***Review article***

# Homeopathic drug standardization through biological evaluations: An untrodden avenue

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## Abstract

**Background:** There is a dearth of chemico-analytical or instrumental methods for standardization and quality control of higher dilutions of homeopathic drugs.

**Aim:** This review highlights the challenges in standardization of anti-inflammatory homeopathic drugs and suggests a battery of biological assays for their standardization.

**Methods:** We retrieved a total 57 scientific reports from the experimental studies and scientific reviews published between January 1999 and June 2014 related to anti-inflammatory homeopathic drugs and their high dilutions. These comprised of 18 reports on preclinical evaluation, 15 on source materials, 9 on isolated constituents and 15 studies on *in-vitro* experiments. Few recent citations which supported the initial studies were added later during the compilation of the manuscript.

**Conclusion:** Standardization and quality control of homeopathic mother tinctures and high dilutions warrants an urgent attention. As biological activities are observed to be attributed to the high dilutions which are practically devoid of active ingredients, their standardization may be done through the suggested battery of biological investigations. It is suggested that the current methods of standardization of homeopathic drugs need to be upgraded to include sensitive, reproducible and relevant biological assays so that the end users are assured of the quality, efficacy, and safety of homeopathic dilutions.

**Keywords:** Homeopathy, Drug standardization, High dilution, Mother tincture, *in vitro* test, *in vivo* test

## Introduction

Homeopathic medicines include any drug which are prepared according to methods endorsed in homeopathic pharmacopeias. Their therapeutic efficacy is established through clinical use, experience as recorded in authoritative homeopathic literature and to some extent by means of research. Homoeopathy is one of the most widespread and controversial form of complementary and alternative medicines.[1,2] Therapy with homeopathic drugs is based on the principle of '*like cures like*', according to which a medicine capable of causing certain



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symptoms in healthy volunteers may be used in minute doses to cure the similar symptoms and signs. Homeopathic medicines are prepared by vigorous agitating/shaking in a step wise manner known as potentization. The process of potentization is supposed to make the drug suitable to be given to an organism.[3] Homeopathic practice includes the use of potentized drugs routinely in high dilutions.[4, 5] Various authorities have proposed certain pathways to explain the action of high dilutions.[6-10]

Apart from controversies related to high dilutions and mechanisms of action, a major concern with homeopathy is lack of strict quality control measures and validated markers which may be correlated with the biological efficacy. The issue of standardization is complicated due to vast diversity of the sources used in the preparation of high dilutions.[11] The monographs included in the pharmacopoeias of various countries prescribe dissimilar specifications and methods of preparation for the same drugs. This further adds to the inconsistency in the quality and efficacy of homeopathic drugs.[12]

For standardization and quality control of the mother tinctures of homeopathic drugs modern analytical methods including chromatographic techniques are used. However, even advanced chemical and analytical assays prove to be incompetent in standardization of the high dilutions devoid of well-defined active principles[13] hence, the standardization of high dilutions becomes an insurmountable challenge. Bioassays are used for the standardization of the drugs for which sensitive chemical or analytical assay methods are unavailable. Certain homeopathic mother tinctures and lower dilutions containing substantial amounts of source material are standardized using bioassays.[14]

Technological advances and deeper understanding of the disease pathogenesis provide an unprecedented opportunity to standardize the homeopathic drugs including high dilutions. In this review, the challenges related to the standardization of homeopathic medicines are summarized. Certain validated biological assay methods are suggested for the biological standardization of anti-inflammatory homeopathic medicines including high dilutions.

## Search Methodology

Database searches using search engines like Google Scholar, Pubmed, and Science Direct were conducted to include the scientific publications starting from year 1999 up to July 2014. The search was limited to English language papers. For data mining, following MeSH words were used: Homeopathy, Homeopathy AND anti-inflammatory, Homeopathic prevalence, Homeopathy AND Medication, Drug standardization, High dilution, Mother tincture, *in vitro* test, *in vivo* test. Animal origin homeopathy, Plant origin homeopathy, Mineral origin homeopathy, Homeopathy AND *Arnica Montana*, *Thuja occidentalis*, *Atropa Belladonna*, *Hamamelis virginiana*, *Aconitum napellus*, *Bryonia alba*, *Asafoetida*, *Ipecacuanha*, *Toxicodendron pubescens*, *Apis mellifica*, *Lachesis muta*, *Arsenicum album*, Phoshorus, Urshiol, α-thujon, β-Thujon, Fenchone, Atropine, Hamamelitannin, Helenalin, Sesquiterpene lactones (SL), Homeopathy PLA254, Homeopathy Bryonin, Homeopathy cucurbitacins. Homeopathy AND animal drug



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standardization, Homeopathy AND plant drug standardization, Homeopathy AND mineral drug standardization.

