Test for Non-Synergistic Interactions in Phytomedicine, Just as You Do for Isolated Compounds

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ABSTRACT: Phytomedicine has often been used as "alternative therapy," which in our opinion is unfortunate as it prevents its main actions being systematically studied, side effects explored, and toxicity tested, like all single-compound-based medicine. Our group is interested in finding which traditional or modern phytomedicines actually work and which are simply "working" through placebo, standardizing phytomedicine preparations, studying their toxicity, and finding active molecules in plants for modification and chemical synthesis as single compounds. Although fluctuation in efficacy due to seasonal and geographical variations in phytomedicine remains a concern, if well regulated, even plant extracts without isolated compounds can serve medicinal needs where single-compound options are currently not great. A potential concern with such phytomedicine is frequent mixing of ingredients in commercial formulations without test of synergism. Our study on the use of 2 traditional plants for Parkinson disease shows a clear lack of synergism, and to study nonsynergism better, we developed a new visualization approach. In this commentary, using our study on Parkinson disease as an example, we make a case for better evaluation of phytomedicines, especially testing for synergistic interactions. We also critique our own exploration of oxidative stress and few behavioral parameters alone to lay grounds for what we and hopefully others can do in future to extract more information from their phytomedicine studies. We hope this commentary acts as a good warning for anyone mixing 2 phytomedicines without testing.

KEYWORDS: phytomedicine, Parkinson disease, dopamine, *Withania somnifera*, *Centella asiatica*, drug discovery, oxidative stress, data visualization

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We strongly discredit the term "alternative medicine" and believe that there is no such entity as "alternative" medicine. Either a single isolated or synthesized chemical or natural product gives effective results, which can be developed as "medicine," or if it does not, then it cannot be developed as "medicine." All variants of medicine, irrespective of their sources, should be subjected to high standards of testing and then only be made available to a broader population. Although phytomedicine has a lot of potential and is widely used by large populations, a bypass of toxicity studies and standardization can cause more harm than good. We published a study on Parkinson disease (PD) and how 2 plant extracts can help ameliorate the symptoms in a nonsynergistic manner.[1] We use this commentary to highlight the 2 major imports of the study—need to study synergism and need for better visualization tools, outline its weakness of being centered around oxidative stress, and comment on the status of the field so that phytopharmaceutical research can be used better for finding medicines for PD.

Ethno- and phytomedicine scientists have generally focused on inflammation and antioxidant characteristics in drug discovery for all diseases. In the light of our advanced knowledge of the pathogenesis of diseases on a systems level, we need to broaden our field to encompass parameters and pathways on molecular, genetic, and epigenetic levels. Our field of phytomedicine should look toward the "omics" approaches and incorporate them to understand the larger picture. As plant-based molecules are usually multitargeted, "omics" studies will aid in decoding the exact molecular mechanisms of their action at different targets.

Loss of dopaminergic (DA) neurons in substantia nigra pars compacta (SNc) and the manifestation of proteinaceous inclusions, called Lewy bodies, leading to akinesia, bradykinesia, and impaired balance, are common features of PD.[2] Multiple animal models are available to study the pathogenesis of PD and aid in the development of therapeutic strategies, although none of them fully mimic PD in humans.[3] 6-hydroxydopamine (6-OHDA), MPTP, paraquat, and rotenone are the most widely used toxin models. 6-hydroxydopamine is a unilateral model, which causes loss of DA neurons in SNc without the formation of Lewy bodies and needs to be injected into the brain. MPTP, rotenone, and paraquat are similar in action as they block the mitochondrial electron transport chain through inhibition of complex I. Although rotenone imitates almost all features of PD and has flexible administration, it is difficult to replicate owing to high mortality. Paraquat forms Lewy bodies and causes loss of DA neurons, but there are contradictory claims regarding its specificity for DA neurons and effects on nigrostriatal DA system. MPTP models produce lesions, DA neuron loss, and nerve terminal degeneration and can cross the blood-brain barrier, but it does not form Lewy bodies and

exhibit typical PD behavior. Drugs inducing antioxidant effects might be a success in the MPTP model. Gene models are generally employed to analyze familial PD, shed light on PD pathways, and use as therapeutic screens. α-Synuclein, PARKIN, LRRK2, DJ-1, and PINK1 are the main PD gene models. Multiple gene functions might be hampered in common PD cases; hence, monogenic models are generally less effective for drug discovery than toxin-induced models. For drug discovery, we use toxin-induced models.

