to know the extent of brain damage. Any change in the nerve cell membrane or other structures in these regions is also an indication of neurotoxicity. The behavioral changes are also associated with histopathological and neurochemical changes in the brain tissue. Accumulation of excessive protein and lysosomal bodies in the nerve cells indicates the neurodegenerative effect. The common neurotoxicity tests are qualitative and semiquantitative, and the results are recorded based on the extent of damage of the brain tissue and represented on a scale from 0 to 5; 0 for no damage, 1 for mild, 2 for moderate, and 3 for severe. Recently argyrophilic staining method was employed to carry out a quantitative analysis of the brain tissue damage with the help of automated computer software-based image analysis, and these types of advanced quantitative studies can be unbiased and are high-throughput to obtain neurotoxic effects quickly.

5.4 Biochemical Tests

Biochemical assays of some of the vital enzymes such as LDH and ions such as Ca^{2+} are measured and represented as indicators of toxicity. Blood samples are collected from the animals fed with polyherbal formulations to quantify biochemical parameters like aspartate transaminase (AST), alanine transaminase (ALT), creatinine level, bilirubin, and urea.

5.5 *Molecular Biology Tests*

Change in gene expression can be studied using the RT-PCR technique and western blotting techniques to know about the upregulation of genes and proteins in the test samples obtained from animals treated with polyherbal formulations. It has been proven that changes in gene expression occur upon neurotoxicity. Some of the most commonly recorded changes in protein expression are overexpression of matrix metalloproteinases (MMPs) and induction of mitogen-activated protein kinases (MAPK) pathway-related proteins. C-jun-N-terminal kinase (JNK) pathway, which is associated with the neurodegenerative disease, can also be studied, which provides more information on the neurotoxic effects of polyherbal formulations.

6 Animal Models for Neurotoxicity Tests

Animal behavioral studies provide scientific data on neurotoxicity and the safety level of polyherbal formulations (Kulig et al. 1996). As per regulatory guidelines, "animals" studies need to be conducted before recommending any polyherbal formulation for human applications. After obtaining toxicological and safety level data of the polyherbal formulation from animal models, the safe concentration of the herbal formulations has to be calculated according to the human body. Most of the time, body weight and body surface are taken into account for scaling the safety level of polyherbal formulation from animal studies. Various factors that affect the therapeutic and toxicity dose of polyherbal formulations are life span, body size, metabolic function, anatomical factors, physiological and pharmacological responses of animals. Hence, it is recommended to calculate the Human Equivalent Dose (HED) of herbal formulations through animal models such as rat, pig, rabbit, dog, and so on (Bae et al. 2015). Interpretation of animal dose and the human equivalent dose is an important criterion to be considered while formulating polyherbal formulations. It has been recommended that bovine serum albumin (BSA) can be taken as a factor to calculate HED.

7 Future Perspective and Conclusion

The use of herbal-based medicines and formulations is increasing in the form of alternative medicine to treat many human diseases. But considering the safety of the polyherbal formulations, further investigations following advanced techniques with sensitive equipment to detect the very low level of the herbal toxins are required. Purification methods need to be employed to remove the potential neurotoxins from the herbal extracts before it is used for preparing formulations. To increase the sensitivity of toxicity testing, a noninvasive method based on in vivo neurotoxicity

animal model is reported by genetically engineered mice with a molecular reporter gene. It may help to test the neurotoxicity of the samples through time-course analysis and also dose-dependent study using a lesser number of test animals. Quantification of heavy metals in traditional herbal preparation should be made compulsory, and the general public should be educated on the severity of heavy metal toxicity on the nervous system. Stringent regulations must be enforced to grant a patent on polyherbal formulation and issuing manufacturing licenses, including Good Manufacturing Practices (GMP).

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