In almost all the cases, the original articles were obtained and the relevant data was extracted. The data was studied to determine current mode of standardization of homeopathic drugs, diversity of source materials of homeopathic drugs, challenges associated with standardization and quality control of the homeopathic drugs sourced from different origins, reports on the biological testing of the anti-inflammatory homeopathic drugs, and the biological assays which can be implemented in the standardization of the homeopathic drugs including their high dilutions. Various aspects of anti-inflammatory homeopathic medicines like presence of active constituents and experimental proving of anti-inflammatory efficacy of homeopathic drugs have been tabulated to highlight the present status of knowledge on anti-inflammatory homeopathic drugs and their standardization.

## Anti-inflammatory homeopathic medicines

Modern anti-inflammatory medicines like non-steroidal anti-inflammatory drugs (NSAID's) are extensively used in the treatment of inflammatory conditions. However, their long-term use produces obnoxious and severe side effects.[15] Interestingly, homeopathy plays a pivotal role in the treatment an anti-inflammatory conditions. Multiple experimental studies have revealed that the homeopathic drugs and their high dilutions possess significant anti-inflammatory effects. Drugs like *Rhus toxicodendron, Arnica montana,* and *Thuja occidentalis* have been proved to possess anti-inflammatory actions in preclinical experimental models.[12] With an increase in the demand of anti-inflammatory homeopathic medicines, health authorities and consumers are concerned about their efficacy and safety.[16] Homeopathic medicines are often contemplated to present no major safety concerns. Still, there are a few aspects of the production of homeopathic medicines that could comprise potential safety concerns.[16]

Table-1 summarizes the homeopathic anti-inflammatory drugs from plant, animal, and mineral origins along with the dilutions for which the activities have been proved *through in vitro* and *in vivo* biological assays. The reproducibility of such experimental evidences has been recently reviewed by Endler et al. 2010. The efficacy of homeopathic *Apis mellifica* as an inhibitor of human mast cell degranulation is repeatedly proved through independent studies and the *in vitro* assay of basophil degranulation is reported to yield reproducible results in 13 out of 17 studies.[17] The data indicates that the basophil degranulation and histamine release assays may have a role in proving the efficacy of not only the homeopathic drug dilutions but also the high dilutions of a structurally well-defined chemical like histamine.[18] However, till date there have not been systematic efforts to use such validated assays in the standardization of homeopathic drugs and their dilutions.

**From plant origin**

| Sr. No | Name of homeopathic drug | Dilution used | Reported biological activity | Assay methods | Biological name and family | References |
|---|---|---|---|---|---|---|