Reviewing the phytomedicine literature, there exist a plethora of studies on plant extracts showing effective amelioration in PD models. The lack of follow-up to these primary studies is a glaring issue that deprives the scientific world of potential breakthroughs in the field of PD drug discovery. There is an immediate need to further delve into these plant extracts to isolate and characterize their active molecules and critical interactions, assuming these scientific studies are in fact making valid claims. Crude extract might perform better than single isolated molecules due to the presence of synergistic or catalytic interactions of multiple molecules in extract; hence, studying and also modeling mechanisms are essential.[4] Due to multitargeting properties of most natural products, there is also a need to develop PD models incorporating multiple genes along with an effective neurotoxin. Other crucial aspects, often neglected in phytomedicine, are toxicity studies and well-structured clinical trials. Natural products have been known to induce genotoxicity and other forms of acute and chronic toxicity.[5,6] Thus, omitting toxicity studies under the garb of apparently "safe" herbal products is a great disservice. Incompatible adjuvants in drug formulation might also induce toxicity or reduce efficiency; hence, appropriate interactions need to be investigated. There is a chasm between the pharmacological studies in drug discovery from plant sources and single-compound work, which causes potentially more effective drugs from plant sources to be discredited as "alternative" therapy. We support the necessity of standardizing all medicines and subjecting them to rigorous examination.

Levodopa, the most widely prescribed drug for PD, poses long-term risks and is unable to stop degeneration of DA nigral neurons.[7] Both *Withania somnifera* and *Centella asiatica* have been studied to alleviate symptoms in different models of PD.[8,9] MPTP damages the nigrostriatal DA pathway. MPTP is a lipophilic precursor of neurotoxin 1-methyl-4-phenylpyridinium (MPP+), which on entering the mitochondria exploits the mitochondrial transmembrane potential and blocks the electron transport chain by inhibiting mitochondrial complex I.[10] We employed MPTP-induced PD model to study the behavioral and antioxidant effects of these plant extracts in isolation and in combination. We used the combination of the extracts to assess additive properties if any. While both drugs worked well individually to restore antioxidant balance and improve motor skills, their combination never outperformed them and even performed worse than the individual components in some parameters, refuting any claims of additive properties. The presence of similar active ingredients, ingredients acting on the same pathway, or counteraction of synergistic action with antagonistic action of their active components could be attributed to this phenomenon. While withaferin and withanolides in *W somnifera* and triterpenes in *C asiatica* are largely believed to be the primary active components for several neuronal effects, we could not carry out molecular investigation in our study. We suspect that we would find novel molecules, which can easily be synthesized and modified into even more effective compounds than found in these plants.

There is a standard practice of comparing each group with a single "control" group, which we have largely modified in the form of complete comparison display (CCD). Stringent statistical one-to-one testing was done to compare probability of each pair to be similar (or different) and assign significant values. This one-to-one comparison should not be confused with group comparison, which involves correction for multiple comparisons. We created a ladder-like visualization of comparisons of all groups one by one with the rest. With the advent of big data and network approaches where multiple groups affect each other, we believe that CCD will empower better analysis of results and effective visual representation.

Our results demonstrating antagonistic effects of the plant extracts warn against random mixing of herbal components in the name of supplements. Combining herbal extracts without in-depth investigation of their components and interactions could pose a health hazard or simply have a placebo effect, which still cheats the consumer physiologically and financially. We want to stress the fact that "natural" does not imply "safe," and it is essential to proceed with caution. Toxicity studies, as carried out with single-molecule drugs, are vital to formulate standardized medicines. The soil content and environmental conditions play a significant role in altering the composition of the plants cultivated, and chemical profiling of the plants with subsequent standardization should be carried out. Structured clinical testing and transparency of data should follow molecular investigations for the natural product–based drug to be widely accepted as a modern drug.

Several medicinal plants are claimed to possess therapeutic properties, and as with modern drugs, their consumption in the absence of disease has deleterious effects.[11] It is thus necessary to proceed with caution in the supplements sector. The trend of passing off phytomedicine as supplements without due toxicity studies and required vigilance puts a large number of consumers at serious risk. We hope drug regulatory agencies of various countries expand their scope to include phytopharmaceuticals. Toxicity studies and regulations are crucial and should be pushed for in the supplements sector with equal force as for the rest of the pharmaceutical industry. Paucity of appropriate investigations by unscrupulous researchers has led to a potential

goldmine for drug discovery to be used inappropriately as unregulated supplements.

Some plant-based substances have exhibited potent anti-PD activity. Levodopa in *Mucuna pruriens*, anticholinergics in *Datura stramonium*, monoamine oxidase (MAO) inhibitor activity in *Banisteria caapi*, and dopamine agonist activity in *Claviceps purpurea* have been identified. Olive oil, curcumin from *Curcuma longa, Convolvulus pluricaulis, Nardostachys jatamansi, Ginkgo biloba*, and *Bacopa monnieri* are some of the promising candidates for anti-PD drug discovery in the light of currently available data.[12] Exploration of marine sources might also be a profitable venture due to highly potent molecules produced by marine organisms owing to the stressful and dynamic conditions in the marine ecosystem.[13] Primary studies for herbal drugs showing significant neuroprotection must be carried forward to identify key molecules and interactions, chemical profiling, toxicity studies, drug formulation, and systematic clinical trials with appropriate permissions to qualify for use as "medicine" and not be lost in the sea of "supplements" or "alternative medicine."

Author Contributions

AP and SK wrote the paper with inputs from the other authors.

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