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| | | | | | | |
|---|---|---|---|---|---|---|
| **1** | *Arnica* | 6cH | Anti-inflammatory | ▪ Carrageenan-induced rat paw edema,<br>▪ *In-vitro* model of Human umbilical Vein endothelial cells and *in vivo* model skeletal muscle regeneration. | *Arnica Montana (Asteraceae)* | [21,22,23] |
| **2** | *Thuja* | 19dH | Anti-inflammatory | ▪ Canova treated human macrophage co-cultured with human lymphocytes (Inhibition of proliferation) | *Thuja occidentalis (Cupressaceae)* | [24] |
| **3** | *Atropine* | 4dH | Anti-inflammatory, Anti-peritonotis | ▪ Carrageenan induced paw edema in rats<br>▪ Experimentally induced peritonitis in mice | *Atropa belladonna (Solanaceae)* | [25,26] |
| **4** | *Hamamelis* | 4dH | Anti-inflammatory | ▪ Carrageenan-induced paw edema in rats | *Hamamelis virginiana (Hamamelidaceae)* | [25] |
| **5** | *Aconite* | 20dH | Immune response Modifiers | ▪ Lymphocyte proliferation stimulated by activated *Cebus paella* | *Aconitum napellus (Ranunculaceae)* | [27] |
| **6** | *Bryonia* (As a component of Canova) | 14dH | Immune response modifiers | ▪ Peritoneal macrophages cell culture<br>▪ Sarcoma 180-bearing mice | *Bryonia alba (Cucurbitaceae)* | [28] |
| **7** | *Asafoetida* (As a component of Canova) | 20dH | Immune response modifiers | ▪ Lymphocyte proliferation stimulated by activated *Cebus paella* | *Ferula foietada (Apiaceae)* | [27] |
| **8** | *Ipecac* (As a component of Canova) | 13dH | Immune response modifiers | ▪ Lymphocyte proliferation stimulated by activated *Cebus paella* | *Ipecacuanha (Rubiaceae)* | [27] |
| **9** | *Rhus Tox* | 4X, 30X, 30cH and 200cH | Anti-inflammatory | ▪ Carrageenan- induced paw edema in rats<br>▪ Mouse articular chondrocytes | *Toxicodendron pubescens (Anacardiaceae)* | [29,30,31] |
| **Animal origin** | | | | | | |
| **10** | *Apis mellifica* Western honey bee, European honey bee | 9cH, 3cH, 5cH, 7cH | Immune response modifiers | ▪ Degranulation of human basophils<br>▪ Gene expression by transcriptomic analysis on RWPE-1 cell line | *Apis mellifica (Apidae)* | [32,33] |
| **11** | Venomous pit viper | *6dH, 30dH* | Anti-inflammatory | ▪ Carrageenan induced edema<br>▪ Autologous blood induced edema | *Lachesis muta (Viperidae)* | [25] |
| **12** | Thymulin | 5cH | Anti-inflammatory | ▪ *Leishmania (L.) amazonensis*- induced inflammation | *Thymus extract* | [34] |
| **From mineral origin** | | | | | | |
| **13** | Arsenic trioxide | 30cH | ROS scavenger | ▪ *In-vitro* antioxidant activity | *Arsenicum album* | [35] |
| **14** | *Phosphorus* | 6x, 30x | Anti-inflammatory | ▪ Carrageenan-induced paw edema in rats<br>▪ Autologous blood induced edema | *Phosphorus* | [25] |
| **15** | *Antimonium crudum* | 30cH | Anti-inflammatory | ▪ *Leishmania* (L.) *amazonensis*-induced inflammation | *Sulphide of Antimony* | [36] |
| **16** | *Causticum* | 6cH, 12cH,30cH | Anti-inflammatory | ▪ Carrageenan-induced rat paw oedema | *Potassium Hydrate* | [37] |
| **17** | *Dexamethasone (synthetic)* | 7cH, 15cH | Anti-inflammatory | ▪ Carrageenan-induced rat paw oedema | - | [38] |



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**Table – 1**: Homeopathic drugs and their dilutions reported to possess anti-inflammatory activity in experimental models

The biological testing of homeopathic drugs and particularly of their high dilutions focuses on the proving of their efficacy and not detailed mechanisms of actions. However, parallel advances in the processes of extraction/isolation technologies, modern analytical methods and sensitive biological assay methods have contributed to the generation of data on the active constituents of source materials used for the preparation of the homeopathic drugs. Table-2 presents examples of the source materials of homeopathic drugs, their active constituents and reports on the experimental proving of the efficacy of such active constituents. It is noteworthy that similar experimental models have revealed the anti-inflammatory activity of the homeopathic high dilutions of these drugs (Table 1, Table 2 and Table 3).

| Plant Name | Active constituents | Quantity reported | Reported Biological Activity |
|---|---|---|---|
| ***Toxicodendron pubescens (RhusTox)*** | Urshiol | 1.2-1.7 g/ pound [39] | Anti-oxidant and natural killer cell activity in non-alcoholic fatty liver disease of rat [40] |
| | | | Involvement of IFN-γ, TNF-α in contact hypersensitivity induced by urushiol [41] Wakabayeshi |
| | | | Contact with Urshiol causes epederma mediated by IL-lα ,IL- 1β and TNFα transcription [42] |
| ***Thuja occidentalis*** | α-thujone, β-Thujone, Fenchone | 65% Thujone and 8% isothujone (Essential oil from leaves) [43,44] | Stimulate the cell-mediated immunological response in normal and tumor bearing Balb/c mice[45] |
| ***Atropa belladonna*** | Atropine | Up to 0.5 % (In leaves) [26] | Atropine inhibited the antibody and T-cell proliferative responses also suppressed the turpentine-induced leukocytic infiltration and tissue injury, inhibited chemotaxis of leukocytes toward chemo-attractant [46] |
| ***Hamamelis virginiana*** | Hamamelitannin | Up to 0.5 % (In leaves) [47] | Inhibition of TNF-α induced cell death [48] |
| | | | Oxygen scavaging activity by protection of cell damage induced by active oxygens [49] |
| | | | Protective activity on cell damage of murine skin fibroblasts induced by UVB irradiation [50] |
| ***Arnica montana*** | Helenalin | 5.2-10.3 mg/g; 0.4% [51] | Inhibition of NF-κB DNA binding in Electrophoretic Mobility shift assay and Inhibition of NF-KB-Dependent Gene Expression [52] |
| | | | Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells [53] |
| | | | Inhibition of human neutrophil migration and chemotaxis [54] |
| | | | Inhibition of Carageenan-induced inflammation and the chronic adjuvant-induced arthritis [55] |
| | | | Anti-inflammatory and cytotoxic effects Jurkat T cells and human peripheral blood cells [56] |
| | | | Anti-inflammatory activity by inhibition Transcription Factor NF-kB by directly targeting p65 in Jurkat T cells [57] |
| ***Lachesis muta*** | $PLA_2$ | 0.8 and 0.2% [58] | Modulation of natural killer activity of lymphocytes as a protein kinase C effector [59] |
| | | | Induction of hind paw edema by Lachesis muta venom [60] |
| ***Bryonia alba*** | Bryonin, cucurbitacins | 0.875% [61] | Inhibition both TNF-α and IL-1 β production in LPS-stimulated RAW 264.7 cells [62] |



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| | |
|---|---|
| | Inhibition of the activation and proliferation of activated mouse lymphocytes and expression of inflammatory cytokine [63] |

**Table – 2**: The active constituent of homeopathic drugs and their quantity reported

| Source | Animal model/ Cell line | References |
|---|---|---|
| ***Toxicodendron pubescens (RhusTox)*** | Mouse articular chondrocytes | [29] |
| | Carrageenan induced paw edema in rats | [ 29, 30] |
| | Freund's adjuvant induced arthritis in rats | [31] |
| ***Thuja occidentalis*** | Lymphocyte proliferation assay | [24] |
| ***Atropa belladona*** | Experimentally induced peritonitis in rats | [26] |
| | Carrageenan induced paw edema in rats | [25] |
| | Turpentine-induced leukocytic infiltration | [46] |
| ***Hamamelis virginiana*** | DPPH (1, I-diphenyl-2-picrylhydrazyl) assay | [66, 49] |
| | TNF-α induced endothelial cell damage | [48] |
| | UVB radiation induced mouse skin fibroblast cell damage assay | [50] |
| ***Arnica montana*** | Inhibition of transcription factor NF-kB by targeting p65 gene and inhibition of neutrophil elastase | [57, 67] |
| | Anti-inflammatory activity in Croton oil induced ear inflammation in mice | [68] |
| | Inhibition of Carrageenan-induced and Nystatin-induced rat paw oedema | [21] |
| | Inhibition of release of inflammatory mediators from J774 murine macrophage cells | [69] |
| | Inhibition of contact hypersensitivity in mice | [70] |
| ***Lachesis muta*** | Inhibition of Irradiation induced scleroderma | [71] |
| | Modulation of natural killer cell activity in human peripheral blood lymphocytes | [59] |
| | *Lachesis muta* venom- induced hind paw oedema | [60, 72] |
| ***Bryonia alba*** | Inhibition of nitric oxide release from mouse peritoneal macrophages culture | [73] |
| | Sarcoma 180-bearing mice | [28] |

**Table 3:** Reports on Anti-inflammatory activity of source materials of homeopathic drugs

The presence of anti-inflammatory constituents in the lower dilutions of anti-inflammatory homeopathic drugs can be easily detected using chemical or instrumental analysis and hence, the efficacy of lower dilutions of these homeopathic drugs can be attributed to the presence of a group of active constituents. However, proving the presence of active constituents only in mother tinctures can't resolve the issue of standardization of homeopathic drugs. The homeopathic drugs are administered in the form of higher dilutions which are devoid of any active principles. To standardize such high dilutions, detection and quantification of the known active principles in them is necessary. However, there is a dearth of chemical or instrumental analytical methods that can detect very minute quantities of the active principles present in homeopathic dilutions beyond 6cH dilution ($10^{-12}$ dilution). As shown in Table 2, the source material of the homeopathic drugs contains substantial amount of active ingredients and at least lower homeopathic dilution, even in very minute doses, may still contain detectable amounts of active principles. There is a possibility that the observed effects of homeopathic medicines may correlate with such small amount of very potent active principles. However, there is a need of further investigations to establish whether the active principle administered at such low doses can exert any significant biological effects.



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In table 2, the approximate content of active constituents of the source materials of respective homeopathic drugs is given. If the source material contains 1% active constituents having a molecular weight of 500 Daltons, then it can be calculated using Avogadro's number that, in 100 ml of 6cH dilution, there are at least $10^6$ molecules of active constituents. Though, it is accepted that the recipients of homeopathic therapy are rarely exposed to quantifiable amounts of the drug dilutions, and hence, it is practically impossible to prove whether even few molecules of the active principle really enter the recipient's body. There are reports on the experimental evaluations of anti-inflammatory activity of the high dilutions of homeopathic medicine. However, the quantification of particular active constituents is not possible in such dilutions. Further, to accept this hypothesis of presence of active components in the high dilutions, there is a need of judicious experimentation to substantiate the reproducibility of the results.

## Current status of homeopathic drug standardization

Homoeopathy is currently used in over 80 countries and in 42 countries it is legally recognized as a system of medicine. In 28 countries, it is regarded as a part of complementary and alternative medicines.[72] Such a vast use of homeopathic drugs highlights the importance of their quality, safety and efficacy related issues.[72] Not all the homeopathic medicines are administered as high dilutions. Sometimes, a lower dilution like mother tincture is also administered to patients. In such cases, the process of manufacturing and the content of harmful ingredients need to be validated to assess the safety of homeopathic drugs.[73] Homeopathic medicines are obtained from a wide range of natural or synthetic sources. In homeopathy, various sources for preparing medicines include plant parts (roots, stems, leaves, flowers, bark, pollen, lichen, moss, ferns, algae etc), microorganisms (fungi, bacteria, viruses and plant parasites), and other sources like animal organs, tissues, secretions and cell lines etc. In certain cases the source may present potential safety hazards even if they are used at high dilutions.[74]

The authenticity, quality and purity of homeopathic drugs are established by reference criteria given in the monographs in the homeopathic pharmacopoeia. These monographs prescribe physical, chemical, biological, standards for the drugs. The important standards mentioned in the Indian Homeopathic pharmacopoeia are shown in figure 1.

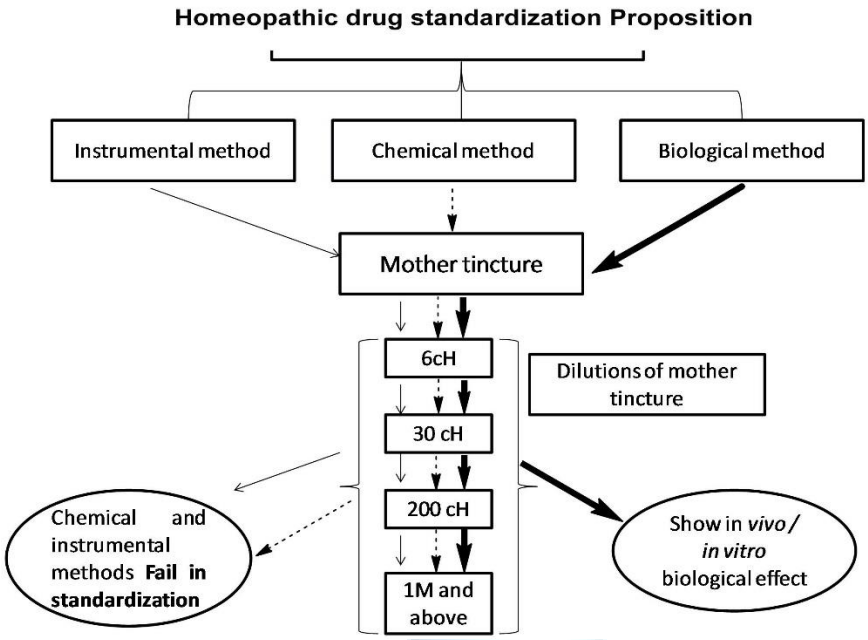


**Figure 1:** Homeopathic Drug Standardization Proposition

## Challenges in the standardization of homeopathic drugs and their dilutions

The skepticisms regarding the quality of homeopathic drugs is reinforced due to intrinsic difficulties in determining the uniformity of contents of homeopathic drugs and their dilutions. Still, the use of homeopathic medicines remains widespread all over the world, including developing and developed countries.[75] Policy-makers, health professionals and the end user question the safety, efficacy, quality, bioavailability and stability of homeopathic drugs alike.[76]

There has been a need of parallel development of standards and assay methods for determining quality, efficacy and safety in case of homeopathy. This is especially true of medicines derived from natural sources, where the effectiveness and quality is influenced by numerous factors.[77] Insufficient research in the homeopathic drug standardization methods and the methods to manufacture high dilutions is proving detrimental to the widespread acceptance of homeopathy as a therapeutic system. This has led to decrease in the interest of modern researchers in the homeopathic drug research.[76] Further, there are discrepancies in the pharmacopoeial monographs and the pharmacopoeias of different countries describe different methods of preparations to achieve the 1x dilution. In the Homeopathic Pharmacopoeia of India, mother tincture itself is considered as 1x dilution whereas, German and France homeopathic Pharmacopoeias describe 1:4 and 1:9 dilution of mother tincture as 1x dilution.[77] WHO has taken up the steps to harmonize the terminology used in homeopathy to tackle with such discrepancies which make the process of standardization complex.[77]

The identity, quality and authenticity of starting material are the major issues related to the homeopathic drugs prepared from the plants and herbs.[78] The natural, biological and geographical variation of starting material is responsible for the variation in the quality of the material derived from natural origins. For homeopathic mother tinctures, the quality of source material is tested through specific chemical tests and chromatographic analysis for the presence of certain chemical markers.[79] The detection and quantification of chemical markers is performed before the processing of the source material for manufacturing. Specific chemical tests and sophisticated instrumental methods like HPLC, HPTLC and GC-MS etc. are inducted in the confirmation of the identity of the source material. These tests also help to determine possible contaminants and toxic constituents in the formulation. The homeopathic pharmacopoeial monographs prescribe determination of physicochemical properties, certain markers and chromatographic techniques for standardization of mother tinctures.[80] However, prior to formulation, the raw material used in homeopathic preparations should be characterized to determine the origin, the history and the nature of the starting material in terms of,

- Identification through morphological and microscopic characteristics (For botanicals)
- Source of origin, if of biological origin, by the physical, anatomical and histological studies (For animal and patient derived medicines) and
- Physical form, physiochemical properties, structural formula and relative molecular mass (For chemical and mineral origins).[77]

## Homeopathic medicines from plant origin

Consistency of composition and reproducibility of biological actions are the prerequisites for safety and therapeutic use of any drug. However, herbal drugs frequently fail to meet these criteria due to difficulties in the identification, genetic variability, variations in environmental conditions, harvesting procedures, and difference in the processing of the extracts. Apart from these factors, the lack of scientifically validated information on active principles mainly hampers the process of quality control.[81]

The use of chromatographic techniques and marker compounds for the standardization of herbal products can ensure batch-to-batch consistency; however, this does not ensure consistent pharmacological activity or stability. The chemical composition of plant material varies according to the age of plant, environmental conditions, geographical locations, and methods of collection and storage.[82] Hence, inadequate quality control measures introduce batch-to-batch variations in the formulations. Even the revised monographs prescribed in the recent versions of homeopathic pharmacopoeias still contain mainly morphological, chemical and chromatographic methods for standardization of drugs. There is a scope for to improvise these monographs by including the detection and quantification of the validated chemical markers of the source material. For example, in case of *Toxicodendron pubescens* (Poison ivy), the proposed active constituent- 'urushiol' itself is a mixture of multiple polyphenolic compounds.[83] It is important to note here that the pharmacopoeial monograph of *Toxicodendron pubescens* prescribes use of



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rutin and quercitrin as biomarkers and not the urushiol.[84] There is a need to substantiate correlation between the content of rutin/ quercitrin with the anti-inflammatory efficacy of *Toxicodendron pubescens.* Such validated chemical markers responsible for biological activity of the drugs may revolutionize the standardization of the homeopathic drugs.

## Homeopathic medicines from animal origin

The use of homeopathic medicines derived from animal or human origins raise serious issues of standardization. It is recognized that the potency of complex biologicals needs to be tested by comparative methods against a stable standards.[85] Standardization of *Apis mellifica, Susscrofa* cartilage and *Lachesis muta* which are sources of anti-inflammatory homeopathic medicines is inevitable.

There have been unsuccessful efforts to correlate the biological activities of drugs from natural origins with their chemical compositions. The best example is of honey and its antioxidant activity. Honey contains at least 200 highly complex phytochemicals, the composition of which depends on floral and geographical origins. Due to such diverse sources, honey can't be standardized in terms of chemical composition as it contains diverse phyto-constituents coming from the plant sources. Still, there is a possibility that its antioxidant capacities might be useful in its standardization. This is how, assay of biological effects can be introduced in drug standardization. [85]

Wild boar or wild pig (*Susscrofa*) is a species of the pig genus *Sus*, a member of the biological family *Suidae*. The cartilage of *Susscrofa*is used in preparation of an anti-inflammatory homeopathic medicine. The species include many subspecies. It is the wild ancestor of the domestic pig, an animal with which it freely hybridizes.[86,87] The genetic composition of *Susscrofa* varies from region to region and obviously, the tissues used from it also vary in their composition.[87] Similar problem is evident in the standardization homeopathic drug derived from the poison of *Lachesis muta*, a venomous pit viper species found in South America.[88] *Lachesis muta* poison is used in high dilutions in the treatment of inflammatory conditions. There is no standardized method for isolation of venom from the *Lachesis muta*. The yield of *Lachesis* venom depends upon the extraction method. It is probable that the toxicity of venom would also get affected by the process of extraction. The poison extraction potency from viper might be altered due to stress.[89, 90]

## Homeopathic medicines from mineral origin

Dependent upon the sources and origin, the mineral drugs are bound to vary in their physicochemical properties and contents of impurities. The homeopathic pharmacopoeias don't specify the origins of mineral medicines. Further, monographs of such drugs of mineral origin lack in specific and sensitive assay strategies to work out their purity and efficacy. The properties of minerals are bound to vary dependent upon their physical structure (amorphous/ crystalline), solubility, and presence of impurities.

## Standardization of high dilutions used in homeopathy

The most controversial issue related to the homeopathic medicines is the use of high dilutions that too beyond an extent where

molecules of source material do not exist in the solution. From simple calculations, it can be substantiated that, at least few molecules of the source material are present in homeopathic dilutions beyond 12cH levels. Interestingly, there are many *in-vitro* studies proving significant and reproducible efficacy of homeopathic drugs even as higher dilutions.[91, 92]

Homeopathic pharmacopoeias throughout the world appear to be silent on the issue of standardization and quality control of the high dilutions used in homeopathic medicines. The quality of such high dilutions is deduced only from the claims of manufacturers regarding their methods of preparation. However, in the absence of any quality control methods and standardization parameters, the quality of highly diluted homeopathic medicines always remains questionable.

## The necessity of biological standardization of homeopathic medicines

In homeopathy, the standardization and quality control on medicinal formulations is an immensely controversial subject. In this review, we have highlighted the inadequacy of the chemical and instrumental analytical assays in standardization and quality control of the homeopathic medicines including high dilutions. There is a dearth of validated markers for the identification of the source materials used in the homeopathic drugs. At present, the use of chemical/ analytical methods as prescribed in the homeopathic pharmacopoeias is imperative due to the scarcity of judicious experimental studies which can reproducibly correlate the presence of active principles with the biological activity of homeopathic drugs. There are very few studies where efforts have been directed to confirm the reproducibility of the biological assays used to prove the efficacy of homeopathic drugs. Even such assays yield variable results if there are minute alterations in the assay conditions.[93]

The degranulation and histamine release assays on human basophils exemplify a couple of methods which may have place in the biological standardization of the homeopathic drugs. There is a need of validating such assays to prove whether the high dilutions of different anti-inflammatory homeopathic drugs mentioned in this review exert reproducible alterations in human basophils degranulation and induced histamine release from them. There is an urgent need of undertaking multi-centered experimental trials using human basophil degranulation assay and histamine release assays for anti-inflammatory homeopathic drugs and their dilutions. If validated, such biological assays not only provide a method for standardization of high dilutions of homeopathic drugs but also provide scientifically acceptable proof of the efficacy of high dilutions of the homeopathic drugs.

It is not feasible to maintain uniformity in the environmental conditions, quality of the raw material and the process of succussion at all the manufacturing units. Even the composition of the material of the containers is bound to vary. Hence, uniformity in the formulations can only be achieved through the in process quality control and testing of finalized products through validated assay methods which can prove the presence of active constituents in the low dilutions and

presence of biological efficacy in the high dilutions.

To support such claims and to bring uniformity in the biological efficacy of these medicines, it is imperative that sensitive biological assays related to the claimed efficacy are used in standardization of these medicines. The biological assays in association with the chromatographic fingerprinting procedure can add to the correlation of the molecular fingerprint with the biological efficacy of mother tinctures. In case of anti-inflammatory activity, numerous validated assay methods are available to test the efficacy of drugs. Table 4 summarizes some of the *in-vitro* and *in-vivo* biological assays which can be employed in the standardization of homeopathic anti-inflammatory drugs. However, there is a need that even these assays are validated and the biological assays which encompass the characteristic biological activity of individual homeopathic drugs are implemented in their standardization.

| Methods | Principle | References |
|---|---|---|
| ***In vitro*** | | |
| **LPS induced cytokine release from blood cells** | Lipopolysaccharide (LPS) stimulated the release of cytokines and inflammatory mediators | 96, 97 |
| **Particulate matter induces release of interleukin (IL)** | Particulate matter induced expression of *IL-8* | 98 |
| **Cyclooxygenase assay (COX) assay** | COX-2 is inducible by cytokines and growth factors, and induction of COX-2 is linked to inflammatory cell types and tissues.<br>COX-2 is believed to be the target for the anti-inflammatory actions of NSAIDs | 99 |
| **Human whole blood cell culture cytokine release assay** | Lipopolysaccharide induced release of cytokine from culture whole blood cells | 100 |
| **In vitro Polymorphonuclear cell (PMN) functions like chemotaxis, phagocytosis and oxidative burst induced by stimulants like lipopolysaccharides** | N-formyl-methionyl-leucyl-phenylalanine (fMLP) induced chemotaxis, and generation of reactive oxygen radicals from PMN cells | 101,102 |
| **Leukotriene B4 assay** | Leukotriene B4, pro-inflammatory mediator synthesized in myeloid cells from arachidonic acid | 103,104 |
| ***In vivo*** | | |
| **Ultraviolet erythema in guinea pigs venules *in vivo*** | UV irradiation induces vasodilation of cutaneous blood vessels resulting in erythema | 105 |
| **Carrageenan-induced paw edema in rats** | Release of inflammatory mediators. Initial phase elevation of histamine and serotonin. Late phase release of prostaglandin, Protease, Lysosome | 106 |
| **Carrageenan-induced Vascular permeability in rats** | Accumulation of prostaglandins (PGs) leads to vascular permeability[94] | 107 |
| **Inhibition of leukocyte adhesion to rat mesenteric venule *in vivo*** | Reactive oxygen metabolites (ROMs) as potential mediators of TNF-□ stimulated neutrophil adhesion to rat mesenteric venules *in vivo* | 108, 109 |
| **Oxazolone-induced ear edema in mice** | Oxazolone-induced delayed contact hypersensitivity in mice can be quantitated and used for gradation of topical and systemic anti-inflammatory activity [97] | 110 |
| **Croton-oil ear edema in rats** | Croton oil contains 12-o-tetracanoilphorbol-13-acetate (TPA). TPA | 111,112 |

| | | |
|---|---|---|
| **and mice.** | activates protein kinase C (PKC) which consequently activates mitogen activated protein kinases (MAPK), phospholipase A2 (PLA2) leading to release of platelet activation factor (PAF) and arachidonic acid | |
| **Granuloma pouch technique** | Subcutaneous air pouch induced granuloma and injection of phlogistic agents into the air pouch provides advantage of quantitation of fluid extravasation, leucocyte migration and inflammatory meditors. | 113 |

**Table 4:** Proposed biological assays for standardization of anti-inflammatory homeopathic drugs

The homeopathic mother tinctures can be standardized using the monographs prescribed in the pharmacopoeias of respective countries. There is a vast scope to modernize these monographs to include more sensitive and specific detection techniques like HPTLC fingerprinting, HPLC, Mass spectroscopy etc. Recent advances in the DNA finger printing can provide gold standard for identification of natural resources. If there are known correlations between biological activity and the chemical constituents then the content of such validated constituents can be used for standardization of mother tinctures. In addition to this, additional testing must be included to determine the safety of these formulations. Particularly, in case of the nosodes which contain dilutions of material collected from pathological secretions/organisms, there should be strict control over the microbial load and the nosode formulations must comply with the specified limits of the microbial contents.

## Conclusion

The proponents of modern medicines and skeptics criticize homeopathy for the lack of validations and non-reproducibility of biological effects. The efficacy of homeopathic medicines is ascribed either to the placebo effect of to the systematic consultations given to the patients by the homeopaths.[112,113]. Lack of faith in homeopathic medication is principally attributable to the shortage of standardized ways to prove the precise therapeutic interpretation. The homeopathic pharmacopoeias prescribe methods of standardization only for mother tinctures. There is no validated method available for standardization of homeopathic drugs except the guideline for the preparation of high dilutions. However, such inadequate specifications create loopholes in the process of standardization of homeopathic drugs and their dilutions.

Recent delineation of molecular mechanisms involved in the biological processes like inflammation help in pinpointing the probable targets of drug actions. For individual drugs, if the affected biological processes are determined through systematic evaluations, a battery of tests can be suggested for standardization of homeopathic drugs including ultra-high dilutions. Numerous reports on proving the biological activities of high dilutions pose a challenge to the pharmacologists to either reproduce such results and confirm the efficacy of the homeopathic drugs or to systematically refute such claims through a series of controlled and validated experiments. In the light of numerous experimental evidencens on the efficacy of homeopathic drugs and their high dilutions, it is suggested that the validated biological assays must be included in the standardization and quality control of the

homeopathic medicines and their high dilutions.

## Conflict of interest

None declared.

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